13 Aromatherapy with Essential Oils

Maria Lis-Balchin

CONTENTS

13.1 INTRODUCTION

13.1.1 Aromatherapy Practice in the United Kingdom and the United States

Aromatherapy has become more of an art than a science. This is mostly due to the health and beauty industries, which have taken over the original concept as a money-spinner in the United Kingdom, United States, and almost all other parts of the world. There are virtually thousands of "aromatherapy" products in pharmacies, high street shops, supermarkets, hair salons, and beauty salons. The products are supposedly made with "essential oils" (which are usually perfumes) and include skin creams, hair shampoos, shower gels, moisturizers, bath salts, lotions, candles, as well as essential oils themselves.

Many aromatherapy products, such as perfumes, are also linked with sexual attractiveness. There are numerous "health and beauty" salons or clinics that offer aromatherapy as part of their "treatments" together with waxing, electrolysis, massage (of various types, including "no-hands massage"), facial treatments including botox, manicures and pedicures, eyes and eyebrow shaping, ear-piercing, tanning, and makeup application. Often hundreds of these "therapies" are offered in one small shop, with aromatherapy thrown in. Most people, especially men, consider aromatherapy to be a sensual massage with some perfumes given all over the body by a young lady. This is often the case, although aromatherapy massage is often provided just on the back or even just on the face and hands for busy people. The use of pure essential oils both in such beauty massage and all the aromatherapy products on sale everywhere is very doubtful (because of the cost) but the purchaser believes the advertisements assuring pure oil usage. Beauty consultants/therapists use massage skills and a nice odor simply for relaxation; they sometimes include beautifying treatments using specific essential oils as initiated by Marguerite Maury (1989). Aromatherapy has thus become an art.

However, aromatherapists (who have studied the "science" for 3 h, a week, a year, or even did a 3-year degree) are keen to bring science into this alternative "treatment." The multitude of books written on the subject, aromatherapy journals, and the web sites all consider that there has been enough proof of the scientific merit of aromatherapy. They quote studies that have shown *no* positive or statistically significant effects as proof that aromatherapy works. The actual validity of these claims will be discussed later and several publications criticized this on scientific grounds. Aromatherapy is often combined with "counseling" by a "qualified" therapist, with no counseling qualifications. Massaging is carried out using very diluted plant essential oils (2–5 drops per 10 mL of carrier oil, such as almond oil) on the skin—that is, in almost homeopathic dilutions! But they believe that the essential oils are absorbed and go straight to the target organ where they exert the healing effect. Many aromatherapists combine their practice with cosmology, crystals, colors, music, and so on. These may also be associated with a commercial sideline in selling "own trademark" essential oils and associated items, including diffusers, scented candles, and scented jewelry.

13.2 DEFINITIONS OF AROMATHERAPY

Aromatherapy is defined as "the use of aromatic plant extracts and essential oils in massage and other treatment" (*Concise Oxford Dictionary*, 1995). However, there is no mention of massage or the absorption of essential oils through the skin and their effect on the target organ (which is the mainframe

of aromatherapy in the United Kingdom and the United States) in *Aromatherapie* (Gattefossé, 1937/1993). This was where the term "aromatherapy" was coined after all, by the "father of aromatherapy"—but was actually based on the odor of essential oils and perfumes and their antimicrobial, physiological, and cosmetological properties (Gattefossé, 1928, 1952, 1937/1993). "Pure" essential oils were of no concern to Gattefossé. Recently, definitions have begun to encompass the effects of aromatherapy on the mind as well as on the body (Lawless, 1994; Worwood, 1996, 1998; Hirsch, 1998).

13.3 INTRODUCTION TO AROMATHERAPY CONCEPTS

The original concept of modern aromatherapy was based on the assumption that the volatile, fat-soluble essential oil was equivalent in bioactivity to that of the whole plant when inhaled or massaged onto the skin. Information about the medicinal and other properties of the plants was taken from old English herbals (e.g., Culpeper, 1653), combined with some more esoteric nuances involving the planets and astrology (Tisserand, 1977).

This notion is clearly flawed. As an example, a whole orange differs from just the essential oil (extracted from the rind alone) as the water-soluble vitamins (thiamine, riboflavin, nicotinic acid, and vitamins C and A) are excluded, as are calcium, iron, proteins, carbohydrates, and water. Substantial differences in bioactivity are found in different fractions of plants, for example, the essential oils of *Pelargonium* species produced a consistent relaxation of the smooth muscle of the guinea pig *in vitro*, whereas the water-soluble extracts did not (Lis-Balchin, 2002b). Botanical misinterpretations are also common in many aromatherapy books, for example, "geranium oil" bioactivity is based on Herb Robert, a hardy *Geranium* species found widely in European hedgerows, whereas geranium oil is distilled from species of the South African genus *Pelargonium* (Lis-Balchin, 2002a).

13.3.1 Aromatherapy, Aromatology, and Aromachology

Aromatherapy can now be divided into three "sciences": aromatherapy, aromatology, and aromachology.

Aromachology [coined by the Sense of Smell Institute (SSI), USA, 1982] is based on the interrelationship of psychology and odor, that is, its effect on specific feelings (e.g., relaxation, exhilaration, sensuality, happiness, and achievement) by its direct effect on the brain.

Aromatherapy is defined by the SSI as "the therapeutic effects of aromas on physical conditions (such as menstrual disorders, digestive problems, etc.) as well as psychological conditions (such as chronic depression)." The odor being composed of a mixture of fat-soluble chemicals may thus have an effect on the brain via inhalation, skin absorption, or even directly via the nose.

Aromatology is concerned with the internal use of oils (SSI). This is similar to the use of aromatherapy in most of Europe, excluding the United Kingdom; it includes the effect of the chemicals in the essential oils via oral intake, or via the anus, vagina, or any other possible opening by medically qualified doctors or at least herbalists, using essential oils as internal medicines.

There is a vast difference between aromatherapy in the United Kingdom and that in continental Europe (aromatology): the former is "alternative" while the latter is "conventional." The "alternative" aromatherapy is largely based on "healing," which is largely based on belief (Millenson, 1995; Benson and Stark, 1996; Lis-Balchin, 1997). This is credited with a substantial placebo influence. However, the placebo effect can be responsible for results in both procedures.

13.3.2 Scientifically Accepted Benefits of Essential Oils versus the Lack of Evidence for Aromatherapy

There is virtually no scientific evidence, as yet, regarding the direct action of essential oils, applied through massage on the skin, on specific internal organs—rather than through the odor pathway leading into the mid-brain's "limbic system" and then through the normal sympathetic and

parasympathetic pathways. This is despite some evidence that certain components of essential oils can be absorbed either through the skin or lungs (Buchbauer et al., 1992; Jager et al., 1992; Fuchs et al., 1997).

Many fragrances have been shown to have an effect on mood and, in general, pleasant odors generate happy memories, more positive feelings, and a general sense of well-being (Knasko et al., 1990; Knasko, 1992; Warren and Warrenburg, 1993) just like perfumes. Many essential oil vapors have been shown to depress contingent negative variation (CNV) brain waves in human volunteers and these are considered to be sedative (Torii et al., 1988). Others increase CNV and are considered stimulants (Kubota et al., 1992). An individual with anosmia showed changes in cerebral blood flow on inhaling certain essential oils, just as in people able to smell (Buchbauer et al., 1993c), showing that the oil had a positive brain effect despite the patient's inability to smell it. There is some evidence that certain essential oils (e.g., nutmeg) can lower high blood pressure (Warren and Warrenburg, 1993). Externally applied essential oils (e.g., tea tree) can reduce/eliminate acne (Bassett et al., 1990) and athlete's foot (Tong et al., 1992). This happens, however, using conventional chemical effects of essential oils rather than aromatherapy.

Most clients seeking out aromatherapy are suffering from some stress-related conditions, and improvement is largely achieved through relaxation. An alleviation of suffering and possibly pain, due to gentle massage and the presence of someone who cares and listens to the patient, could be beneficial in such cases as in cases of terminal cancer; the longer the time spent by the therapist with the patient, the stronger the belief imparted by the therapist and the greater the willingness of the patient to believe in the therapy, the greater the effect achieved (Benson and Stark, 1996). There is a need for this kind of healing contact, and aromatherapy with its added power of odor fits this niche, as the main action of essential oils is probably on the primitive, unconscious, limbic system of the brain (Lis-Balchin, 1997), which is not under the control of the cerebrum or higher centers and has a considerable subconscious effect on the person. However, as mood and behavior can be influenced by odors, and memories of past odor associations could also be dominant, aromatherapy should not be used by aromatherapists, unqualified in psychology, and so on in the treatment of Alzheimer's or other diseases of aging (Lis-Balchin, 2006).

Proven uses of essential oils and their components are found in industry, for example, foods, cosmetic products, household products, and so on. They impart the required odor or flavor to food, cosmetics and perfumery, tobacco, and textiles. Essential oils are also used in the paint industry, which capitalizes on the exceptional "cleaning" properties of certain oils. This, together with their embalming properties, suggests that essential oils are very potent and dangerous chemicals—not the sort of natural products to massage into the skin!

Why, therefore, should essential oils be of great medicinal value? They are, after all, just chemicals. However, essential oils have many functions in everyday life ranging from their use in dentistry (e.g., cinnamon and clove oils), as decongestants (e.g., *Eucalyptus globulus*, camphor, peppermint, and cajuput) to their use as mouthwashes (e.g., thyme), also external usage as hyperemics (e.g., rosemary, turpentine, and camphor) and anti-inflammatories (e.g., German chamomile and yarrow). Some essential oils are used internally as stimulants of digestion (e.g., anise, peppermint, and cinnamon) and as diuretics (e.g., buchu and juniper oils) (Lis-Balchin, 2006).

Many plant essential oils are extremely potent antimicrobials *in vitro* (Deans and Ritchie, 1987; Bassett et al., 1990; Lis-Balchin, 1995; Lis-Balchin et al., 1996; Deans, 2002). Many are also strong antioxidant agents and have recently been shown to stop some of the symptoms of aging in animals (Dorman et al., 1995a, 1995b). The use of camphor, turpentine oils, and their components as rubefacients, causing increased blood flow to a site of pain or swelling when applied to the skin, is well known and is the basis of many well-known medicaments such as Vicks VapoRub and Tiger Balm. Some essential oils are already used as orthodox medicines: peppermint oil is used for treating irritable bowel syndrome and some components of essential oils, such as pinene, limonene, camphene, and borneol, given orally have been found to be effective against certain internal ailments,

such as gallstones (Somerville et al., 1985) and ureteric stones (Engelstein et al., 1992). Many essential oils have been shown to be active on many different animal tissues *in vitro* (Lis-Balchin et al., 1997b). There are many examples of the benefits of using essential oils by topical application for acne, Alopecia areata, and Athlete's foot (discussed later in Section 13.21), but this is a treatment using chemicals rather than aromatherapy treatment.

Future scientific studies, such as those on Alzheimer's syndrome (Perry et al., 1998, 1999), may reveal the individual benefits of different essential oils for different ailments, but in practice this may not be of utmost importance as aromatherapy massage for relief from stress. Aromatherapy has had very little scientific evaluation to date. As with so many alternative therapies, the placebo effect may provide the largest percentage benefit to the patient (Benson and Stark, 1996). Many aromatherapists have not been greatly interested in scientific research and some have even been antagonistic to any such research (Vickers, 1996; Lis-Balchin, 1997). Animal experiments, whether maze studies using mice or pharmacology using isolated tissues, are considered unacceptable and only essential oils that are "untested on animals" are acceptable, despite all essential oils having been already tested on animals (denied by assurances of essential oil suppliers) because this is required by law before they can be used in foods.

The actual mode of action of essential oils *in vivo* is still far from clear, and clinical studies to date have been scarce and mostly rather negative (Stevenson, 1994; Dunn et al., 1995; Brooker et al., 1997; Anderson et al., 2000). The advent of scientific input into the clinical studies, rather than aromatherapist-led studies, has recently yielded some more positive and scientifically acceptable data (Smallwood et al., 2001; Ballard et al., 2002; Burns et al., 2000; Holmes et al., 2002; Kennedy et al., 2002). The main difficulty in clinical studies is that it is virtually impossible to do randomized double-blind studies involving different odors as it is almost impossible to provide an adequate control as this would have to be either odorless or else of a different odor, neither of which is satisfactory. In aromatherapy, as practiced, there is a variation in the treatment for each client, based on "holistic" principles, and each person can be treated by an aromatherapist with one to five or more different essential oil mixtures on subsequent visits, involving one to four or more different essential oils in each mixture. This makes scientific evaluation almost useless, as seen by studies during childbirth (Burns and Blaney, 1994; see also Section 13.19). There is also the belief among alternative medicine practitioners that if the procedure "works" in one patient, there is no need to study it using scientific double-blind procedures. There is therefore a great bias when clinical studies in aromatherapy are conducted largely by aromatherapists.

Recent European regulations (the seventh Amendment to the European Cosmetic Directive 76/768/EEC, 2002; see Appendices 27 and 28) have listed 26 sensitizers found in most of the common essential oils used: this could be a problem for aromatherapists as well as clients, both in possibly causing sensitization and also resulting in legal action regarding such an eventuality in the case of the client. Care must be taken regarding the sensitization potential of the essential oils, especially when massaging patients with cancer or otherwise sensitive skin. It should also be borne in mind when considering the use of essential oils during childbirth and in other clinical studies (Burns and Blaney, 1994; Burns et al., 2000) that studies in animals have indicated that some oils cause a decrease in uterine contractions (Lis-Balchin and Hart, 1997).

13.4 HISTORICAL BACKGROUND TO AROMATHERAPY

The advent of "aromatherapy" has been attributed to both the Ancient Egyptians and Chinese over 4500 years ago, as scented plants and their products were used in religious practices, as medicines, perfumes, and embalming agents (Manniche, 1989, 1999), and to bring out greater sexuality (Schumann Antelme and Rossini, 2001). But essential oils as such were unlikely to have been used. In Ancient Egypt, crude plant extracts of frankincense, myrrh, or galbanum, and so on were used in an oily vegetable or animal fat that was massaged onto the bodies of workers building the pyramids or the rich proletariat after their baths (Manniche, 1999). These contained essential oils, water-soluble

extractives, and pigments. Incense smoke from resinous plant material provided a more sacrosanct atmosphere for making sacrifices, both animal and human, to the gods. The incense was often mixed with narcotics like cannabis to anesthetize the sacrificial animals, especially with humans (Devereux, 1997). The frankincense extract in oils (citrusy odor) was entirely different to that burnt (church-like) in chemical composition (Arctander, 1960), and therefore would have entirely different functions.

13.4.1 Scented Plants used as Incense in Ancient Egypt

Frankincense (*Boswellia carterii*; *Boswellia thurifera*) (Burseraceae), Myrrh (*Commiphora myrrha; Balsamodendron myrrha; Balsamodendron opobalsamum*) (Burseraceae), Labdanum (*Cistus ladaniferus*), Galbanum (*Ferula galbaniflua*), Styrax (*Styrax officinalis*), or *Liquidambar orientalis*, Balm of Gilhead (*Commiphora opobalsamum*), Sandalwood (*Santalum album*), and Opoponax (*Opoponax chironium*).

Uses included various concoctions of kyphi, burnt three times a day to the sun god Ra: morning, noon, and sunset, in order for him to come back. The ingredients included raisins, juniper, cinnamon, honey, wine, frankincense, myrrh, burnt resins, cyperus, sweet rust, sweet flag, and aspalanthus in a certain secret proportion (Loret, 1887; Manniche, 1989; Forbes, 1955), as shown on the walls of the laboratory in the temples of Horus at Edfu and Philae. Embalming involved odorous plants such as juniper, cassia, cinnamon, cedarwood, and myrrh, together with natron to preserve the body and ensure safe passage to the afterlife. The bandages in which the mummy was wrapped were drenched in stacte (oil of myrrh) and sprinkled with other spices (for further descriptions and uses, see Lis-Balchin, 2006).

The Chinese also used an incense, *hsiang*, meaning "aromatic," made from a variety of plants, with sandalwood being particularly favored by Buddhists. In India, fragrant flowers including jasmine and the root of spikenard giving a sweet scent were used. The Hindus obtained cassia from China and were the first to organize trading routes to Arabia where frankincense was exclusively found. The Hebrews traditionally used incense for purification ceremonies. The use of incense probably spread to Greece from Egypt around the eighth century BC. The Indians of Mesoamerica used copal, a hard, lustrous resin, obtained from pine trees and various other tropical trees by slicing the bark (*Olibanum americanum*). Copal pellets bound to corn-husk tubes would be burnt in hollows on the summits of holy hills and mountains, and these places, blackened by centuries of such usage, are still resorted to by today's Maya in Guatemala (Janson, 1997) and used medicinally to treat diseases of the respiratory system and the skin.

Anointing also involves incense (Unterman, 1991). Queen Elizabeth II underwent the ritual in 1953 at her coronation, with a composition of oils originated by Charles I: essential oils of roses, orange blossom, jasmine petals, sesame seeds, and cinnamon combined with gum benzoin, musk, civet, and ambergris were used (Ellis, 1960). Similarly, musk, sandalwood, and other fragrances were used by the Hindus to wash the effigies of their gods, and this custom was continued by the early Christians. This probably accounts for the divine odor frequently reported when the tombs of early Christians were opened (Atchley and Cuthbert, 1909). The Christian Church was slow to adopt the use of incense until medieval times, when it was used for funerals (Genders, 1972). The reformation reversed the process as it was considered to be of pagan origin but it still survives in the Roman Catholic Church. Aromatic substances were also widely used in magic (Pinch, 1994).

13.5 PERFUME AND COSMETICS: PRECURSORS OF COSMETOLOGICAL AROMATHERAPY

The word "perfume" is derived from the Latin *per fumare*: "by smoke." The preparation of perfumes in Ancient Egypt was done by the priests, who passed on their knowledge to new priests (Manniche, 1989, 1999). Both high-class people like Nefertiti and Cleopatra used huge amounts of

fragranced materials as unguents, powders, and perfumes and the workers building the great pyramids, who even went on strike when they were denied their allocation of "aromatherapy massage oil" (Manniche, 1999).

13.5.1 Three Methods of Producing Perfumed Oils by the Egyptians

Enfleurage involved steeping the flowers or aromatics in oils or animal fats (usually goat) until the scent from the materials was imparted to the fat. The impregnated fat was often molded into cosmetic cones and used for perfuming hair wigs, worn on festive occasions, which could last for 3 days; the fat would soften and start melting, spreading the scented grease not only over the wig, but also over the clothes and body—more pleasing than the stench of stale wine, food, and excrement (Manniche, 1999).

Maceration was used principally for skin creams and perfumes: flowers, herbs, spices, or resins were chopped up and immersed in hot oils. The oil was strained and poured into alabaster (calcite) containers and sealed with wax. These scented fatty extracts were also massaged onto the skin (Manniche, 1999).

Expression involved putting flowers or herbs into bags or presses, which extracted the aromatic oils. Expression is now only used for citrus fruit oils (Lis-Balchin, 1995). Wine was often included in the process and the resulting potent liquid was stored in jars. These methods are still used today.

Megaleion, an Ancient Greek perfume described by Theophrastus who believed it to be good for wounds, was made of burnt resins and balanos oil, and boiled for 10 days before adding cassia, cinnamon, and myrrh (Groom, 1992). Rose, marjoram, sage, lotus flower, and galbanum perfumes were also made. Apart from these, aromatic oils from basil, celery, chamomile, cumin, dill, fenugreek, fir, henna, iris, juniper, lily, lotus, mandrake, marjoram, myrtle, pine, rose, rue, and sage were sometimes used in perfumes or as medicines taken internally and externally.

Dioscorides, in his *De Materia Medica*, discussed the components of perfumes and their medicinal properties, providing detailed perfume formulae. Alexandrian chemists were divided into three schools, one of which was the school of Maria the Jewess, which produced pieces of apparatus for distillation and sublimation, such as the *bain Marie*, useful for extracting the aromatic oils from plant material. Perfumes became more commonly known in medieval Europe as knights returning from the Crusades brought back musk, floral waters, and a variety of spices.

13.6 MEDICINAL USES: PRECURSORS OF AROMATOLOGY OR "CLINICAL" AROMATHERAPY

The ancient use of plants, not essential oils, can be found in fragments of Egyptian herbals. The names of various plants, their habitats, characteristics, and the purposes for which they were used are included in the following: *Veterinary papyrus* (*ca.* 2000 B.C.), *Gynaecological papyrus* (*ca.* 2000 B.C.), *Papyrus Edwin Smith* (an army surgeon's manual, *ca.* 1600 B.C.), *Papyrus Ebers* (includes remedies for health, beauty, and the home, *ca.* 1600 B.C.), *Papyrus Hearst* (with prescriptions and spells, *ca.* 1400 B.C.), and *Demotic medical papyri* (second century B.C. to first century A.D.).

Magic was often used as part of the treatment and gave the patient the expectation of a cure and thus provided a placebo effect (Pinch, 1994). The term "placing the hand" appears frequently in a large number of medical papyri; this probably alludes to the manual examination in order to reach a diagnosis but could also imply cure by the "laying on of hands," or even both (Nunn, 1997). This could be the basis of modern massage (with or without aromatherapy). It is certainly the basis of many alternative medicine practices at present (Lis-Balchin, 1997).

Plants were used in numerous ways. Onions were made into a paste with wine and inserted into the vagina to stop a woman menstruating. Garlic ointment was used to keep away serpents and

snakes, heal dog-bites, and bruises; raw garlic was given to asthmatics; fresh garlic and coriander in wine was a purgative and an aphrodisiac! Juniper mixed with honey and beer was used orally to encourage defecation; and origanum was boiled with hyssop for a sick ear (Manniche, 1989).

Egyptians also practiced inhalation by using a double-pot arrangement whereby a heated stone was placed in one of the pots and a liquid herbal remedy poured over it. The second pot, with a hole in the bottom through which a straw was inserted, was placed on top of the first pot, allowing the patient to breathe in the steaming remedy (Manniche, 1989), that is, aromatherapy by inhalation.

13.6.1 Middle Ages: Use of Aromatics and Quacks

In the twelfth century, the Benedictine Abbess Hildegard of Bingen (1098–1179) was authorized by the Church to publish her visions on medicine (*Causae et Curae*), dealing with the causes and remedies for illness (Brunn and Epiney-Burgard, 1989). The foul smell of refuse in European towns in the seventeenth century was thought to be the major cause of disease, including the plague (Classen et al., 1994), and aromatics were used for both preventing and in some cases curing diseases; herbs such as rosemary were in great demand and sold for exorbitant prices as a prophylactic against the plague (Wilson, 1925). People forced to live near victims of the plague would carry a pomander, which contained a mixture of aromatic plant extracts. Medical practitioners carried a small cassolette or "perfume box" on the top of their walking sticks, when visiting contagious patients, which was filled with aromatics (Rimmel, 1865). Some physicians wore a device filled with herbs and spices over their nose when they examined plague patients (Wilson, 1925). These became known as "beaks" and it is from this that the term "quack" developed.

Apothecaries were originally wholesale merchants and spice importers, and in 1617 the Worshipful Society of Apothecaries was formed, under the control of the London Royal College of Physicians, which produced an "official" pharmacopoeia specifying the drugs the apothecaries were allowed to dispense. The term "perfumer" occurs in some places instead of "apothecary" (Rimmel, 1865).

John Gerard (1545–1612) and Nicholas Culpeper (1616–1654) were two of the better-known apothecaries of their time. Nicholas Culpeper combined healing herbs with astrology as he believed that each plant, like each part of the body, and each disease, was governed or under the influence of one of the planets: rosemary was believed to be ruled by the Sun, lavender by Mercury, and spearmint by Venus. Culpeper also adhered to the Doctrine of Signatures, introduced by Paracelsus in the sixteenth century, and mythology played a role in many of the descriptive virtues in Culpeper's herbal. This astrological tradition is carried through by many aromatherapists today, together with other innovations such as ying and yang, crystals, and colors.

Culpeper's simple or distilled waters and oils (equivalent to the present hydrosols) were prepared by the distillation of herbs in water in a pewter still, and then fractionating them to separate out the essential or "chymical" oil from the scented plants. The plant waters were the weakest of the herbal preparations and were not regarded as being beneficial. Individual plants such as rose or elderflower were used to make the corresponding waters, or else mixtures of herbs were used to make compound waters (Culpeper, 1826/1981; Tobyn, 1997). Essential oils of single herbs were regarded by Culpeper as too strong to be taken alone, due to their vehement heat and burning, but had to be mixed with other medicinal preparations. Two or three drops were used in this way at a time. Culpeper mentioned the oils of wormwood, hyssop, marjoram, the mints, oregano, pennyroyal, rosemary, rue, sage, thyme, chamomile, lavender, orange, and lemon. Spike lavender, not *Lavandula angustifolia*, is used in aromatherapy nowadays. Herbs such as dried wormwood and rosemary were also steeped in wine and set in the sun for 30–40 days to make a "physical wine." The "herbal extracts" mentioned in the herbals were mostly water soluble and at best, alcoholic extracts, none of which are equivalent to essential oils, which contain many potent chemical components are not found in essential oils.

13.7 MODERN PERFUMERY

In the fourteenth century, alcohol was used for the extraction and preservation of plants, and oleum mirable, an alcoholic extract of rosemary and resins, was later popularized as "Hungary water," without the resins (Müller et al., 1984).

In the sixteenth century, perfumes were made using animal extracts, which were the base notes or fixatives, and made the scent last longer (Piesse, 1855). Among these ingredients were ambergris, musk, and civet.

Perfumes came into general use in England during the reign of Queen Elizabeth (1558–1603). Many perfumes, such as rose water, benzoin, and storax, were used for sweetening the heavy ornate robes of the time, which were impossible to wash. Urinals were treated with orris powder, damask rose powder, and rose water. Bags of herbs, musk, and civet were used to perfume bath water.

Elizabeth I carried a pomander filled with ambergris, benzoin, civet, damask rose, and other perfumes (Rimmel, 1865) and used a multitude of perfumed products in later life. Pomanders, from the French *pomme d'amber* ("ball of ambergris"), were originally hung in silver perforated balls from the ceiling to perfume the room. The ingredients such as benzoin, amber, labdanum, storax, musk, civet, and rose buds could be boiled with gum tragacanth and kneaded into balls; the small ones were made into necklaces.

Various recipes were used for preparing aromatic waters, oils, and perfumes. Some of these were for perfumes and some undoubtedly for alcoholic beverages, as one of the major ingredients for many concoctions was a bottle or two of wine, which when distilled produced a very alcoholic brew.

Ambergris, musk, and civet went out of fashion, as the excremental odors could not be reconciled with modesty (Corbin, 1986). The delicate floral perfumes became part of the ritual of bodily hygiene, gave greater variety, and allowed Louis XV a different perfume every day. Today the sentiment "odours are carried in bottles, for fear of annoying those who do not like them" (Dejeans, 1764) is reemerging as more and more people are becoming sensitive to odors, giving them headaches, asthma, and migraines.

The Victorians liked simple perfumes made of individual plant extracts. Particular favorites were rose, lavender, and violet. These would be steam distilled or extracted with solvents. The simple essential oils produced would often be blended together to produce perfumes like eau de Cologne (1834).

The first commercial scent production was produced in the United Kingdom, in Mitcham, Surrey, in the seventeenth century, using lavender (Festing, 1989). In 1865, cinnamaldehyde, the first synthetic, was made. Adulteration and substitution by the essential oil or component of another plant species became rampant. Aroma chemicals synthesized from coal, petroleum by-products, and terpenes are much cheaper than the equivalent plant products, so perfumes became cheap.

The way was now open for the use of scent in the modern era. It seems therefore a retrograde step to use pure essential oils in "aromatherapy," especially as the "father of aromatherapy," René-Maurice Gattefossé, used scents or deterpenated essential oils.

13.8 AROMATHERAPY PRACTICE

Aromatherapists usually treat their clients (patients) after an initial full consultation, which usually involves taking down a full medical case history. The aromatherapist then decides what treatment to give, which usually involves massage with three essential oils, often one each chosen from those with top, middle, and base perfumery notes, which balances the mixture. Sometimes only "specific" essential oils for the "disease" are used. Most aromatherapists arrange to see the client 3–5 times and the mixture will often be changed on the next visit, if not on each visit, in order to treat all the possible symptoms presented by the client (holistically), or simply as a substitute when no improvement was initially obtained. Treatment may involve other alternative medicine procedures, including chakras.

Many aromatherapists offer to treat any illness, as they are convinced that essential oils have great powers. They embark on the treatment of endometriosis, infertility, asthma, diabetes, and arthritis, even cancer, as they are convinced of the therapeutic nature of essential oils, but are often without the necessary scientific and medical knowledge. "Psychoneuroimmunology" treatment is the current buzzword.

Although aromatherapists consider themselves professionals, there is no Hippocratic oath involved. The aromatherapist, being nonmedically qualified, may not even be acquainted with most of the illnesses or symptoms, so there could be a very serious mistake made as potentially serious illnesses could be adversely affected by being "treated" by a layperson. Some, but not all, aromatherapists ask the patients to tell their doctor of the aromatherapy treatment. Counseling is greatly recommended by aromatherapy schools. Aromatherapists are not necessarily, however, trained in counseling, and with few exceptions could do more damage than good, especially when dealing with psychiatric illness, cases of physical or drug abuse, people with learning difficulties, and so on, where their "treatment" should only be complementary and under a doctor's control (Lis-Balchin, 2006).

13.8.1 Methods of Application of Aromatherapy Treatment

Various methods are used to apply the treatment in aromatherapy. The most usual methods are the following:

- A diffuser, usually powered by electricity, giving out a fine mist of the essential oil.
- A burner, with water added to the fragrance to prevent burning of the essential oil. About 1–4 drops of essential oil are added to about 10 mL water. The burner can be warmed by candles or electricity. The latter would be safer in a hospital or a children's room or even a bedroom.
- Ceramic or metal rings, placed on an electric light bulb with a drop or two of essential oil. This results in a rapid burnout of the oil and lasts for a very short time due to the rapid volatilization of the essential oil in the heat.
- A warm bath with drops of essential oil added. This results in the slow volatilization of the essential oil, and the odor is inhaled via the mouth and nose. Any effect is not likely to be through the absorption of the essential oil through the skin as stated in aromatherapy books, as the essential oil does not mix with water. Droplets either form on the surface of the water, often coalescing, or else the essential oil sticks to the side of the bath. Pouring in an essential oil mixed with milk serves no useful purpose as the essential oil still does not mix with water, and the premixing of the essential oil in a carrier oil, as for massage, just results in a nasty oily scum around the bath.
- A bowl of hot water with drops of essential oil, often used for soaking feet or used as a bidet. Again the essential oil does not mix with the water. This is, however, a useful method for inhaling essential oils in respiratory conditions and colds; the essential oil can be breathed in when the head is placed over the container and a towel placed over the head and container. This is an established method of treatment and has been used successfully with Vicks VapoRub, obas oil, and *Eucalyptus* oils for many years, so it is not surprising that it works with aromatherapy essential oils!
- Compresses using essential oil drops on a wet cloth, either hot or cold, to relieve inflammation, treat wounds, and so on. Again, the essential oil is not able to mix with the water and can be concentrated in one or two areas, making it a possible health hazard.
- Massage of hands, feet, back, or all over the body using 2–4 drops of essential oil (single essential oil or mixture) diluted in 10 mL carrier oil (fixed, oily), for example, almond oil or jojoba oil, grapeseed, wheat-germ oils, and so on. The massage applied is usually by gentle effleurage with some petrissage (kneading), with and without some shiatsu, lymph

drainage in some cases, and is more or less vigorous, according to the aromatherapist's skills and beliefs.

- Oral intake is more like conventional than "alternative" usage of essential oils. Although it is practiced by a number of aromatherapists, this is not to be condoned unless the aromatherapist is medically qualified. Essential oil drops are "mixed" in a tumbler of hot water or presented on a sugar cube or "mixed" with a teaspoonful of honey and taken internally. The inability of the essential oil to mix with aqueous solutions presents a health hazard, as do the other methods, as such strong concentrations of essential oils are involved.

13.9 MASSAGE USING ESSENTIAL OILS

The most popular method of using aromatherapy is through massage. The first written records referring to massage date back to its practice in China more than 4000 years and in Egypt. Hippocrates, the father of modern medicine, wrote, "the physician must be experienced in many things, but most assuredly in rubbing."

Massage has been used for centuries in Ayurvedic medicine in India as well as in China and shiatsu, acupressure, reflexology, and many other contemporary techniques have their roots in these sources. Massage was used for conventional therapeutic purposes in hospitals before World War II and is still used by physiotherapists for various conditions including sports injuries.

René-Maurice Gattefossé, credited as being the founding father of modern aromatherapy, never made a connection between essential oils and massage. It was Marguerite Maury who advocated the external use of essential oils combined with carrier oils (Maury, 1989). She used carefully selected essential oils for cleansing the skin, including that in acne, using a unique blend of oils for each client created specifically for the person's temperament and health situation. Maury's main focus was on rejuvenation; she was convinced that aromas could be used to slow down the aging process if the correct oils were chosen. In recent experiments on animals, it has been shown that the oral intake of some antioxidant essential oils can appear to defer aging, as indicated by the composition of membranes in various tissues (Youdim and Deans, 2000).

Massage *per se* can be a relaxing experience and can help to alleviate the stresses and strains of daily life. In a review of the literature on massage, Vickers (1996) found that in most studies massage had no psychological effect, in a few studies there was arousal, and in an even smaller number of studies there was sedation; some massage has both local and systemic effects on blood flow and possibly on lymph flow and reduction of muscle tension.

It may be that these variable responses are directly related to the variability of massage techniques, of which there are over 200. Massage can be given over the whole body or limited to the face, neck, or just hands, feet, legs—depending on the patient and his or her condition or illness, for example, patients with learning disabilities and many psychiatric patients are often only able to have limited body contact for a short time.

13.9.1 Massage Techniques

Massage is customarily defined as the manual manipulation of the soft tissues of the body for therapeutic purposes, using strokes that include gliding, kneading, pressing, tapping, and/or vibrating (Tisserand, 1977; Price and Price, 1999). Massage therapists may also cause movement within the joints, apply heat or cold, use holding techniques, and/or advise clients on exercises to improve muscle tone and range of motion. Some common massage techniques include Swedish massage, acupressure, craniosacral therapy, deep tissue massage, infant massage, lymph system massage, polarity therapy, reflexology, reiki, rolfing, shiatsu, and therapeutic touch.

Massage usually involves the use of a lubricating oil to help the practitioner's hands glide more evenly over the body. The addition of perfumed essential oils further adds to its potential to relax.

In most English-speaking countries, massage is nowadays seen as an alternative or complementary treatment. However, before World War II, it was regarded as a conventional treatment (Goldstone, 1999, 2000), as it is now in continental Europe. In Austria, for example, most patients with back pain receive (and are usually reimbursed for) massage treatment (Ernst, 2003a).

Not all massage treatments are free of risk. Too much force can cause fractures of osteoporotic bones, and even rupture of the liver and damage to nerves have been associated with massage (Ernst, 2003b). These events are rarities, however, and massage is relatively safe, provided that well-trained therapists observe the contraindications: phlebitis, deep vein thrombosis, burns, skin infections, eczema, open wounds, bone fractures, and advanced osteoporosis (Ernst et al., 2001).

It is not known exactly how massage works, although many theories abound (Vickers, 1996; Ernst et al., 2001). The mechanical action of the hands on cutaneous and subcutaneous structures enhances circulation of blood and lymph, resulting in increased supply of oxygen and removal of waste products or mediators of pain (Goats, 1994). Certain massage techniques have been shown to increase the threshold for pain (Dhondt et al., 1999). Also, most importantly from the standpoint of aromatherapy, a massage can relax the mind and reduce anxiety, which could positively affect the perception of pain (Vickers, 1996; Ernst, 2003a). Many studies have been carried out, most of which are unsatisfactory. It appears that placebo-controlled, double-blind trials may not be possible, yet few randomized clinical trials have been forthcoming.

Different client groups require proper recognition before aromatherapy trials are started or aromatherapy massage is given. For example, for cancer patients, guidelines must be observed (Wilkinson et al., 1999): special care must be taken for certain conditions such as autoimmune disease (where there are tiny bruises present); low blood cell count, which makes the patient lethargic and needing nothing more than very gentle treatment; and lymphoedema, which should not be treated unless the therapist has special knowledge and where enfleurage toward the lymph nodes should not be used.

Recent individual studies to investigate the benefit of massage for certain complaints have given variable results. Many are positive, although the standard of the studies has, in general, been poor (Vickers, 1996). The most successful applications of massage or aromatherapy massage have been in cancer care, and about a third of patients with cancer use complementary/alternative medicine during their illness (Ernst and Cassileth, 1998). Massage is commonly provided within UK cancer services (Kohn, 1999), and although only anecdotal and qualitative evidence is available, it is considered by patients to be beneficial. Only a few small-scale studies among patients with cancer have identified short-term benefits from a course of massage, mainly in terms of reduced anxiety (Corner et al., 1995; Kite et al., 1998; Wilkinson et al., 1999). These studies have been criticized by scientists; however, as they were either nonrandomized, had inadequate control groups or were observational in design (Cooke and Ernst, 2000). Complementary therapy practitioners have criticized medical research for not being sufficiently holistic in approach, focusing on efficacy of treatments in terms of tumor response and survival, rather than quality of life (Wilkinson, 2003).

A general study of the clinical effectiveness of massage by Ernst (1994) used numerous trials, with and without control groups. A variety of control interventions were used in the controlled studies including placebo, analgesics, transcutaneous electrical nerve stimulation (TENS), and so on. There were some positive effects of vibrational or manual massage, assessed as improvements in mobility, Doppler flow, expiratory volume, and reduced lymphoedema in controlled studies. Improvements in musculoskeletal and phantom limb pain, but not cancer pain, were recorded in controlled studies. Uncontrolled studies were invariably positive. Adverse effects included thrombophlebitis and local inflammation or ulceration of the skin.

Different megastudies included massage for delayed-onset muscle soreness—seven trials were included with 132 patients in total (Ernst, 1998); effleurage backrub for relaxation—nine trials were included with a total of 250 patients (Labyak and Metzger, 1997), and massage for low back pain (Ernst, 1999a, 1999b). All gave positive and negative outcomes.

13.10 AROMATHERAPY: BLENDING OF ESSENTIAL OILS

There are numerous suggestions for the use of particular essential oils for treating specific illnesses in books on aromatherapy. However, when collated, each essential oil can treat each illness (Vickers, 1996; compare also individual essential oil monographs in Lis-Balchin, 2006).

A few drops of the essential oil or oils chosen are always mixed with a carrier oil before being applied to the skin for an aromatherapy massage. The exact dilution of the essential oils in the carrier oil is often controversial and can be anything from 0.5% to 20% and more. Either 5, 10, or 20 mL of carrier oil is first poured into a (usually brown) bottle with a stoppered dropper. The essential oil is then added dropwise into the carrier oil, either as a single essential oil or as a mixture of 2–3 different essential oils, and then stoppered.

Volumes of essential oils used for dilutions vary widely in different aromatherapies and the fact that even the size of a "dropper" varies raised the question of possible safety problems (Lis-Balchin, 2006), and a recent article in a nursing journal makes a request for standardization of the measurement of the dropper size (Ollevant et al., 1999).

13.10.1 Fixed Oils

Many fixed oils are used for dilution and all provide a lubricant; many have a high vitamin E and A content. By moistening the skin, they can assist in a variety of mild skin conditions especially where the skin is rough, cracked, or dry (Healey and Aslam, 1996).

Almond (*Prunus amygdalus* var. dulcis)—sweet, cheapest, and most commonly used. Others include apricot kernel (*Prunus armeniaca*), borage seed (*Borago officinalis*), calendula (*Calendula officinalis*), coconut oil (*Cocos nucifera*), evening primrose (*Oenothera biennis)*, grapeseed (*Vitis vinifera*), macadamia nut (*Macadamia integrifolia*), olive (*Olea europaea*), rose hip seed (*Rosa mosqueta*, etc.), soya bean (*Glycine soya*), sunflower (*Helianthus annuus*), wheatgerm (*Triticum vulgare*), and jojoba (*Simmondsia californica*). The latest oil in vogue is emu oil (*Dromiceius novaehol-landiae*), which comes from a thick pad of fat on the bird's back. For centuries, the aborigines of Australia have been applying emu oil to their wounds with excellent results. It is now found in muscle pain relievers, skin care products, and natural soaps.

The exact method of mixing is controversial, but most aromatherapists are taught not to shake the bottle containing the essential oil(s) and the diluent fixed oils, but to gently mix the contents by turning the bottle in the hand. Differences in the actual odor and thereby presumable benefits of the diluted oils made by different aromatherapists can just be due to the different droppers (Lis-Balchin, 2006).

13.11 INTERNAL USAGE OF ESSENTIAL OILS BY AROMATHERAPISTS

Oral intake of essential oils is not true "aromatherapy" as the odor has virtually no effect past the mouth and the effect of the chemical components takes over as odors cannot influence the internal organs (Lis-Balchin, 1998a). Therapy with essential oils is dealt with in another chapter. Most aromatherapists consider that essential oils should only be prescribed by primary care practitioners such as medical doctors or medical herbalists who have intimate knowledge of essential oil toxicology (Tisserand and Balacs, 1995). In the United Kingdom, such "clinical aromatherapy" is rare, unlike on the continent. Maladies treated include arthritis, bronchitis, rheumatism, chilblains, eczema, high blood pressure, and venereal diseases. In clinical aromatherapy, there is a real risk of overdosage due to variable droppers on bottles, which can differ by as much as 200% (Lis-Balchin, 2006); this may be the cause of asphyxiation of a baby, as already shown by peppermint oil (Bunyan, 1998). It is possible that aromatherapists would not be covered by their insurance if there were adverse effects. However, most of us ingest small amounts of essential oils and their components daily in almost all processed foods and drinks, but it does not make us all healthy.

Conventional drugs involving essential oils and their components have been used internally for a long time, for example, decongestants containing menthol, camphor and pine, and various throat drops containing components from essential oils such as lemon, thyme, peppermint, sage, and hyssop.

Essential oils in processed foods are used in very minute amounts of 10 ppm, but can be 1000 ppm in mint confectionery or chewing gum (Fenaroli, 1997). This contrasts greatly with the use of drops of undiluted essential oils on sugar lumps for oral application, or on suppositories in anal or vaginal application. Damage to mucous membranes could result due to the high concentration of the essential oils in certain areas of the applicator.

Essential oils and their components are incorporated into enterically coated capsules to prevent damage and used for treating irritable bowel syndrome (peppermint in Colpermin), a mixture of monoterpenes for treating gallstones (Rowatol) and ureteric stones (Rowatinex); these are under product licenses as medicines (Somerville et al., 1984, 1985; Engelstein et al., 1992).

Some aromatherapists support the use of essential oils in various venereal conditions. However, aromatherapists are either qualified to treat venereal disease conditions, nor can make an accurate diagnosis in the first place, unless they are also medically qualified. Tea tree oil (2–3 drops undiluted) was used on a tampon for candidiasis with apparently very encouraging results (Zarno, 1994). *Candida* treatments also include chamomile, lavender, bergamot, and thyme (Schnaubelt, 1999). Essential oils used in this way, sometimes for months, often produced extremely painful reactions and putrid discharges due to damage to delicate mucosal membranes.

13.12 USE OF PURE OR SYNTHETIC COMPONENTS

Does it really matter whether the essential oil is pure or a synthetic mixture as long as the odor is the same? The perfumers certainly do not see any difference, and even prefer the synthetics as they remain constant. Many of the so-called pure essential oils used today are, however, adulterated (Which Report, 2001; Lis-Balchin et al., 1996, 1998). There is often a difference in the proportion of different enantiomers of individual components that often have different odors and different biological properties (Lis-Balchin, 2002a, 2002b). This was not, however, appreciated by Gattefosse (1937/1993), who worked with perfumes and not with the "pure plant essential oils" (*Formulaires de Parfumerie Gattefossé*, 1906). He studied the antimicrobial and wound-healing properties of essential oils on soldiers during World War I (Arnould-Taylor, 1981). He later worked in hospitals on the use of perfumes and essential oils as antiseptics and other (unstated) applications, and also in dermatology, which led to advances in the development of beauty products and treatments and the publication of *Physiological Aesthetics and Beauty Products* in 1936 (Gattefosse, 1992).

Gattefossé promoted the deterpenization of essential oils because, being a perfumer, he was aware that his products must be stable, have a long shelf-life, and not go cloudy when diluted in alcohol. Terpenes also oxidize rapidly, often giving rise to toxic oxidation products (e.g., limonene of citrus essential oils). But this goes against the use of pure essential oils, as their wholeness or natural synergy is apparently destroyed (Price, 1993). Bergamot and other citrus essential oils obtained by expression are therefore recommended, despite their phototoxicity (Price and Price, 1999). There is no reason why a toxic essential oil should be preferentially used if the nontoxic furanocoumarin-free (FCF) alternative is available. If adverse effects resulted, it is possible that there could be legal implications for the therapist.

13.13 THERAPEUTIC CLAIMS FOR THE APPLICATION OF ESSENTIAL OILS

There are a wide range of properties ascribed to each essential oil in aromatherapy books, without any scientific proof of effectiveness (Vickers, 1996; Lis-Balchin, 2006). The following are a few examples.

Diabetes can be treated by eucalyptus, geranium, and juniper (Tisserand, 1977); clary sage, eucalyptus, geranium, juniper, lemon, pine, red thyme, sweet thyme, vetiver, and ylang ylang (Price,

1993); eucalyptus, geranium, juniper, and onion (Valnet, 1982); and eucalyptus, geranium, cypress, lavender, hyssop, and ginger (Worwood, 1991).

Allergies can be treated by immortelle, chamomile, balm, and rose (Fischer-Rizzi, 1990); lemon balm, chamomile (German and Roman), helichrysum, true lavender, and spikenard (Lawless, 1992); and chamomile, jasmine, neroli, and rose (Price, 1983).

No botanical names are, however, given in the lists, even when there are several possible species. No indication is provided as to why these particular essential oils are used and how they are supposed to affect the condition. Taking the case of diabetes, where there is a lack of the hormone insulin, it is impossible to say how massage with any given essential oil could cure the condition, without giving the hormone itself in juvenile-type diabetes or some blood glucose-decreasing drugs in late-onset diabetes. Unfortunately, constant repetition of a given statement often lends it credence—at least to the layperson, who does not require scientific evidence of its validity.

13.13.1 False Claims Challenged in Court

The false promotion of products for treating not only medical conditions but also well-being generally is now being challenged in the law courts. For example, in 1997, Los Angeles attorney Morsé Mehrban charged that Lafabre and Aroma Vera had violated the California Business and Professions Code by advertising that their products could promote health and well-being, relax the body, relax the mind, enhance mood, purify the air, are antidotes to air pollution, relieve fatigue, tone the body, nourish the skin, promote circulation, alleviate feminine cramps, and do about 50 other things (Barrett, 2000). In September 2000, the case was settled out of court with a $5700 payment to Mehrban and a court-approved stipulation prohibiting the defendants from making 57 of the disputed claims in advertising within California (Horowitz, 2000).

13.14 PHYSIOLOGICAL AND PSYCHOLOGICAL RESPONSES TO ESSENTIAL OILS AND PSYCHOPHYSIOLOGY

Many examples of essential oil effects abound in animal studies, for example, the sedative action of lavender on the overall activity of mice decreased when exposed to lavender vapor (*Lavandula angustifolia* P. Miller); its components linalool and linalyl acetate showed a similar effect (Buchbauer et al., 1992). A possible explanation for the observed sedative effects was shown by Linalool, which produced a dose-dependent inhibition of the binding of glutamate (an excitatory neurotransmitter in the brain) to its receptors on membranes of the rat cerebral cortex (Elisabetsky et al., 1995). More recently, this action was related to an anticonvulsant activity of linalool in rats (Elisabetsky et al., 1999). Other oils with sedative activity were found to be neroli and sandalwood; active components included citronellal, phenylethyl acetate, linalool, linalyl acetate, benzaldehyde, -terpineol, and isoeugenol (in order of decreasing activity).

Stimulant oils included jasmine, patchouli, ylang ylang, basil, and rosemary; active components included fenchone, 1,8-cineole, isoborneol, and orange terpenes (Lis-Balchin, 2006). There was considerable similarity in the sedative and stimulant effects of some essential oils studied physiologically (e.g., their effect on smooth muscle of the guinea pig *in vitro*) and in various psychological assessments, mostly on humans (Lis-Balchin, 2006).

1,8-Cineole when inhaled, showed a decreased blood flow through the brain (measured using computerized tomography) although no changes were found with lavender oil or linalyl acetate (Buchbauer et al., 1993c). Changing electrical activity, picked up by scalp electrodes, in response to lavender odors was considered a measure of brain activity (EEG) (Van Toller et al., 1993). The most consistent responses to odors were in the theta band (Klemm et al., 1992). Many essential oil vapors have been shown to depress CNV brain waves (an upward shift in EEG waves that occurs when people are expecting something to happen) in human volunteers and these are considered to be sedatives; others increase CNV and are considered stimulants: lavender was found to have a sedative

effect on humans (Torii et al., 1988; Kubota et al., 1992; Manley, 1993) and had a "positive" effect on mood, EEG patterns, and maths computations (Diego et al., 1998). It also caused reduced motility in mice (Kovar et al., 1987; Ammon, 1989; Buchbauer et al., 1992, 1993a, 1993b, 1993c; Jaeger et al., 1992). However, Karamat et al. (1992) found that lavender had a stimulant effect on decision times in human experiments.

A large workplace in Japan with odorized air via the whole building showed that citrus smells refreshed the workers first thing in the morning and after the lunch break, and floral smells improved their concentration in between. In the lunch break and during late afternoon, woodland scents were circulated to relax the workers and this increased productivity (Van Toller and Dodd, 1991). It is also possible that the use of a general regime of odorants could have very negative effects on some members of the workforce or on patients in hospital wards, where the use of pleasant odors could mask the usual unpleasant odors providing the smell of fear. Ambient odors have an effect on creativity, mood, and perceived health (Knasco, 1992, 1993) and so does feigned odor (Knasco et al., 1990).

It is very difficult to make simple generalizations concerning the effects of any fragrance on psychological responses, which are based on the immediate perceptual effects, rather than the longer term pharmacological effects because the pharmacological effect is likely to affect people similarly, but the additional psychological mechanisms will create complex effects at the individual level. Odors are perceptible even during sleep, as shown in another experiment; college students were tested with fragrances during the night and the day (Badia, 1991).

Various nonscientific studies have been published in perfumery journals on the treatment of psychiatric patients by psychoaromatherapy in the 1920s (Gatti and Cajola, 1923a, 1923b, 1929; Tisserand, 1997) but there was virtually no information on their exact illnesses. Sedative essential oils or essences were identified as chamomile, melissa, neroli, petitgrain, opoponax, asafoetida, and valerian. Stimulants were angelica, cardamom, lemon, fennel, cinnamon, clove, and ylang ylang. Many aromatherapists have also written books on the effect of essential oils on the mind, giving directives for the use of specific plant oils for treating various conditions, without any scientific proof (Lawless, 1994; Worwood, 1996, 1998; Hirsch, 1998).

13.15 PLACEBO EFFECT OF AROMATHERAPY

The placebo effect is an example of a real manifestation of mind over matter. It does not confine itself to alternative therapies, but there is a greater likelihood of the placebo effect accounting for over 90% of the effect in the latter (Millenson, 1995). Reasons for the potency of the placebo effect are either the patient's belief in the method; the practitioner's belief in the method; or the patient and practitioner's belief in each other, that is, the strength of their relationship (Weil, 1983).

Placebo effects have been shown to relieve postoperative pain, induce sleep or mental awareness, bring about drastic remission in both symptoms and objective signs of chronic diseases, initiate the rejection of warts, and other abnormal growths, and so on (Weil, 1983). Placebo affects headaches, seasickness, and coughs, as well as have beneficial effects on pathological conditions such as rheumatoid and degenerative arthritis, blood cell count, respiratory rates, vasomotor function, peptic ulcers, hay fever, and hypertension (Cousins, 1979). There can also be undesirable side effects, such as nausea, headaches, skin rashes, allergic reactions, and even addiction, that is, a nocebo effect. This is almost akin to voodoo death threats or when patients are mistakenly told that their illness is hopeless—both are said to cause death soon after.

Rats were found to have increased levels of opioids in their brains after inhaling certain essential oils. Opioids are a factor in pain relief (Lis-Balchin, 1998b) and can be increased in the body by autosuggestion, relaxation, belief, and so on.

The use of aromatherapy for pain relief is best achieved through massage, personal concern and touch of the patient, and also listening to their problems. The extra benefit of real "healers" found among aromatherapists is an added advantage.

13.16 SAFETY ISSUE IN AROMATHERAPY

Many aromatherapists and laymen consider natural essential oils to be completely safe. This is based on the misconception that all herbs are safe—because they are "natural," which is a fallacy. The toxicity of essential oils can also be entirely different to that of the herb, not only because of their high concentration, but also because of their ability to pass across membranes very efficiently due to their lipophilicity.

Some aromatherapists erroneously believe that aromatherapy is self-correcting, unlike conventional therapy with medicines, and if errors are made in aromatherapy, they may be resolved through discontinuation of the wrongful application of the oil (e.g., Schnaubelt, 1999).

Many essential oils are inherently toxic at very low concentrations due to very toxic components; these are not normally used in aromatherapy. Many essential oils that are considered to be nontoxic can have a toxic effect on some people; this can be influenced by previous sensitization to a given essential oil, a group of essential oils containing similar components, or some adulterant in the essential oil. It can also be influenced by the age of the person; babies and young children are especially vulnerable and so are very old people. The influence of other medicaments, both conventional and herbal, is still in the preliminary stages of being studied. It is possible that these medicaments, and also probably household products, including perfumes and cosmetics, can influence the adverse reactions to essential oils.

Aromatherapists themselves have also been affected by sensitization (Crawford et al., 2004); in a 12-month period under study, prevalence of hand dermatitis in a sample of massage therapists was 15% by self-reported criteria and 23% by a symptom-based method and included the use of aromatherapy products in massage oils, lotions, or creams. In contrast, the suggestion that aromatherapists have any adverse effects to long-term usage of essential oils was apparently disproved by a nonscientific survey (Price and Price, 1999).

As most essential oils were tested over 30 years ago, the toxicity data may now be meaningless, as different essential oils are now used, some of which contain different quantities of many different synthetic components (Lis-Balchin, 2006).

The major drawbacks of trying to extrapolate toxicity studies in animals to humans concern feelings—from headaches to splitting migraines; feeling sick, vertigo, profound nausea; tinnitus; sadness, melancholia, suicidal thoughts; feelings of hate—which are clearly impossible to measure in animals (Lis-Balchin, 2006). The toxicity of an individual essential oil/component is also tested in isolation in animals and disregards the possibility of modification by other substances, including food components and food additive chemicals, the surrounding atmosphere with gaseous and other components, fragrances used in perfumes, domestic products, in the car, in public transport (including the people), workplace, and so on. These could cause modification of the essential oil/component, its bioavailability, and possibly the enhancement or loss of its function. The detoxification processes in the body are all directed to the production of a more polar product(s), which can be excreted mainly by the kidneys regardless of whether this/these are more toxic or less toxic than the initial substance and differ in different animals.

Most essential oils have GRAS (generally recognized as safe) status granted by the Flavor and Extract Manufacturers Association (FEMA) and approved by the US Food and Drug Administration (FDA) for food use, and many appear in the food chemical codex. This was reviewed in 1996 after evaluation by the expert panel of the FEMA. The assessment was based on data of exposure, and as most flavor ingredients are used at less than 100 ppm, predictions regarding their safety can be assessed from data on their structurally related group(s) (Munro et al., 1996). The no-observed-adverse-effect levels (NOELs) are more than 100,000 times their exposure levels from use as flavor ingredients (Adams et al., 1996). Critical to GRAS assessment are data of metabolic fate and chronic studies rather than acute toxicity. Most essential oils and components have an LD50 of 1–20 g/kg body weight or roughly 1–20 mL/kg, with a few exceptions as follows: Boldo leaf oil 0.1/0.9 (oral/dermal); Calamus 0.8–9/5; Chenopodium 0.2/0.4; Pennyroyal 0.4/4; and Thuja 0.8/4.

Research Institute for Fragrance Materials (RIFM) testing is generally limited to acute oral and dermal toxicity, irritation and dermal sensitization, and phototoxicity of individual materials, and there is little effort to address synergistic and modifying effects of materials in combination (Johansen et al., 1998).

Many materials that were widely used for decades in the past had severe neurotoxic properties and accumulated in body tissues (Spencer et al., 1979; Furuhashi et al., 1994) but most fragrance materials have never been tested for neurological effects, despite the fact that olfactory pathways provide a direct route to the brain (Hastings et al., 1991).

13.17 TOXICITY IN HUMANS

The most recent clinical review of the adverse reactions to fragrances (de Groot and Frosch, 1997) showed many examples of cutaneous reactions to essential oils reported elsewhere (Guin, 1982, 1995). In the United States, about 6 million people have a skin allergy to fragrance and this has a major impact on their quality of life. Symptoms include headaches, dizziness, nausea, fatigue, shortness of breath, and difficulty in concentrating. Fragrance materials are readily absorbed into the body via the respiratory system and once absorbed they cause systemic effects.

Migraine headaches are frequently triggered by fragrances that can act on the same receptors in the brain as alcohol and tobacco, altering mood and function [Institute of Medicine USA, sponsored by the Environmental Protection Agency (EPA)]. Perfumes and fragrances are recognized as triggers for asthma by the American Lung Association. The vast majority of materials used in fragrances are respiratory irritants and there are a few that are known to be respiratory sensitizers. Most have *not* been evaluated for their effects on the lungs and the respiratory system.

Respiratory irritants are known to make the airways more susceptible to injury and allergens, as well as to trigger and exacerbate conditions such as asthma, allergies, sinus problems, and other respiratory disorders. In addition, there is a subset of asthmatics that is specifically triggered by fragrances (Shim and Williams, 1986; Bell et al., 1993; Baldwin et al., 1999), which suggests that fragrances not only trigger asthma, they may also cause it in some cases (Millqvist and Lowhagen, 1996). Placebo-controlled studies using perfumes to challenge people with asthma-like symptoms showed that asthma could be elicited with perfumes without the presence of bronchial obstruction and these were not transmitted by the olfactory nerve as the patients were unaware of the smell (Millqvist and Lowhagen, 1996).

Adverse reactions to fragrances are difficult or even impossible to link to a particular chemical—often due to secrecy rules of the cosmetic/perfumery companies and the enormous range of synthetic components, constituting about 90% of flavor and fragrance ingredients (Larsen, 1998). The same chemicals are used in foods and cosmetics—there is, therefore, a greater impact due to the three different modes of entry: oral, inhalation, and skin.

13.17.1 Increase in Allergic Contact Dermatitis in Recent Years

A study of 1600 adults in 1987 showed that 12% reacted adversely to cosmetics and toiletries, 4.3% of which were used for their odor (i.e., they contained high levels of fragrances). Respiratory problems worsened with prolonged fragrance exposure (e.g., at cosmetic/perfumery counters) and even in churches. In another study, 32% of the women tested had adverse reactions and 80% of these had positive skin tests for fragrances (deGroot and Frosch, 1987). Problems with essential oils have also been increasing. For example, contact dermatitis and allergic contact dermatitis (ACD) caused by tea tree oil has been reported, which was previously considered to be safe (Carson and Riley, 1995). It is unclear whether eucalyptol was responsible for the allergenic response (Southwell, 1997); out of seven patients sensitized to tea tree oil, six reacted to limonene, five to α-terpinene and aromadendrene, two to terpinen-4-ol, and one to *p*-cymene and α-phellandrene (Knight and Hausen, 1994).

Many studies on ACD have been done in different parts of the world (deGroot and Frosch, 1987) and recently more studies have appeared:

- Japan (Sugiura et al., 2000): The patch test with lavender oil was found to be positive in increased numbers and above that of other essential oils in 10 years.
- Denmark (Johansen et al., 2000): There was an 11% increase in the patch test in the last year and of 1537 patients, 29% were allergic to scents.
- Hungary (Katona and Egyud, 2001): Increased sensitivity to balsams and fragrances was noted.
- Switzerland (Kohl et al., 2002): ACD incidence has increased over the years and recently 36% of 819 patch tests were positive to cosmetics.
- Belgium (Kohl et al., 2002): Increased incidence of ACD has been noted.

Occupational increases have also been observed. Two aromatherapists developed ACD: one to citrus, neroli, lavender, frankincense, and rosewood and the other to geraniol, ylang ylang, and angelica (Keane et al., 2000). Allergic airborne contact dermatitis from the essential oils used in aromatherapy was also reported (Schaller and Korting, 1995). ACD occurred in an aromatherapist due to French marigold essential oil, *Tagetes* (Bilsland and Strong, 1990). A physiotherapist developed ACD to eugenol, cloves, and cinnamon (Sanchez-Perez and Garcia Diez, 1999).

There is also the growing problem that patients with eczema are frequently treated by aromatherapists using massage with essential oils. A possible allergic response to a variety of essential oils was found in children with atopic eczema, who were massaged with or without the oils. At first, both massages proved beneficial, though not significantly different; but on reapplying the essential oil massage after a month's break, there was a notable adverse effect on the eczema, which could suggest sensitization (Anderson et al., 2000).

13.17.2 Photosensitizers

Berlocque dermatitis is frequently caused by bergamot or other citrus oil applications on the skin (often due to their inclusion in eau de Cologne) followed by exposure to UV light. This effect is caused by psolarens or furanocoumarins (Klarmann, 1958). Citrus essential oils labeled FCF have no phototoxic effect, but are suspected carcinogens (Young et al., 1990). Other phototoxic essential oils include yarrow and angelica, neroli, petitgrain, cedarwood, rosemary, cassia, calamus, cade, eucalyptus (species not stated), orange, anise, bay, bitter almond, ylang ylang, carrot seed, and linaloe (the latter probably due to linalool, which, like citronellol, has a sensitizing methylene group exposed) (Guin, 1995). Photosensitizer oils include cumin, rue, dill, sandalwood, lemon (oil and expressed), lime (oil and expressed), opoponax, and verbena (the latter being frequently adulterated) (Klarmann, 1958). Even celery soup eaten before UV irradiation has been known to cause severe sunburn (Boffa et al., 1996).

Many of these photosensitizers are now banned or restricted. New International Fragrance Research Association (IFRA) proposals for some phototoxic essential oils include rue oil to be 0.15% maximum in consumer products, marigold oil and absolute to be 0.01%, and petitgrain mandarin oil to be 0.165%.

13.17.3 Commonest Allergenic Essential Oils and Components

The most common fragrance components causing allergy are cinnamic alcohol, hydroxycitronellal, musk ambrette, isoeugenol, and geraniol (Scheinman, 1996). These are included in the eight commonest markers used to check for ACD, usually as a 2% mix. Other components considered allergenic are benzyl salicylate, sandalwood oil, anisyl alcohol, benzyl alcohol, and coumarin.

IFRA and RIFM have forbidden the use of several essential oils and components, including costus root oil, dihydrocoumarin, musk ambrette, and balsam of Peru (Ford, 1991); a concentration

limit is imposed on the use of isoeugenol, cold-pressed lemon oil, bergamot oil, angelica root oil, cassia oil, cinnamic alcohol, hydroxycitronellal, and oakmoss absolute. Cinnamic aldehyde, citral, and carvone oxide can only be used with a quenching agent.

Photosensitivity and phototoxicity occurs with some allergens such as musk ambrette and 6-methyl coumarin that are now removed from skin care products. Children were often found to be sensitive to Peru balsam, probably due to the use of baby-care products containing this (e.g., talcum powder used on nappy rash).

Fragrance materials have been found to interact with food flavorings, for example, a "balsam of Peru-free diet" has been devised in cases where cross reactions are known to occur (Veien et al., 1985). "Newer" sensitizers include ylang ylang (Romaguera and Vilplana, 2000), sandalwood oil (Sharma et al., 1994) but much of this essential oil is adulterated or completely synthetic, lyral (Frosch et al., 1999; Hendriks et al., 1999), and eucalyptol (Vilaplana and Romaguera, 2000).

Some sensitizers have been shown to interact with other molecules. For example, cinnamaldehyde interacts with proteins (Weibel et al., 1989), indicating how the immunogenicity occurs.

There have been very few published reports on neurotoxic aromachemicals such as musk ambrette (Spencer et al., 1984), although many synthetic musks took over as perfume ingredients when public opinion turned against the exploitation of animal products. Musk ambrette was found to have neurotoxic properties in orally fed mice in 1967 and was readily absorbed through the skin. A similar story occurred with acetylethyltetramethyltetralin (AETT), another synthetic musk, also known as versalide, patented in the early 1950s. During routine tests for irritancy in 1975, it was noted that with repeated applications, the skin of the mice turned bluish and they exhibited signs of neurotoxicity. The myelin sheath was damaged irreversibly in a manner similar to that which occurs with multiple sclerosis. Musk xylene, one of the commonest fragrance materials, is found in blood samples from the general population (Kafferlein et al., 1998) and bound to human hemoglobin (Riedel et al., 1999). These musk products have been found to have an effect on the life stages of experimental animals such as the frog, *Xenopus laevis*, the zebra fish, *Danio rerio* (Chou and Dietrich, 1999), and the rat (Christian et al., 1999). The hepatotoxic effect of musks is under constant study (Steinberg et al., 1999).

13.17.4 Toxicity in Young Children: A Special Case

Many aromatherapy books give dangerous advice on the treatment of babies and children, for example, 5–10 drops of "chamomile oil" three times a day in a little warmed milk given to their babies to treat colic with no indication as to which of the three commercially available chamomile oils is to be used and because, depending on the dropper size, the dose could easily approach the oral LD50 for the English and German chamomile oils, this could result in a fatality. Peppermint, often mentioned, could possibly be given by mothers in the form of oil, and has been known to kill a 1-week-old baby (*Evening Standard*, 1998). Dosages given in terms of drops can vary widely according to the size of the dropper in an essential oil.

Many "cosmetics" designed for use by children contain fragrance allergens (Rastogi et al., 1999). In Denmark, samples of children's cosmetics were found to contain geraniol, hydroxycitronellol, isoeugenol, and cinnamic alcohol (Rastogi et al., 1999). Children are more susceptible than adults to any chemical, so the increase in childhood asthma reported in recent years could be caused by fragrance components also found in fast foods. Aromatherapy therefore could be dangerous.

13.17.5 Selected Toxicities of Common Essential Oils and Their Components

Limonene and Linalool are found in a multitude of the commonest aromatherapy oils.

Limonene is a common industrial cleaner and is also the main citrus oil component, which causes ACD, particularly when aged (Chang et al., 1997; Karlberg and Dooms-Goossens, 1997). The major volatile component of lactating mothers' milk in the USA was found to contain *d*-limonene and the component is used as a potential skin penetration promoter for drugs such as indometacin,

especially when mixed with ethanol (Falk-Filipsson et al., 1993). Lastly, cats and dogs are very susceptible to insecticides and baths containing *d*-limonene, giving rise to neurological symptoms including ataxia, stiffness, apparent severe CNS depression, tremors, and coma (von Burg, 1995; see also Beasley, 1999).

Linalool, when oxidized for just 10 weeks, the linalool content fell to 80% and the remaining 20% consisted of a range of breakdown chemicals including linalool hydroperoxide, which was confirmed as a sensitizing agent. The fresh linalool was not a sensitizer; therefore, the EC regulations that are warnings about sensitization potential are looking for potential harm even on storage (Skoeld et al., 2002a, 2002b).

Most cosmetics and perfumes are tested on human "guinea pigs" using similar tests to those described for animals. These are demanded by the RIFM as a final test before marketing a product. Further data are accumulated from notifications from disgruntled consumers who report dermatitis, itching, or skin discoloration after use. These notifications can result in legal claims, although most cases are probably settled out of court and not reported to the general public.

The internet has made it possible for a trusting, although often ill-informed, public to purchase a wide range of dubious plant extracts and essential oils. Even illegal essential oils can now be obtained. Furthermore, unqualified people can offer potentially dangerous advice, such as internal usage or the use of undiluted essential oils on the skin for "mummification," or in order to rid the body of toxic waste. The latter can result in excruciating pain from the burns produced and the subsequent loss of layers of skin.

There is a recipe for suntan oil, including bergamot, carrot seed, and lemon essential oils (all phototoxic) in an aromatherapy book (Fischer-Rizzi, 1990). The author then advises that bergamot oil is added to suntan lotion to get the bonus of the substance called "furocumarin," which lessens the skin's sensitivity to the sun while it helps one to tan quickly. This could cause severe burns. Elsewhere, sassafras (*Ocotea pretiosa*) was said to be only toxic for rats, due to its metabolism and not dangerous to humans (Pénoel, 1991a, 1991b) and a 10% solution in oil was suggested for treating muscular and joint pain and sports injuries. Safrole (and sassafras oil) is, however, controlled under the Controlled Drugs Regulations (1993) and listed as a Category 1 substance, as it is a precursor to the illicit manufacture of hallucinogenic, narcotic, and psychotropic drugs like ecstasy.

French practitioners and other therapists have apparently become "familiar" with untested oils (Guba, 2000). The use of toxicologically untested Nepalese essential oils and the like includes lichen resinoids, sugandha kokila oil, jatamansi oil, and Nepalese lemongrass (*Cymbopogon flexuosa*), also *Tagetes* oil (Basnyet, 1999). *Melaleuca rosalina* (*Melaleuca ericifolia*), 1,8-cineole 18–26%, is apparently especially useful for the respiratory system (Pénoel, 1998), but it is untested and could be a sensitizer.

The Medicines and Healthcare Products Regulatory Agency in the United Kingdom may bring about changes in aromatherapy practice similar to their threat on herbal remedies. Aromatherapists are now using some potentially harmful products in their therapy. This immediately places them at serious risk if there is any untoward reaction to their specific treatment. It virtually means that bottles and containers of essential oils now rank with domestic bleach for labeling purposes and companies are now obliged to self-classify their essential oils on their labels and place them in suitable containers; this applies both to large distributing companies as well as individual aromatherapists reselling essential oils under their own name. Finally, new legislation has gone to the Council of Ministers and may imply that only qualified people will be able to use essential oils, and retail outlets for oils will be pharmacies. Their definition of "qualified" is limited to academic qualifications—doctors or pharmacists.

13.18 CLINICAL STUDIES OF AROMATHERAPY

Very few scientific clinical studies on the effectiveness of aromatherapy have been published to date. Perhaps the main reason is that until recently scientists were not involved and people engaging

in aromatherapy clinical studies had accepted the aromatherapy doctrine in its entirety, precluding any possibility of a nonbiased study. This has been evident in the design and execution of the studies; the main criterion has usually been the use of massage with essential oils and not the effect of the odorant itself. The latter is considered by most aromatherapists as irrelevant to clinical aromatherapy, which implies that it is simply the systemic action of essential oils absorbed through the skin that exerts an effect on specific organs or tissues. Odorant action is considered to be just "aromachology," despite its enormous psychological and physiological impact (Lis-Balchin, 2006). In some studies, attempts are even made to bypass the odorant effect entirely by making the subjects wear oxygen masks throughout (Dunn et al., 1995).

The use of particular essential oils for certain medical conditions is also adhered to, despite the wide assortment of supposed functions for each essential oil claimed by different aromatherapy source materials. In many studies, it is even unclear exactly which essential oil was used; as often the correct nomenclature, chemical composition, and exact purity are not given.

Many aromatherapists feel that they know that aromatherapy works as they have enormous numbers of case studies to prove it. But the production of lists of "positive" results on diverse clients, with diverse ailments, using diverse essential oils in the treatments, and diverse methods of application (which also frequently change from visit to visit for the same client) does not satisfy scientific criteria.

Negative results must surely be among the positive ones, due to the change in essential oils during the course of the treatment, which suggests that they did not work, but these are never stated. There are also no controls in case studies and no attempt to control the bias of the individual aromatherapist and clients.

Double-blind studies are *not* possible in *individual case* studies. Physiological or psychological changes due to the treatment are not properly defined and loose phrases such as "the client felt better" or "happier" are inappropriate for a scientific study.

These faults in the design and interpretation of results of aromatherapy research have been pointed out many times, for example, in Vickers (1996) Kirk-Smith (1996a), Nelson (1997), and Lis-Balchin (2002b). However, the lack of statistically significant results does not prevent many aromatherapists from accepting vaguely positive clinical research results and numerous poor-grade clinical studies are now quoted as factual confirmations that aromatherapy works.

It is almost impossible to do a double-blind study using odorants, as the patient and treatment provider would experience the odor differences and would inevitably react knowingly or unknowingly to that factor alone. The psychological effect(s) could be very diverse, as recall of odors can bring about very acute reactions in different people, depending on the individual's past experiences and on the like (Lis-Balchin, 2006). Lastly, there is potential bias as patients receiving aromatherapy treatment could be grateful for the attention given to them and, not wanting to upset the givers of such attention, would state that they were better and happier than before.

13.19 RECENT CLINICAL STUDIES

13.19.1 Aromatherapy in Dementia

A meticulously conducted double-blind study involved 72 dementia patients with clinically significant agitation treated with *melissa* oil (Ballard et al., 2002). Agitation included anxiety and irritability, motor restlessness, and abnormal vocalization—symptoms that often lead to disturbed behaviors such as pacing, wandering, aggression, shouting, and night-time disturbance, all characterized by appropriate inventories.

Ten percent (by weight) melissa oil (active) or sunflower oil (placebo), combined with a base lotion (*Prunus dulcis* oil, glycerine, stearic acid, cetearyl alcohol, and tocopheryl acetate), was dispensed in metered doses and applied to the face and both arms twice daily for 4 weeks by a care

assistant, the process taking 1–2 min. The patients also received neuroleptic treatment and other conventional treatments when necessary; this was therefore a study of complementary aromatherapy treatment—not an alternative treatment.

The "*melissa* group" showed a higher significant improvement in reducing aggression than the control group by week 4; the total Cohen–Mansfield Agitation Inventory (CMAI) scores had decreased significantly in both groups, from a mean of 68 to 45 (35%; $P < .0001$) in the treatment group and from 61 to 53 (11%; $P < .005$) in the placebo group. Clinically significant reduction in agitation occurred in 60% of the melissa group compared with 14% of placebo responders ($P < .0001$). Neuropsychiatric Inventory (NPI) scores also declined with *melissa* treatment, and quality of life was improved, with less social isolation and more involvement in activities. The latter was in contrast to the usual neuroleptic treatment effects.

The authors concluded that the *melissa* treatment was successful, but pointed out that there was also a significant, but lower, improvement in the control group and suggested that a stronger odor should have been used.

The effect of the *melissa* oil was probably on cholinergic receptors as shown by previous *in vitro* studies (Perry et al., 1999; Wake et al., 2000). The authors also concluded that as most people with severe dementia have lost any meaningful sense of smell, a direct placebo effect due to a pleasant-smelling fragrance, although possible, is an unlikely explanation for the positive effects of *melissa* in this study but others may disagree with this conclusion as it has been shown that subliminal odors can have an effect. The fragrance may have had some impact upon the care staff, and influenced ratings to some degree on the informant schedules.

A further recent study found no support for the use of a purely olfactory form of aromatherapy to decrease agitation in severely demented patients using lavender and thyme oil (Snow et al., 2004).

Other research (Burns et al., 2002) suggested that aromatherapy and light therapy were more effective and gentler alternatives to the use of neuroleptics in patients with dementia. Three studies were analyzed in each category; in the aromatherapy section, it included the study above, plus the use of 2% lavender oil via inhalation in a double-blind study for 10 days (Holmes et al., 2002) and a 2-week single-blind study using either aromatherapy plus massage, aromatherapy plus conversation or massage alone (Smallwood et al., 2001). All of the interventions in the aromatherapy groups proved significantly beneficial. However, so did the light treatment, where patients sat in front of a light box that beamed out 10,000 lux of artificial light, which adjusts the body's melatonin levels, affects the body clock, and is used in the treatment of SAD (seasonal affective disorder).

13.20 PAST CLINICAL STUDIES

In contrast to more recent studies, past clinical trials were often very defective in design and also outcomes. In a recent review, Cooke and Ernst (2000) included only those aromatherapy trials that were randomized and included human patients; they excluded those with no control group or if only local effects (e.g., antiseptic effects of tea tree oil) or preclinical studies on healthy volunteers occurred. The six trials included massage with or without aromatherapy (Buckle, 1993; Stevenson, 1994; Corner et al., 1995; Dunn et al., 1995; Wilkinson, 1995; Wilkinson et al., 1999) and were based on their relaxation outcomes. The authors concluded that the effects of aromatherapy were probably not strong enough for it to be considered for the treatment of anxiety or for any other indication.

A further study included trials with no replicates, and contained six studies. It showed that in five out of six cases the main outcomes were positive; however, these were limited to very specific criteria, such as small airways resistance for common colds (Cohen and Dressler, 1982), prophylaxis of bronchi for bronchitis (Ferley et al., 1989), lessening smoking withdrawal symptoms (Rose and Behm, 1993, 1994), relief of anxiety (Morris et al., 1995), and treatment of alopecia areata (Hay et al., 1998). The alleviation of perineal discomfort (Dale and Cornwell, 1994) was not significant.

Psychological effects, which include inhalation of essential oils and behavioral changes, were already discussed.

13.20.1 Critique of Selected Clinical Trials

The following clinical studies attempted to show that aromatherapy was more efficient than massage alone but they showed mainly negative results; however, in some cases, the authors clearly emphasized some very small positive results and this was then accepted and the report was welcomed in aromatherapy journals as a positive trial that supported aromatherapy.

Massage, aromatherapy massage, or a period of rest in 122 patients in an intensive care unit (ICU) (Dunn et al., 1995) showed no difference between massage with or without lavender oil and no treatment in the physiological parameters and all psychological parameters showed no effects throughout, bar a significantly greater improvement in mood and in anxiety levels between the rest group and essential oil massage group after the first session. The trial had a large number of changeable parameters: it involved patients in the ICU for about 5 days (age range 2–92 years), who received 1–3 therapy sessions in 24 h given by six different nurses. Massage was performed on the back or outside of limbs or scalp for 15–30 min with lavender (*Lavandula vera* at 1% in grapeseed oil, which was the only constant parameter). The patients wore oxygen masks, for some of the time. It seems unlikely that confused patients in ICU could remember the massage or its effects and a child of 2 years could not be expected to answer any pertinent questions.

Massage with and without Roman chamomile in 51 palliative care patients (Wilkinson, 1995) showed that both groups experienced the same decrease in symptoms and severity after three full body massages in 3 weeks. There was, however, a statistically significant difference between the two groups after the first aromatherapy massage and also an improvement in the "quality of life" from pre- to postmassage. German chamomile was likely to have been used, not Roman chamomile as stated, according to the chemical composition and potential bioactivity given.

Aromatherapy with and without massage, and massage alone on disturbed behavior in four patients with severe dementia (Brooker et al., 1997), was an unusual single-case study evaluating the use of "true" aromatherapy (using inhaled lavender oil) for 10 treatments of each, randomly given to each patient over a 3-month period and assessed against 10 no-treatment periods. Two patients became more agitated following their treatment sessions and only one patient seemed to have benefited. According to the staff providing the treatment, however, the use of all the treatments seemed to have been beneficial to the patients, suggesting pronounced bias.

An investigation of the psychophysiological effects of aromatherapy massage following cardiac surgery (Stevenson, 1994) showed experimenter bias due to the statement that "neroli is also especially valuable in the relief of anxiety, it calms palpitations, has an antispasmodic effect and an anti-inflammatory effect … it is useful in the treatment of hysteria, as an antidepressant and a gentle sedative." None of this has been scientifically proven, but as this was not a double-blind study and presumably the author did the massaging, communicating, and collating information alone, bias is probable. Statistical significances were not shown, nor the age ranges of the 100 patients, and no differences between the aromatherapy-only and massage-only groups were shown, except for an immediate increase in respiratory rate when the two control groups (20 min chat or rest) were compared with the aromatherapy massage and massage-only groups.

Atopic eczema in 32 children treated by massage with and without essential oils (Anderson et al., 2000) in a single-case experimental design across subjects showed that this complementary therapy provided no statistically significant differences between the two groups after 8 weeks of treatment. This indicated that massage and thereby regular parental contact and attention showed positive results, which was expected in these children. However, a continuation of the study, following a 3-month period of rest, using only the essential oil massage group showed a possible sensitization effect, as the symptoms worsened.

Massage using two different types of lavender oil on postcardiotomy patients (Buckle, 1993) was proclaimed to be a "double-blind" study but had no controls and the results by the author did not appear to be assessed correctly (Vickers, 1996). The author attempted to show that the "real" lavender showed significant benefits in the state of the patients compared with the other oil. However,

outcome measures were not described and the chemical composition and botanical names of the "real" and "not real" lavender remains a mystery, as three lavenders were stated in the text. Although the results were insignificant, this paper is quoted widely as proof that only "real" essential oils work through aromatherapy massage.

Aromatherapy trails in childbirth have been of dubious design and low scientific merit and, not surprisingly, have yielded confusing results (Burns and Blaney, 1994), mainly due to the numerous parameters incorporated. In the study by Burns and Blaney (1994), many different essential oils were used in various uncontrolled ways during childbirth and assessed using possibly biased criteria as to their possible benefits to the mother and midwife. The first pilot study used 585 women in a delivery suite over a 6-month period using lavender, clary sage, peppermint, eucalyptus, chamomile, frankincense, jasmine, lemon, and mandarin. These oils were either used singly or as part of a mixture where they could be used as the first, second, third, or fourth essential oil. The essential oils were applied in many different ways and at different times during parturition, for example, sprayed in a "solution" in water onto a face flannel, pillow, or bean bag; in a bath; foot bath; an absorbent card for inhalation; or in almond oil for massage. Peppermint oil was applied as an undiluted drop on the forehead and frankincense onto the palm.

Midwives and mothers filled in a form as to the effects of the essential oils including their relaxant value, effect on nausea and vomiting, analgesic action, mood enhancer action, accelerator, or not of labor. The results were inconclusive and there was a bias toward the use of a few oils, for example, lavender was stated to be "oestrogenic and used to calm down uterine tightenings if a woman was exhausted and needed sleep" and clary sage was given to "encourage the establishment of labor." This shows complete bias and a belief in unproven clinical attributes by the authors and presumably those carrying out the study. Which of the lavender, peppermint, eucalyptus, chamomile, or frankincense species were used remains a mystery.

The continuation of this study (Burns et al., 2000) on 8058 mothers during childbirth was intended to show that aromatherapy would "relieve anxiety, pain, nausea and/or vomiting, or strengthen contractions." Data from the unit audit were compared with those of 15,799 mothers not given aromatherapy treatment. The results showed that 50% of the aromatherapy group mothers found the intervention "helpful" and only 14% "unhelpful." The use of pethidine over the year declined from 6% to 0.2% by women in the aromatherapy group. The study also (apparently) showed that aromatherapy may have the potential to augment labor contractions for women in dysfunctional labor, in contrast to scientific data showing that the uterine contractions decrease due to administration of any common essential oils (Lis-Balchin and Hart, 1997).

It is doubtful whether a woman would in her first labor, or in subsequent ones, be able to judge whether the contractions were strengthened or the labor shortened due to aromatherapy. It seems likely that there was some placebo effect (itself a very powerful effector) due to the bias of the experimenters and the "suggestions" made to the aromatherapy group regarding efficacy of essential oils, which were obviously absent in the case of the control group.

Lavender oil (volatilized from a burner during the night in their hospital room) has been successful in replacing medication to induce sleep in three out of four geriatrics (Hardy et al., 1995). There was a general deterioration in the sleep patterns when the medication was withdrawn, but lavender oil seemed to be as good as the original medication. However, the deterioration in the sleep patterns (due to "rebound insomnia"?) may simply have been due to recovery of normal sleep patterns when lavender was given (Vickers, 1996).

The efficacy of peppermint oil was studied on postoperative nausea in 18 women after gynecological operations (Tate, 1997) using peppermint oil or a control, peppermint essence (obviously of similar odor). A statistically significant difference was found between the controls and the test group. The test group required less antiemetics and received less opioid analgesia. However, the use of a peppermint essence as a control seems rather like having two test groups as inhalation was used.

A group of 313 patients undergoing radiotherapy were randomly assigned to receive either carrier oil with fractionated oils, carrier oil only, or pure essential oils of lavender, bergamot, and

cedarwood administered by inhalation concurrently with radiation treatment. There were no significant differences in Hospital Anxiety and Depression Score (HADS) and other scores between the randomly assigned groups. Aromatherapy, as administered in this study, was not found to be beneficial (Graham et al., 2003).

Heliotropin, a sweet, vanilla-like scent, reduced anxiety during magnetic resonance imaging (Redd and Manne, 1991), which causes distress to many patients as they are enclosed in a "coffin"-like apparatus. Patients experienced approximately 63% less overall anxiety than a control group of patients.

A double-blind randomized trial was conducted on 66 women undergoing abortions (Wiebe, 2000). Ten minutes were spent sniffing a numbered container with either a mixture of the essential oils (vetivert, bergamot, and geranium) or a hair conditioner (placebo). Aromatherapy involving essential oils was no more effective than having patients sniff other pleasant odors in reducing preoperative anxiety.

An audit into the effects of aromatherapy in palliative care (Evans, 1995) showed that the most frequently used oils were lavender, marjoram, and chamomile. These were applied over a period of 6 months by a therapist available for 4 h on a weekly basis in the ward. Relaxing music was played throughout, each session to allay fears of the hands-on massage. The results revealed that 81% of the patients stated that they either felt "better" or "very relaxed" after the treatment; most appreciated the music greatly. The researchers themselves confessed that it is uncertain whether the benefits were the result of the patient being given individual attention, talking with the therapist, the effects of touch and massage, the effects of the aromatherapy essential oils, or the effects of the relaxation music.

Aromatherapy massage studied in eight cancer patients did not show any psychological benefit. However, there was a statistically significant reduction in all of the four physical parameters, which suggests that aromatherapy massage affects the autonomic nervous system, inducing relaxation. This finding was supported by the patients themselves, all of whom stated during interview that they felt "relaxed" after aromatherapy massage (Hadfield, 2001).

Forty-two cancer patients were randomly allocated to receive weekly massages with lavender essential oil in carrier oil (aromatherapy group), carrier oil only (massage group), or no intervention for 4 weeks (Soden et al., 2004). Outcome measures included a visual analogue scale (VAS) of pain intensity, the Verran and Snyder–Halpern Sleep Scale (VSH), the Hospital Anxiety and Depression Scale (HADS), and the Rotterdam Symptom Checklist (RSCL). No significant long-term benefits of aromatherapy or massage in terms of improving pain control, anxiety, or quality of life were shown. However, sleep scores improved significantly in both the massage and the combined massage (aromatherapy and massage) groups. There were also statistically significant reductions in depression scores in the massage group. In this study of patients with advanced cancer, the addition of lavender essential oil did not appear to increase the beneficial effects of massage.

A randomized controlled pilot study was carried out to examine the effects of adjunctive aromatherapy massage on mood, quality of life, and physical symptoms in patients with cancer attending a specialist unit (Wilcock et al., 2004). Patients were randomized to conventional day care alone, or day care plus weekly aromatherapy massage using a standardized blend of oils for 4 weeks. At baseline and at weekly intervals, patients rated their mood, quality of life, and the intensity and bother of two symptoms most important to them. However, although 46 patients were recruited to the study, only 11 of 23 (48%) patients in the aromatherapy group and 18 of 23 (78%) in the control group completed all 4 weeks. Mood, physical symptoms, and quality of life improved in both groups but there was *no* statistically significant difference between groups, but all patients were satisfied with the aromatherapy and wished to continue it.

Aromatherapy sessions in deaf and deaf–blind people became an accepted, enjoyable, and therapeutic part of the residents' lifestyle in an uncontrolled series of case studies. It appeared that this gentle, noninvasive therapy could benefit deaf and deaf–blind people, especially as their intact senses can be heightened (Armstrong and Heidingsfeld, 2000).

A scientifically unacceptable study of the effect of aromatherapy on endometriosis, reported only at an aromatherapy conference (Worwood, 1996), involved 22 aromatherapists who treated a total of 17 women in two groups over 24 weeks. One group was initially given massage with essential oils and then not "touched" for the second period, while the second group had the two treatments reversed. Among the many parameters measured were constipation, vaginal discharge, fluid retention, abdominal and pelvic pain, degree of feeling well, renewed vigor, depression, and tiredness. The data were presented as means (or averages, possibly, as this was not stated) but without standard errors of mean (SEM) and lacked any statistical analyses. Unfortunately, the study has been accepted by many aromatherapists as being a conclusive proof of the value in treating endometriosis using aromatherapy.

In all the trials above, there was a more positive outcome for aromatherapy if there were no stringent scientific double-blind and randomized control measures, suggesting that in the latter case, bias is removed.

13.21 USE OF ESSENTIAL OILS MAINLY AS CHEMICAL AGENTS AND NOT FOR THEIR ODOR

The efficacy and safety of capsules containing peppermint oil (90 mg) and caraway oil (50 mg), when studied in a double-blind, placebo-controlled, multicenter trial in patients with nonulcer dyspepsia was shown by May et al. (1996). Intensity of pain was significantly improved for the experimental group compared with the placebo group after 4 weeks.

Six drops of pure lavender oil included in the bath water for 10 days following childbirth was assessed against "synthetic" lavender oil and a placebo (distilled water containing an unknown GRAS additive) for perineal discomfort (Cornwell and Dale, 1995). No significant differences between groups were found for discomfort, but lower scores in discomfort means for days 3 and 5 for the lavender group were seen. This was very unsatisfactory as a scientific study, mainly because essential oils do not mix with water and there was no proof whether the lavender oil itself was pure.

Alopecia areata was treated in a randomized trial using "aromatherapy" carried out over 7 months. The test group massaged a mixture of 2 drops of *Thymus vulgaris*, 3 drops *Lavandula angustifolia*, 3 drops of *Rosmarinus officinalis*, and 2 drops of *Cedrus atlantica* in 3 mL of jojoba and 20 mL grapeseed oil into the scalp for 2 min minimum every night. The control group massaged the carrier oils alone (Hay et al., 1998). There was a significant improvement in the test group (44%) compared with the control group (15%). The smell of the essential oils (psychological/physiological) and/or their chemical nature on the scalp may have achieved these long-term results. On the other hand, the scalp may have healed naturally anyway after 7 months.

Ureterolithiasis was treated with Rowatinex, a mixture of terpenes smelling like Vicks VapoRub in 43 patients against a control group treated with a placebo. The overall expulsion rate of the ureteric stones was greater in the Rowatinex group (Engelstein et al., 1992). Similar mixes have shown both positive and negative results on gallstones over the years.

In a double-blind, placebo-controlled, randomized crossover study involving 332 healthy subjects, four different preparations were used to treat headaches (Gobel et al., 1994). Peppermint oil, eucalyptus oil (species not stated), and ethanol were applied to large areas of the forehead and temples. A combination of the three increased cognitive performance, muscle relaxation, and mental relaxation, but had no influence on pain. Peppermint oil and ethanol decreased the headache. The reason for the success could have been the intense coldness caused by the application of the latter mixture, which was followed by a warming up as the peppermint oil caused counterirritation on the skin; the essential oils were also inhaled.

A clinical trial on 124 patients with acne, randomly distributed to a group treated with 5% tea tree oil gel or a 5% benzoyl peroxide lotion group (Bassett et al., 1990), showed improvement in both groups and fewer side effects in the tea tree oil group. The use of tea tree oil has, however, had detrimental effects in some people (Lis-Balchin, 2006, Chapter 7).

A 10% tea tree oil was used on 104 patients with athlete's foot *Tinea pedis*) in a randomized double-blind study against 1% tolnaflate and placebo creams. The tolnaflate group showed a better effect; tea tree oil was as effective in improving the condition, but was no better than the placebo at curing it (Tong et al., 1992). Surprisingly, tea tree oil is sold as a *cure* for athlete's foot.

13.21.1 Single-Case Studies

In the past few years, the theme of the case studies (reported mainly in aromatherapy journals) has started to change and most of the aromatherapists are no longer announcing that they are "curing" cancer and other serious diseases. Emphasis has swung toward real complementary treatment, often in the area of palliative care. However, the so-called clinical aromatherapists persist in attempting to cure various medical conditions using high doses of oils mainly by mouth, vagina, anus, or on the skin. Many believe that healing wounds using essential oils is also classed as aromatherapy (Guba, 2000) despite the evidence that odor does not kill germs and any effect is due to the chemical activity alone.

Because of the lack of scientific evidence in many studies, we could assume that aromatherapy is mainly based on faith; it works because the aromatherapist believes in the treatment and because the patient believes in the supposed action of essential oils, that is, the placebo effect.

Decreased smoking withdrawal symptoms in 48 cigarette smokers were achieved by black pepper oil puffed out of a special instrument for 3 h after an overnight cigarette deprivation against mint/menthol or nothing (Rose and Behm, 1994).

Chronic respiratory infection was successfully treated when the patient was massaged with tea tree, rosemary, and bergamot oils while on her second course of antibiotics and taking a proprietary cough medicine. She also used lavender and rosemary oils in her bath, a drop of eucalyptus oil and lavender oil on her tissue near the pillow at night, 3 drops of eucalyptus and ginger for inhalations daily, and reduced her dairy products and starches. In a week, her cough was better and by 3 weeks, it had gone (Laffan, 1992). It is unclear which treatment actually helped the patient, and as it took a long time, the infection may well have gone away by then, or sooner, without any medicinal aid.

After just one treatment of aromatherapy massage using rose oil, bergamot, and lavender at 2.5% in almond oil, a 36-year-old woman managed to get pregnant after being told she was possibly infertile following the removal of her right fallopian tube (Rippon, 1993)!

Aromatherapy can apparently help patients with multiple sclerosis, especially for relaxation, in association with many other changes in the diet and also use of conventional medicines (Barker, 1994). French basil, black pepper, and true lavender in evening primrose oil with borage oil was used to counteract stiffness and also to stimulate; this mixture was later changed to include relaxing and sedative oils such as Roman chamomile, ylang ylang, and melissa.

Specific improvements in clients given aromatherapy treatment in dementia include increased alertness, self-hygiene, contentment, initiation of toileting, sleeping at night, and reduced levels of agitation, withdrawal, and wandering. Family carers reported less distress, improved sleeping patterns, and calmness (Kilstoff and Chenoweth, 1998). Other patients with dementia were monitored over a period of 2 months, and then for a further 2 months during which they received aromatherapy treatments in a clinical trial; they showed a significant improvement in motivational behavior during the period of aromatherapy treatment (MacMahon and Kermode, 1998).

13.22 CONCLUSION

Aromatherapy, using essential oils as an odorant by inhalation or massage onto the skin, has not been shown to work better than massage alone or a control. No failures have, however, been reported, although treatment is invariably changed on each visit. Many patients feel better, even if their disease is getting worse, due to their belief in an alternative therapist and this is a good example of "mind over matter," that is, the placebo effect. This effect has been recommended by some members

of the House of Lords Select Committee on Science and Technology, Sixth Report (2000), as a good basis for retaining complementary and alternative medicine, but other members argued that scientific proof of effects is necessary.

It is hoped that aromatherapists do not try to convince their patients of a cure, especially in the case of serious ailments such as cancer, which often recede naturally for a time on their own. Conventional treatment should always be advised in the first instance and retained during aromatherapy treatment with the consent of the patient's primary healthcare physician or consultant. Aromatherapy can provide a useful complementary medical service both in healthcare settings and in private practice, and should not be allowed to become listed as a bogus cure in alternative medicine.

REFERENCES

Adams, T.B., et al., 1996. The FEMA GRAS assessment of alicyclic substances used as flavour ingredients. *Food Chem. Toxicol.*, 34: 763–828.

Ammon, H.P.T., 1989. Phytotherapeutika in der Kneipp-therapie. *Therapiewoche*, 39: 117–127.

Anderson, C., et al., 2000. Evaluation of massage with essential oils on childhood atopic eczema. *Phytother. Res*., 14: 452–456.

Arctander, S., 1960. *Perfume and Flavor Materials of Natural Origin*. Elizabeth, NJ: S Arctander.

Armstrong, F. and V. Heidingsfeld, 2000. Aromatherapy for deaf and deafblind people living in residential accommodation. *Compl. Ther. Nurs. Midwifery*, 6: 180–188.

Arnould-Taylor, W.E., 1981. *A Textbook of Holistic Aromatherapy*. London: Stanley Thornes.

Atchley, E.G. and F. Cuthbert, 1909. *A History of the Use of Incense in Divine Worship*. London: Longmans, Green and Co.

Badia, P., 1991. Olfactory sensitivity in sleep: The effects of fragrances on the quality of sleep: A summary of research conducted for the fragrance research fund. *Perf. Flav.*, 16: 33–34.

Baldwin, C.M., et al., 1999. Odor sensitivity and respiratory complaint profiles in a community-based sample with asthma, hay fever, and chemical intolerance. *Toxicol. Ind. Health*., 15: 403–409.

Ballard, C., et al., 2002. Aromatherapy as a safe and effective treatment for the management of agitation in severe dementia: A double-blind, placebo-controlled trial with Melissa. *J. Clin. Psychiatry*, 63: 553–558.

Barker, A., 1994. Aromatherapy and multiple sclerosis. *Aromather. Q*, 4–6.

Barrett, S., 2000. Aromatherapy company sued for false advertising. *Quackwatch*, September 25, http://www.quackwatch.com/01QuackeryRelatedTopics/aroma.html, accessed 8 August 2009.

Basnyet, J., 1999. Tibetan essential oils. *IFA J.*, 43: 12–13.

Bassett, I.B., et al., 1990. A comparative study of tea tree oil versus benzoylperoxide in the treatment of acne. *Med. J. Aust.*, 153: 455–458.

Beasley, V., ed., 1999. Toxicants that cause central nervous system depression. *Veterinary Toxicology*. Ithaca, NY: International Veterinary Information Service (www.ivis.org), A2608.0899.

Bell, I.R., et al., 1993. Self-reported illness from chemical odors in young adults without clinical syndromes or occupational exposures. *Arch. Environ. Health*, 48: 6–13.

Benson, H. and M. Stark, 1996. *Timeless Healing. The Power and Biology of Belief*. London: Simon and Schuster.

Bilsland, D. and A. Strong, 1990. Allergic contact dermatitis from the essential oil of French Marigold (*Tagetes patula*) in an aromatherapist. *Cont. Dermat.*, 23: 55–56.

Boffa, M.J., et al., 1996. Celery soup causes severe phototoxicity during PUVA therapy. *Br. J. Dermatol*., 135: 334.

Brooker, D.J.R., et al., 1997. Single case evaluation of the effects of aromatherapy and massage on disturbed behaviour in severe dementia. *Br. J. Clin. Psychol.*, 36: 287–296.

Brunn, E.Z. and G. Epiney-Burgard, 1989. *Women Mystics in Medieval Europe*. New York: Paragon House.

Buchbauer, G., et al., 1992. Passiflora and limeblossom: Motility effects after inhalation of the essential oils and of some of the main constituents in animal experiments. *Arch. Pharm.* (*Weinheim*), 325: 247–248.

Buchbauer, G., et al., 1993a. Fragrance compounds and essential oils with sedative effects upon inhalation. *J. Pharm. Sci.*, 82: 660–664.

Buchbauer, G., et al., 1993b. Therapeutic properties of essential oils and fragrances. In: *Bioactive Volatile Compounds from Plants*, R. Teranishi, et al., eds, pp. 159–165. Washington, DC: American Chemical Society.

Buchbauer, G., et al., 1993c. New results in aromatherapy research. Paper presented at the *24th Int. Symp. on Essential Oils*, Berlin.
Buckle, J., 1993. Does it matter which lavender oil is used? *Nurs. Times*, 89: 32–35.
Bunyan, N., 1998. Baby died after taking mint water for wind. *Daily Telegraph*, May 21.
Burns, E. and C. Blaney, 1994. Using aromatherapy in childbirth. *Nurs. Times*, 90: 54–58.
Burns, E., et al., 2000. An investigation into the use of aromatherapy in intrapartum midwifery practice. *J. Alt. Compl. Med.*, 6: 141–147.
Carson, C.F. and T.V. Riley, 1995. Toxicity of the essential oil of *Melaleuca alternifolia* or tea tree oil. *J. Toxicol. Clin. Toxicol.*, 33: 193–194.
Chang Y.-C., et al., 1997. Allergic contact dermatitis from oxidised *d*-limonene. *Cont. Dermat.*, 37: 308–309.
Chou, Y.J. and D.R. Dietrich, 1999. Toxicity of nitromusks in early life-stages of South African clawed frog, *Xenopus laevis* and zebra-fish, *Danio rerio*. *Toxicol. Lett.*, 111: 17–25.
Christian, M.S., et al., 1999. Developmental toxicity studies of four fragrances in rats. *Toxicol. Lett.*, 111: 169–174.
Classen, C., et al., 1994. *Aroma*. London: Routledge.
Cohen, B.M. and W.E. Dressler, 1982. Acute aromatics inhalation modifies the airways. Effects of the common cold. *Respiration*, 43: 285–293.
Concise Oxford Dictionary, 1995. 9th Edition, Della Thompson, Ed., Oxford University Press, Oxford.
Cooke, B. and E. Ernst, 2000. Aromatherapy: A systematic review. *Br. J. Gen. Pract.*, 50: 493–496.
Corbin, A., 1986. *The Foul and the Fragrant*. Cambridge, MA: Harvard University Press.
Corner, J., et al., 1995. An evaluation of the use of massage and essential oils on the well-being of cancer patients. *Int. J. Palliat. Nurs.*, 1: 67–73.
Cornwell, S. and A. Dale, 1995. Lavender oil and perineal repair. *Mod. Midwifery*, 5: 31–33.
Cousins, N., 1979. *Anatomy of an Illness*. New York: WW Norton.
Crawford, G.H., et al., 2004. Use of aromatherapy products and increased risk of hand dermatitis in massage therapists. *Arch. Dermatol.*, 140: 991–996.
Culpeper, N., 1653/1995. *Culpeper's Complete Herbal and The English Physician and Family Dispensatory.* Facsimile: Wordsworth editions, 1995.
Culpeper, N., 1826/1981. *Culpeper's Complete Herbal and English Physician*. Facsimile: Pitman Press Ltd., Bath, 1981.
Dale, A. and S. Cornwell, 1994. The role of lavender oil in relieving perineal discomfort following childbirth: A blind randomized clinical trial. *J. Adv. Nurs.*, 19: 89–96.
De Groot, A.C. and P.J. Frosch, 1997. Adverse reactions to fragrances. A clinical review. *Cont. Dermat.*, 36: 57–86.
Deans, S.G., 2002. Antimicrobial properties of lavender volatile oil. In: *Genus Lavandula, Aromatic and Medicinal Plants—Industrial Profiles*, M. Lis-Balchin, ed. London: Taylor & Francis.
Deans, S.G. and G. Ritchie, 1987. Antibacterial properties of plant essential oils. *Int. J. Food Microbiol.*, 5: 165–180.
Dejeans, M. (Antoine de Hornot), 1764. *TraitŽ des Odeurs*. Paris: Nyon/Guillyn/Saugrain.
Devereux, P., 1997. *The Long Trip*. New York: Penguin.
Dhondt, W., et al., 1999. Pain threshold in patients with rheumatoid arthritis and effect of manual oscillations. *Scand. J. Rheumatol.*, 28: 88–93.
Diego, M.A., et al., 1998. Aromatherapy positively affects mood, EEG patterns of alertness and math computations. *Int. J. Neurosci.*, 96: 217–224.
Dorman, H.J.D., et al., 1995a. Antioxidant-rich plant volatile oils: In vitro assessment of activity. Paper presented at the *26th Int. Symp. on Essential Oils*, Hamburg, Germany, 10–13 September.
Dorman, H.J.D., et al., 1995b. Evaluation in vitro of plant essential oils as natural antioxidants. *J. Essent. Oil Res.*, 7: 645–651.
Dunn, C., et al., 1995. Sensing an improvement: An experimental study to evaluate the use of aromatherapy, massage and periods of rest in an intensive care unit. *J. Adv. Nurs.*, 21: 34–40.
Elisabetsky, E., et al., 1995. Effects of linalool on glutamatergic system in the rat cerebral cortex. *Neurochem. Res.*, 20: 461–465.
Elisabetsky, E., et al., 1999. Anticonvulsant properties of linalool in glutamate-related seizure models. *Phytomedicine*, 6: 107–113.
Ellis, A., 1960. *The Essence of Beauty*. London: Secker and Warburg.
Engelstein, E., et al., 1992. Rowatinex for the treatment of ureterolithiasis. *J. Urol.*, 98: 98–100.
Ernst, E., 1994. Clinical effectiveness of massage—a critical review. *Forsch Komplementärmed*, 1: 226–232.

Ernst, E., 1998. Does post-exercise massage treatment reduce delayed onset muscle soreness? *Br. J. Sports Med.*, 32: 212–214.
Ernst, E., 1999a. Abdominal massage for chronic constipation: A systematic review of controlled clinical trials. *Forsch Komplementärmed*, 6: 149–151.
Ernst, E., 1999b. Massage therapy for low back pain: A systematic review. *J. Pain. Sympt. Manage.*, 17: 56–69.
Ernst, E., 2003a. Massage treatment for back pain. *BMJ*, 326: 562–563.
Ernst, E., 2003b. The safety of massage therapy. *Rheumatology*, 42: 1101–1106.
Ernst, E. and B.R. Cassileth, 1998. The prevalence of complementary/alternative medicine in cancer: A systematic review. *Cancer*, 83: 777–782.
Ernst, E., et al., 2001. *The Desktop Guide to Complementary and Alternative Medicine*. Edinburgh: Mosby.
Evans, B., 1995. An audit into the effects of aromatherapy massage and the cancer patient in palliative and terminal care. *Compl. Ther. Med.*, 3: 239–241.
Falk-Filipsson, A., et al., 1993. *d*-Limonene exposure to humans by inhalation: Uptake, distribution, elimination, and effects on the pulmonary function. *J. Toxicol. Environ. Health*, 38: 77–88.
Fenaroli, G., 1997. *Handbook of Flavour Ingredients*, 3rd ed., Vol. 1. London: CRC Press.
Ferley, J.P., et al., 1989. Prophylactic aromatherapy for supervening infections in patients with chronic bronchitis. Statistical evaluation conducted in clinics against a placebo. *Phytother. Res.*, 3: 97–100.
Festing, S., 1989. *The Story of Lavender*. Surrey: Heritage in Sutton Leisure.
Fischer-Rizzi, S., 1990. *Complete Aromatherapy Handbook*. New York: Sterling Publishing.
Forbes, R.J., Ed. 1955. Cosmetics and perfumes in antiquity. In: *Studies in Ancient Technology*, Vol. III. Leiden: EJ Brill.
Ford, R.A., 1991. The toxicology and safety of fragrances. In: *Perfumes, Art, Science and Technology*, P.M. Muller and D. Lamparsky, eds, pp. 442–463. New York: Elsevier.
Frosch, P.J., et al., 1999. Lyral¨ is an important sensitizer in patients sensitive to fragrances. *Br. J. Dermatol.*, 141: 1076–1083.
Fuchs, N., et al., 1997. Systemic absorption of topically applied carvone: Influence of massage technique. *J. Soc. Cosmet. Chem.*, 48: 277–282.
Furuhashi, A., et al., 1994. Effects of AETT-induced neuronal ceroid lipofuscinosis on learning ability in rats. *Jpn. J. Psychiatry Neurol.*, 48: 645–653.
Gattefossé, M., 1992. Rene-Maurice Gattefossé, The father of modern aromatherapy. *Int. J. Aromather.*, 4: 18–20.
Gattefossé, R.M., 1928. *Formulaire du chimiste-Parfumeur et du Savonnier* [Formulary of cosmetics]. Paris: Librairie des Sciences.
Gattefossé, R.M., 1937/1993. *Gattefossé's Aromatherapy*, R. Tisserand, ed. Saffron Walden: CW Daniel Co.
Gattefossé, R.M., 1952. *Formulary of Perfumery and of Cosmetology*. London: Leonard Hill.
Gatti, G. and R. Cajola, 1923a. L'Azione delle essenze sul systema nervoso. *Riv Ital Della Essenze e Profumi*, 5: 133–135.
Gatti, G. and R. Cajola, 1923b. L'Azione terapeutica degli olii essenziali. *Riv Ital Della Essenze e Profumi*, 5: 30–33.
Gatti, G. and R. Cajola, 1929. L'essenza di valeriana nella cura delle malattie nervose. *Riv Ital Della Essenze e Profumi*, 2: 260–262.
Genders, R., 1972. *A History of Scent*. London: Hamish Hamilton.
Goats, G.C., 1994. Massage—the scientific basis of an ancient art: Parts 1 and 2. *Br. J. Sports Med.* (*UK*), 28: 149–152, 153–156.
Gobel, H., et al., 1994. Effect of peppermint and eucalyptus oil preparations on neurophysiological and experimental algesimetric headache parameters. *Cephalagia*, 14: 228–234.
Goldstone, L., 1999. From orthodox to complementary: The fall and rise of massage, with specific reference to orthopaedic and rheumatology nursing. *J. Orthop. Nurs.*, 3: 152–159.
Goldstone, L., 2000. Massage as an orthodox medical treatment, past and future. *Compl. Ther. Nurs. Midwifery*, 6: 169–175.
Graham, P.H., et al., 2003. Inhalation aromatherapy during radiotherapy: Results of a placebo-controlled double-blind randomized trial. *J. Clin. Oncol.*, 15: 2372–2376.
Groom, N., 1992. *The Perfume Handbook*, 2nd ed. London: Chapman & Hall.
Guba, R., 2000. Toxicity myths: The actual risks of essential oil use. *Perf. Flav.*, 25: 10–28.
Guin, J.D., 1982. History, manufacture and cutaneous reaction to perfumes. In: *Principles of Cosmetics for the Dermatologist*, P. Frost and S.W. Horwitz, eds. St. Louis: The CV Mosby Co.
Guin, J.D., 1995. *Practical Contact Dermatitis*. New York: McGraw-Hill.

Hadfield, N., 2001. The role of aromatherapy massage in reducing anxiety in patients with malignant brain tumours. *Int. J. Palliat. Nurs.*, 7: 279–285.

Hardy, M., et al., 1995. Replacement of drug treatment for insomnia by ambient odour. *Lancet*, 346: 701.

Hastings, L., et al., 1991. Olfactory primary neurons as a route of entry for toxic agents into the CNS. *Neurotoxicology*, 12: 707–714.

Hay, I.C., et al., 1998. Randomized trial of aromatherapy. Successful treatment for Alopecia areata. *Arch. Dermatol.*, 134: 1349–1352.

Healey, M.A. and M. Aslam, 1996. Aromatherapy. In: *Trease & Evans' Pharmacognosy*, W.C. Evans, ed., 14th ed. London: WB Saunders.

Hendriks, S.A., et al., 1999. Allergic contact dermatitis from the fragrance ingredient Lyral¨ in underarm deodorant. *Cont. Dermat.*, 41: 119.

Hirsch, A., 1998. *Sensational Sex: The Secret to Using Aroma for Arousal*. Boston, MA: Element.

Holmes, C., et al., 2002. Lavender oil as a treatment for agitated behaviour in severe dementia: A placebo controlled study. *Int J. Geriatr. Psychiatry*, 17: 305–308.

Horowitz, D.A., 2000. Judgement (pursuant to stipulation). National Council against Health Fraud, Inc, v. Aroma Vera, Inc, et al. Superior Court No. BC183903, October 11.

Jager, W., et al., 1992. Percutaneous absorption of lavender oil from a massage oil. *J. Soc. Cosmet. Chem.*, 43: 49–54.

Jager, W., et al., 1996. Pharmokinetic studies of the fragrance compound 1,8-cineole in humans during inhalation. *Chem. Senses*, 21: 477–480.

Janson, T., 1997. *Mundo Maya*. Guatemala: Editorial Artemis Edinter.

Johansen, J.D., et al., 1998. Allergens in combination have a synergistic effect on the elicitation response: A study of fragrance-sensitized individuals. *Br. J. Dermatol.*, 139: 264–270.

Johansen, J.D., et al., 2000. Rash related to use of scented products. A questionnaire study in the Danish population. Is the problem increasing? *Cont. Dermat.*, 42: 222–226.

Kafferlein, H.U., et al., 1998. Musk xylene: Analysis, occurrence, kinetics and toxicology. *Crit. Rev. Toxicol*, 28: 431–476.

Karamat, R., et al., 1992. Excitatory and sedative effects of essential oils on human reaction time performance. *Chem. Senses*, 17: 847.

Karlberg, A.-T. and A. Dooms-Goossens, 1997. Contact allergy to oxidised *d*-limonene among dermatitis patients. *Cont. Dermat.*, 36: 201–206.

Katona, M. and K. Egyud, 2001. [Increased sensitivity to balsams and fragrances among our patients.] *Orv. Hetil.*, 142: 465–466.

Keane, F.M., et al., 2000. Occupational allergic contact dermatitis in two aromatherapists. *Cont. Dermat.*, 43: 49–51.

Kennedy, D.O., et al., 2002. Modulation of mood and cognitive performance following acute administration of *Melissa officinalis* (lemon balm. *Pharmacol. Biochem. Behav.*, 72: 953–964.

Kilstoff, K. and L. Chenoweth, 1998. New approaches to health and well-being for dementia day-care clients, family carers and day-care staff. *Int. J. Nurs. Pract.*, 4: 70–83.

Kirk-Smith, M., 1996a. Clinical evaluation: Deciding what questions to ask. *Nurs. Times*, 92: 34–35.

Kite, S.M., et al., 1998. Development of an aromatherapy service at a Cancer Centre. *Palliat. Med.*, 12: 171–180.

Klarmann, E.G., 1958. Perfume dermatitis. *Ann. Allergy*, 16: 425–434.

Klemm, W.R., et al., 1992. Topographical EEG maps of human responses to odors. *Chem. Senses*, 17: 347–361.

Knasko, S.C., 1992. Ambient odours effect on creativity, mood and perceived health. *Chem. Senses*, 17: 27–35.

Knasko, S.C., 1993. Performance, mood and health during exposure to intermittent odours. *Arch. Environ. Health*, 48: 3058.

Knasko, S.C., et al., 1990. Emotional state, physical well-being and performance in the presence of feigned ambient odour. *J. Appl. Soc. Psychol.*, 20: 1345–1347.

Knight, T.E. and B.M. Hausen 1994. Melaleuca oil (tea tree oil) dermatitis. *J. Am. Acad. Dermatol.*, 30: 423–427.

Kohl, L., et al., 2002. Allergic contact dermatitis from cosmetics: Retrospective analysis of 819 patch-tested patients. *Dermatology*, 204: 334–337.

Kohn, M., 1999. Complementary therapies in cancer care. Macmillan Cancer Relief Report.

Kovar, K.A., et al., 1987. Blood levels of 1,8-cineole and locomotor activity of mice after inhalation and oral administration of rosemary oil. *Planta Med.*, 3: 315–318.

Kubota, M., et al., 1992. Odor and emotion-effects of essential oils on contingent negative variation. In: *Proc. 12th Int. Congr. on Flavours, Fragrances and Essential Oils*, pp. 456–461. Vienna, Austria, October 4–8.
Labyak, S.E. and B.L. Metzger, 1997. The effects of effleurage backrub on the physiological components of relaxation: A meta-analysis. *Nurs. Res.*, 46: 59–62.
Laffan, G., 1992. Chronic respiratory infection. *Int. J. Aromather.*, 4: 17.
Larsen, W., 1998. A study of new fragrance mixtures. *Am. J. Contact Dermat.*, 9: 202–206.
Lawless, J., 1992. *The Encyclopedia of Essential Oils*. Shaftesbury: Element Books.
Lawless, J., 1994. *Aromatherapy and the Mind*. London: Thorsons.
Lis-Balchin, M. 1995. *Aroma Science: The Chemistry and Bioactivity of Essential Oils*, Surrey: Amberwood Publishing Ltd.
Lis-Balchin, M., 1997. Essential oils and 'aromatherapy': Their modern role in healing. *J. R. Soc. Health*, 117: 324–329.
Lis-Balchin, M., 1998a. Aromatherapy versus the internal intake of odours. *NORA Newsletter*, 3: 17.
Lis-Balchin, M., 1998b. Aromatherapy for pain relief. *Pain Concern Summer*, 12–15. (Now available as a pamphlet from the organisation.)
Lis-Balchin, M., 2006. *Aromatherapy Science*. London: Pharmaceutical Press.
Lis-Balchin, M., ed., 2002a. *Geranium and Pelargonium Genera Geranium and Pelargonium: Medicinal and Aromatic Plants—Industrial Profiles*. London: Taylor & Francis.
Lis-Balchin, M., ed., 2002b. *Genus Lavandula: Medicinal and Aromatic Plants—Industrial Profiles*. London: Taylor & Francis.
Lis-Balchin, M., S. Hart, S.G. Deans, and E. Eaglesham, 1996. Comparison of the pharmacological and antimicrobial action of commercial plant essential oils. *Journal of Herbs, Spices and Medicinal Plants*, 4: 69–86.
Lis-Balchin, M., et al., 1996. Bioactivity of commercial geranium oil from different sources. *J. Essent. Oil Res.*, 8: 281–290.
Lis-Balchin, M., et al., 1998. Relationship between the bioactivity and chemical composition of commercial plant essential oils. *Flav. Fragr. J.*, 13: 98–104.
Lis-Balchin, M. and S. Hart, 1997. The effect of essential oils on the uterus compared to that on other muscles. In: *Proc. 27th Int. Symp. Essential Oils*, Ch, Franz A. Mathé, and G. Buchbauer, eds. Vienna, Austria, pp. 29–32, September 8–11, 1996. Carol Stream, IL: Allured Publishing.
Lis-Balchin, M. and S. Hart, 1997c. A preliminary study of the effect of essential oils on skeletal and smooth muscles in vitro. *J. Ethnopharmacol.*, 58: 183–187.
Loret, V., 1887. Le kyphi, parfum sacre des anciens egyptiens. *J. Asiatique*, 10: 76–132.
MacMahon, S. and S. Kermode 1998. A clinical trial of the effect of aromatherapy on motivational behaviour in a dementia care setting using a single subject design. *Aust. J. Holist. Nurs.*, 5: 47–49.
Manley, C.H., 1993. Psychophysiological effect of odor. *Crit. Rev. Food Sci. Nutr.*, 33: 57–62.
Manniche, L., 1989. *An Ancient Egyptian Herbal*. London: British Museum Publications.
Manniche, L., 1999. *Sacred Luxuries. Fragrance, Aromatherapy and Cosmetics in Ancient Egypt*. London: Opus Publishing.
Maury, M., 1989. *Marquerite Maury's Guide to Aromatherapy*. Saffron Walden: CW Daniel Co.
May, B., et al., 1996. Efficacy of a fixed peppermint oil/caraway combination in non-ulcer dyspepsia. *Arzneimettelforsch*, 146: 1149–1153.
Millenson, J.R., 1995. *Mind Matters: Psychological Medicine in Holistic Practice*. Seattle, WA: Eastland Press.
Millqvist, E. and O. Lowhagen 1996. Placebo-controlled challenges with perfume in patients with asthma-like symptoms. *Allergy*, 51: 434–439.
Morris, N., et al., 1995. Anxiety reduction by aromatherapy: Anxiolytic effects of inhalation of geranium and rosemary. *Int. J. Aromather.*, 7: 33–39.
Munro, I.C., et al., 1996. Correlation of structural class with no-observed-effect levels: A proposal for establishing a threshold of concern. *Food Cosmet. Toxicol.*, 34: 829–867.
Müller, J., et al., 1984. *The H&R Book of Perfume*. London: Johnson Publications.
Nelson, N.J., 1997. Scents or nonsense: Aromatherapy's benefits still subject to debate. *J. Natl. Cancer Inst.*, 89: 1334–1336.
Nunn, J.F., 1997. *Ancient Egyptian Medicine*. London: British Museum Press.
Ollevant, N.A., et al., 1999. How big is a drop? A volumetric assay of essential oils. *J. Clin. Nurs.*, 8: 299–304.
Perry, E.K., et al., 1998. Medicinal plants and Alzheimer's disease. *J. Alt. Compl. Med.*, 4: 419–428.

Perry, E.K., et al., 1999. Medicinal plants and Alzheimer's disease: From ethnobotany to phytotherapy. *J. Pharm. Pharmacol.*, 51: 527–534.
Piesse, S.G.W., 1855. *The Art of Perfumery*. London: Longman, Brown, Green.
Pinch, G., 1994. *Magic in Ancient Egypt*. London: British Museum Press.
Price, S., 1983. *Practical Aromatherapy*. Welling-borough: Thorsons.
Price, S., 1993. *Aromatherapy Workbook*. London: Thorsons.
Price, S. and L. Price, 1999. *Aromatherapy for Health Professionals*, 2nd ed. London: Churchill Livingstone.
Pénoel, D., 1991a. *Médecine Aromatique, Médecine Planétaire*. Limoges: Roger Jollois.
Pénoel, D., 1991b. *Cf. les travaux de J.-A. Giralt-Gonzalez*. Limoges: Roger Jollois.
Pénoel, D., 1998. *A Natural Home Health Care using Essential Oils*. Hurricane, UT: Essential Science Publishing.
Rastogi, S.C., et al., 1999. Contents of fragrance allergens in children's cosmetics and cosmetic-toys. *Cont. Dermat.*, 41: 84–88.
Redd, W. and S. Manne, 1991. Fragrance reduces patient anxiety during stressful medical procedures. *Focus on Fragrance Summer*, 1: 1.
Riedel, J., et al., 1999. Haemoglobin binding of a musk xylene metabolite in man. *Xenobiotica*, 29: 573–582.
Rimmel, E., 1865. *The Book of Perfumes*. London: Chapman & Hall.
Rippon Mandy, 1993. Infertility and stress. *Int. J. Aromather.*, 5: 33.
Romaguera, C. and J. Vilplana, 2000. Occupational contact dermatitis from ylang-ylang oil. *Cont. Dermat.*, 43: 251.
Rose, J.E. and F.M. Behm, 1994. Inhalation of vapour from black pepper extract reduces smoking withdrawal symptoms. *Drug Alcohol Depend.*, 34: 225–229.
Sanchez-Perez, J. and A. Garcia-Diez, 1999. Occupational allergic contact dermatitis from eugenol, oil of cinnamon and oil of cloves in a physiotherapist. *Cont. Dermat.*, 41: 346–347.
Schaller, M. and H.C. Korting, 1995. Allergic airborne contact dermatitis from essential oils used in aromatherapy. *Clin. Exp. Dermatol.*, 20: 143–145.
Scheinman, P.L., 1996. Allergic contact dermatitis to fragrance. *Am. J. Cont. Dermatol.*, 7: 65–76.
Schnaubelt, K., 1999. *Medical Aromatherapy—Healing with Essential Oils*. Berkeley, CA: Frog Ltd.
Schumann Antelme, R. and S. Rossini, 2001. *Sacred Sexuality in Ancient Egypt*. Vermont: Inner Traditions International.
Sharma, J.N., et al., 1994. Suppressive effects of eugenol and ginger oil on arthritic rats. *Pharmacology*, 49: 314–318.
Shim, C. and M.H. Williams Jr., 1986. Effect of odors in asthma. *Am. J. Med.*, 80: 18–22.
Skoeld, M., et al., 2002a. Sensitization studies on the fragrance chemical linalool, with respect to autooxidation. Abstract. *Cont. Dermat.*, 46(Suppl 4): 20.
Skoeld, M., et al., 2002b. Studies on the auto-oxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. *Cont. Dermat.*, 46: 267–272.
Smallwood, J., et al., 2001. Aromatherapy and behaviour disturbances in dementia: A randomized controlled trial. *Int. J. Geriatr. Psychiatry*, 16: 1010–1013.
Snow, A.L., et al., 2004. A controlled trial of aromatherapy for agitation in nursing home patients with dementia. *J. Alt. Compl. Med.*, 10: 431–437.
Soden, K., et al., 2004. A randomized controlled trial of aromatherapy massage in a hospice setting. *Palliat. Med.*, 18: 87–92.
Somerville, K.W., et al., 1984. Delayed release peppermint oil capsules (Colpermin) for the spastic colon syndrome—a pharmaco-kinetic study. *Br. J. Clin. Pharm.*, 18: 638–640.
Somerville, K.W., et al., 1985. Stones in the common bile duct: Experience with medical dissolution therapy. *Postgrad. Med. J.*, 61: 313–316.
Southwell, I.A., 1997. Skin irritancy of tea tree oil. *J. Essent. Oil Res.*, 9: 47–52.
Spencer, P.S., et al., 1979. Neurotoxic fragrance produces ceroid and myelin disease. *Science*, 204: 633–635.
Spencer, P.S., et al., 1984. Neurotoxic properties of musk ambrette. *Toxicol. Appl. Pharmacol.*, 75: 571–575.
Steinberg, P., et al., 1999. Acute hepatotoxicity of the polycyclic musk 7-acetyl-1,1,3,4,4,6-hexamethyl1,2,3,4-tetrahydronaphthaline (AHTN. *Toxicol. Lett.*, 111: 151–160.
Stevenson, C., 1994. The psychophysiological effects of aromatherapy massage following cardiac surgery. *Comp. Ther. Med.*, 2: 27–35.
Sugiura, M., et al., 2000. Results of patch testing with lavender oil in Japan. *Cont. Dermat.*, 43: 157–160.
Tate, S, 1997. Peppermint oil: A treatment for postoperative nausea. *J. Adv. Nurs*., 26: 543–549.
Tisserand, R., 1977. *The Art of Aromatherapy*. Saffron Walden: CW Daniel Co.
Tisserand, R. and T. Balacs 1995. *Essential Oil Safety—A Guide for Health Care Professionals*. Edinburgh: Churchill Livingstone.

Tobyn, G., 1997. *Culpeper's Medicine: A Practice of Holistic Medicine*. Shaftesbury: Element Books.
Tong, M.M., et al., 1992. Tea tree oil in the treatment of tinea pedis. *Aust. J. Dermatol.*, 33: 145–149.
Torii, S., et al., 1988. Contingent negative variation and the psychological effects of odor. In: *Perfumery: The Psychology and Biology of Fragrance*, S. Toller and G.H. Dodd, eds. New York: Chapman & Hall.
Unterman, A., 1991. *Dictionary of Jewish Lore & Legend*. London: Thames and Hudson.
Valnet, J., 1982. *The Practice of Aromatherapy*. Saffron Walden: CW Daniel Co.
Van Toller, S. and G.H. Dodd 1991. *Perfumery: The Psychology and Biology of Fragrance*. New York: Chapman & Hall.
Van Toller, S. et al., 1993. An analysis of spontaneous human cortical EEG activity to odours. *Chem. Senses*, 18: 1–16.
Veien, N.K., et al., 1985. Reduction of intake of balsams in patients sensitive to balsam of Peru. *Cont. Dermat.*, 12: 270–273.
Vickers, A., 1996. *Massage and Aromatherapy*. London: Chapman & Hall.
Vilaplana, J. and C. Romaguera 2002. Contact dermatitis from the essential oil of tangerine in fragrance. *Cont. Dermat.*, 46: 108.
von Burg, R., 1995. Toxicology update. *J. Appl. Toxicol.*, 15: 495–499.
Wake, G., et al., 2000. CNS acetylcholine receptor activity in European medicinal plants traditionally used to improve failing memory. *J. Ethnopharmacology*, 69: 105–114.
Warren, C. and S. Warrenburg, 1993. Mood benefits of fragrance. *Perf. Flav.*, 18: 9–16.
Weibel, H. et al., 1989. Cross-sensitization patterns in guinea pigs between cinnamaldehyde, cinnamyl alcohol and cinnamic acid. *Acta Dermatol. Venereol.*, 69: 302–307.
Weil, A., 1983. *Health and Healing*. Boston, MA: Houghton Mifflin.
Which?, 2001. Essential oils. Health Which? *February*, 17–19.
Wiebe, E., 2000. A randomized trial of aromatherapy to reduce anxiety before abortion. *Eff. Clin. Pract.*, 3: 166–169.
Wilcock, A., et al., 2004. Does aromatherapy massage benefit patients with cancer attending a specialist palliative care day centre? *Palliat. Med.*, 18: 287–290.
Wilkinson, S., 1995. Aromatherapy and massage in palliative care. *Int. J. Palliat. Care*, 1: 21–30.
Wilkinson, S., et al., 1999. An evaluation of aromatherapy massage in palliative care. *Palliat. Med.*, 13: 409–417.
Wilkinson, S.M., 2003. Evaluating the efficacy of massage in cancer care. *BMJ*, 326: 562–563.
Wilson, F.P., 1925. *The Plague Pamphlets of Thomas Dekker*. Oxford: Clarendon Press.
Worwood, V., 1991. *The Fragrant Pharmacy*. London: Bantam Books.
Worwood, V., 1996. *The Fragrant Mind*. London: Doubleday.
Worwood, V., 1998. *The Fragrant Heavens. The Spiritual Dimension of Fragrance and Aromatherapy*. Novato, CA: New World Library.
Youdim, K.A. and S.G. Deans, 2000. Effect of thyme oil and thymol dietary supplementation on the antioxidant status and fatty acid composition of the ageing rat brain. *Br. J. Nutr.*, 83: 87–93.
Young, A.R., et al., 1990. Phototumorigenesis studies of 5-methoxypsoralen in bergamot oil: Evaluation and modification of risk of human use in an albino mouse skin model. *J. Phytochem. Photobiol.*, 7: 231–250.
Zarno, V., 1994. Candidiasis: A holistic view. *Int. J. Aromather.*, 6(2): 20–23.

14 Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals

Yoshiaki Noma and Yoshinori Asakawa

CONTENTS

14.1 INTRODUCTION

A large number of monoterpenoids have been detected in or isolated from essential oils and solvent extracts of fungi, algae, liverworts, and higher plants, but the presence of monoterpenoids in fern is negligible. Vegetables, fruits, and spices contain monoterpenoids; however, their fate in human and other animal bodies has not yet been fully investigated systematically. The recent development of analytical instruments makes it easy to analyze the chemical structures of very minor components, and the essential oil chemistry field has dramatically developed.

Since monoterpenoids, in general, show characteristic odor and taste, they have been used as cosmetic materials; food additives; and often for insecticides, insect repellents, and attractant drugs. In order to obtain much more functionalized substances from monoterpenoids, various chemical reactions and microbial transformations of commercially available and cheap synthetic monoterpenoids have been carried out. On the other hand, insect larva and mammals have been used for direct biotransformations of monoterpenoids to study their fate and safety or toxicity in their bodies.

The biotransformation of α-pinene (**4**) by using the black fungus *Aspergillus niger* was reported by Bhattacharyya et al. (1960) half a century ago. During that period, many scientists studied the biotransformation of a number of monoterpenoids by using various kinds of bacteria, fungi, insects, mammals, and cultured cells of higher plants. In this chapter, the microbial transformation of monoterpenoids using bacteria and fungi is discussed. Furthermore, the biotransformation by using insect larva, mammals, microalgae, as well as suspended culture cells of higher plants is also summarized. In addition, several biological activities of biotransformed products are also represented. At the end of this chapter, the metabolite pathways of representative monoterpenoids for further development on biological transformation of monoterpenoids are demonstrated.

14.2 METABOLIC PATHWAYS OF ACYCLIC MONOTERPENOIDS

14.2.1 Acyclic Monoterpene Hydrocarbons

14.2.1.1 Myrcene

The microbial biotransformation of myrcene (**302**) was described with *Diplodia gossypina* ATCC 10936 (Abraham et al., 1985). The main reactions were hydroxylation, as shown in Figure 14.1. On oxidation, myrcene (**302**) gave the diol (**303**) (yield up to 60%) and also a side-product (**304**) that possesses one carbon atom less than the parent compound, in yields of 1–2%.

One of the publications dealing with the bioconversion of myrcene (Busmann and Berger, 1994) described its transformation to a variety of oxygenated metabolites, with *Ganoderma applanatum*, *Pleurotus flabellatus*, and *Pleurotus sajor-caju* possessing the highest transformation activities. One of the main metabolites was myrcenol (**305**) (2-methyl-6-methylene-7-octen-2-ol), which gives a fresh, flowery impression and dominates the sensory impact of the mixture (see Figure 14.1).

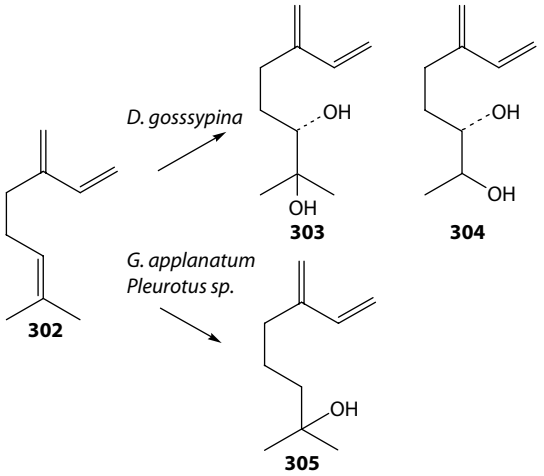


FIGURE 14.1 Biotransformation of myrcene (**302**) by *Diplodia gossypina* (Abraham et al., 1985), *Ganoderma applanatum*, and *Pleurotus* sp. (Modified from Busmann, D. and R.G. Berger, 1994. *J. Biotechol.*, 37: 39–43.)

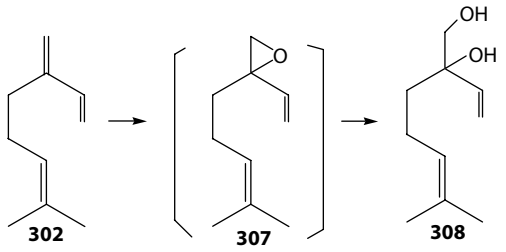


FIGURE 14.2 Biotransformation of myrcene (**302**) by *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1998. *Proc. 42nd TEAC*, pp. 123–125.)

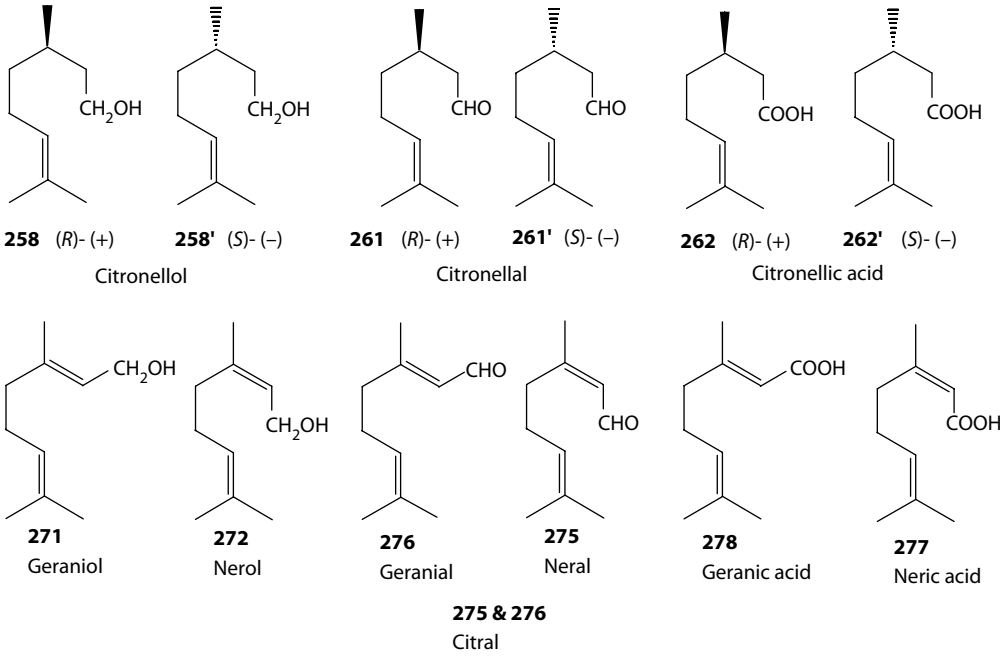
β-Myrcene (**302**) was converted by common cutworm larvae, *Spodoptera litura*, to give myrcene-3,(10)-glycol (**308**) via myrcene-3,(10)-epoxide (**307**) (Figure 14.2) (Miyazawa et al., 1998).

14.2.1.2 Citronellene

(−)-Citronellene (**309**) and (+)-citronellene (**309′**) were biotransformed by the cutworm *Spodoptera litura* to give (3*R*)-3,7-dimethyl-6-octene-1,2-diol (**310**) and (3*S*)-3,7-dimethyl-6-octene-1,2-diol (**310′**), respectively (Takeuchi and Miyazawa, 2005) (Figure 14.3).

14.2.2 Acyclic Monoterpene Alcohols and Aldehydes

14.2.2.1 Geraniol, Nerol, (+)- and (−)-Citronellol, Citral, and (+)- and (−)-Citronellal



The microbial degradation of the acyclic monoterpene alcohols citronellol (**258**), nerol (**272**), geraniol (**271**), citronellal (**261**), and citral (equal mixture of **275** and **276**) was reported in the early part of 1960 (Seubert and Remberger, 1963; Seubert et al., 1963; Seubert and Fass, 1964a, 1964b). *Pseudomonas citronellolis* metabolized citronellol (**258**), citronellal (**261**), geraniol (**271**), and geranic acid (**278**). The metabolism of these acyclic monoterpenes is initiated by the oxidation of the

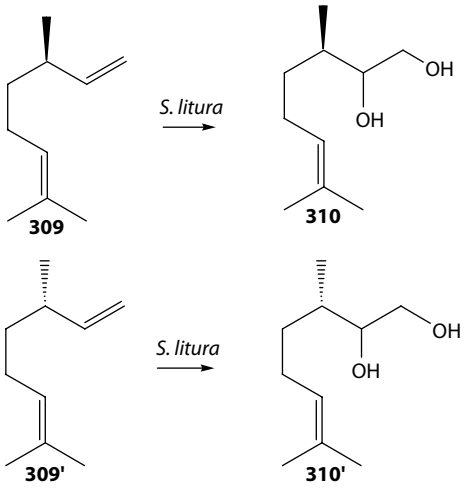


FIGURE 14.3 Biotransformation of (–)-citronellene (**309**) and (+)-citronellene (**309′**) by *Spodoptera litura*. (Modified from Takeuchi, H. and M. Miyazawa, 2005. *Proc. 49th TEAC*, pp. 426–427.)

primary alcohols group to the carboxyl group, followed by the carboxylation of the C-10 methyl group (β-methyl) by a biotin-dependent carboxylase (Seubert and Remberger, 1963). The carboxymethyl group is eliminated at a later stage as acetic acid. Further degradation follows the β-oxidation pattern. The details of the pathway are shown in Figure 14.4 (Seubert and Fass, 1964a).

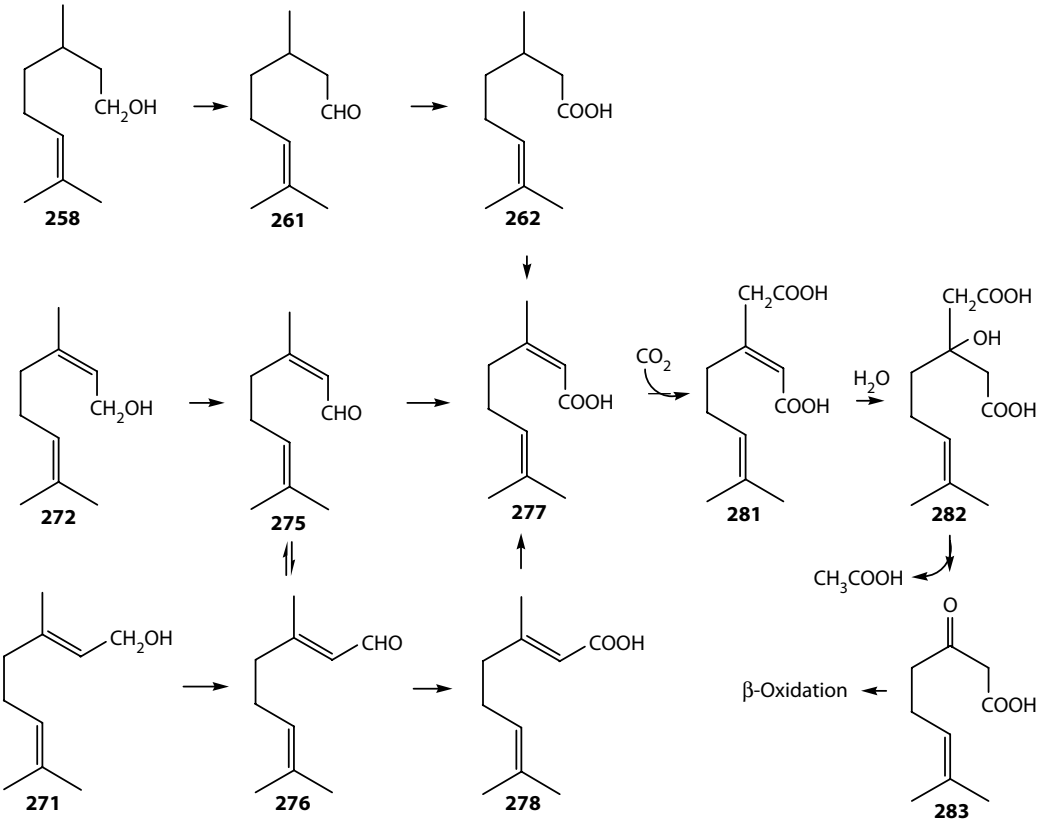


FIGURE 14.4 Biotransformation of citronellol (**258**), nerol (**272**), and geraniol (**271**) by *Pseudomonas citronellolis*. [Modified from Madyastha, K.M. 1984. *Proc. Indian Acad. Sci.* (*Chem. Sci.*), 93: 677–686.]

The microbial transformation of citronellal (**261**) and citral (**275** and **276**) was reported by way of *Pseudomonas aeruginosa* (Joglekar and Dhavlikar, 1969). This bacterium, capable of utilizing citronellal (**261**) or citral (**275** and **276**) as the sole carbon and energy source, has been isolated from soil by the enrichment culture technique. It metabolized citronellal (**261**) to citronellic acid (**262**) (65%), citronellol (**258**) (0.6%), dihydrocitronellol (**259**) (0.6%), 3,7-dimethyl-1,7-octanediol (**260**) (1.7%), and menthol (**137**) (0.75%) (Figure 14.5). The metabolites of citral (**275** and **276**) were geranic acid (**278**) (62%), 1-hydroxy-3,7-dimethyl-6-octen-2-one (**279**) (0.75%), 6-methyl-5-heptenoic acid (**280**) (0.5%), and 3-methyl-2-butenoic acid (**286**) (1%) (Figure 14.5). In a similar way, *Pseudomonas convexa* converted citral (**275** and **276**) to geranic acid (**278**) (Hayashi et al., 1967). The biotransformation of citronellol (**258**) and geraniol (**271**) by *Pseudomonas aeruginosa*, *Pseudomonas citronellolis*, and *Pseudomonas mendocina* was also reported by another group (Cantwell et al., 1978).

A research group in Czechoslovakia patented the cyclization of citronellal (**261**) with subsequent hydrogenation to menthol by *Penicillium digitatum* in 1952. Unfortunately the optical purities of the intermediates pulegol and isopulegol were not determined and presumably the resulting menthol was a mixture of enantiomers. Therefore, it cannot be excluded that this extremely interesting cyclization is the result of a reaction primarily catalyzed by the acidic fermentation conditions and only partially dependent on enzymatic reactions (Babcka et al., 1956) (Figure 14.6).

Based on previous data (Madyastha et al., 1977; Rama and Bhattacharyya, 1977a), two pathways for the degradation of geraniol (**271**) were proposed by Madyastha (1984) (Figure 14.7). Pathway A involves an oxidative attack on the 2,3-double bond, resulting in the formation of an epoxide. Opening of the epoxide yields the 2,3-dihydroxygeraniol (**292**), which upon oxidation forms 2-oxo, 3-hydroxygeraniol (**293**). The ketodiol (**293**) is then decomposed to 6-methyl-5-hepten-2-one (**294**) by an oxidative process. Pathway B is initiated by the oxidation of the primary alcoholic group to geranic acid (**278**) and further metabolism follows the mechanism as proposed earlier for *Pseudomonas citronellolis* (Seubert and Remberger, 1963; Seubert et al., 1963). In the case of nerol (**272**), the *Z*-isomer of geraniol (**271**), degradative pathways analogous to pathways A and B as in geraniol (**271**) are observed. It was also noticed that *Pseudomonas incognita* metabolizes acetates of geraniol (**271**), nerol (**272**), and citronellol (**258**) much faster than their respective alcohols (Madyastha and Renganathan, 1983).

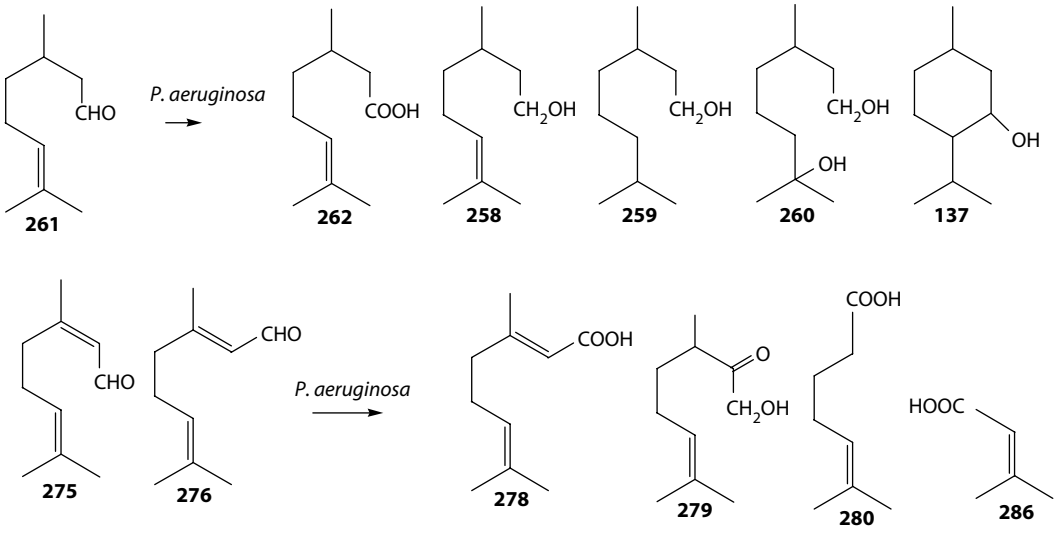


FIGURE 14.5 Biotransformation of citronellal (**261**) and citral (**275** and **276**) by *Pseudomonas aeruginosa*. (Modified from Joglekar, S.S. and R.S. Dhavlikar, 1969. *Appl. Microbiol.*, 18: 1084–1087.)

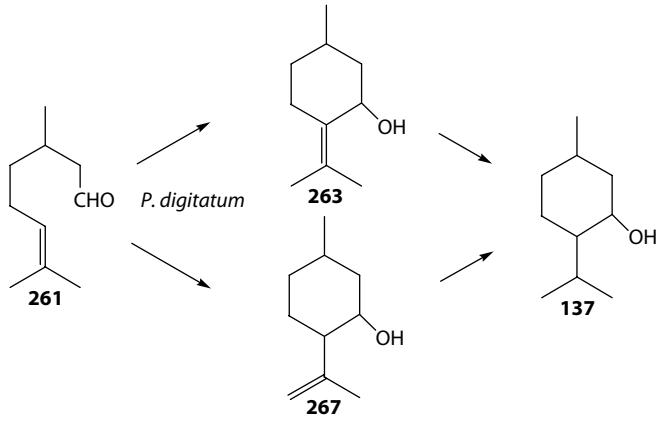


FIGURE 14.6 Biotransformation of citronellal to menthol by *Penicillium digitatum*. (Modified from Babcka, J. et al., 1956. Patent 56-9686b.)

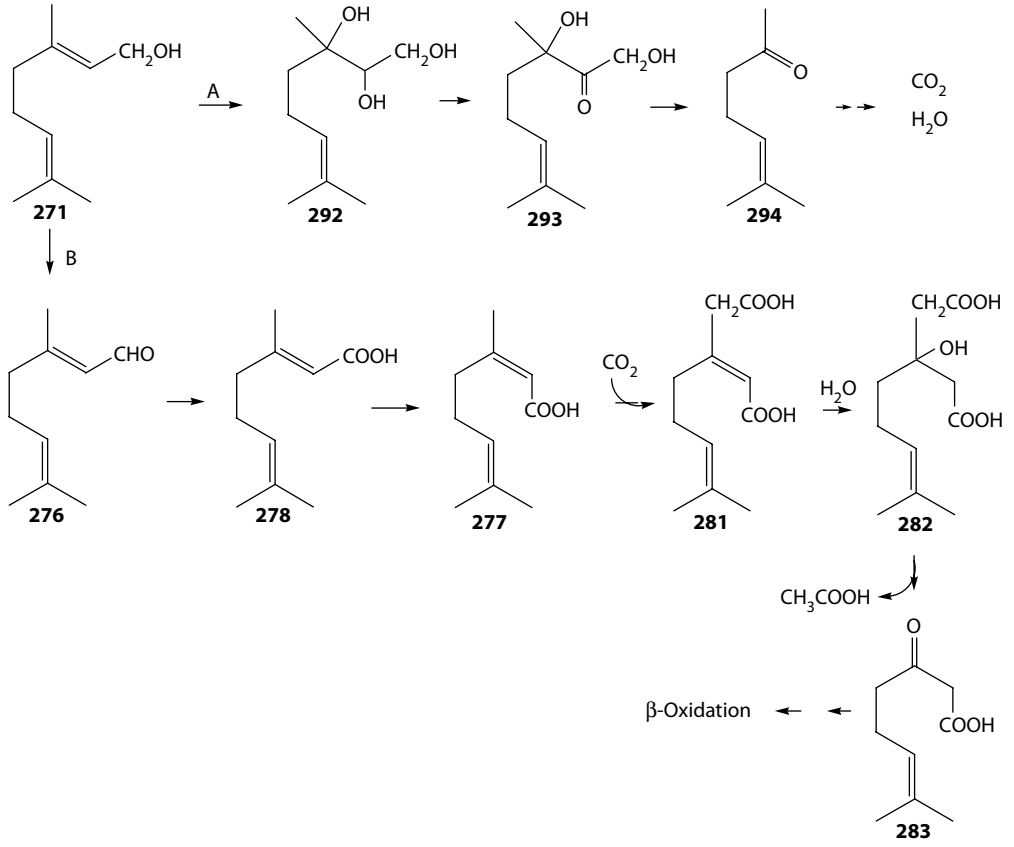


FIGURE 14.7 Metabolism of geraniol (**271**) by *Pseudomonas incognita*. [Modified from Madyastha, K.M. 1984. *Proc. Indian Acad. Sci.* (*Chem. Sci.*), 93: 677–686.]

Euglena gracilis Z converted citral (**275** and **276**, 56:44, peak area in GC) to geraniol (**271**) and nerol (**272**), respectively, of which geraniol (**271**) was further transformed to (+)- and (−)-citronellol (**258** and **258′**). On the other hand, when either geraniol (**271**) or nerol (**272**) was added, both compounds were isomerized to each other and, then, geraniol (**271**) was transformed to citronellol. These results showed that *Euglena* could distinguish between the stereoisomers geraniol (**271**) and nerol (**272**) and hydrogenated geraniol (**271**) selectively. (+)-, (−)-, and (±)-Citronellal (**261**, **261′**, and

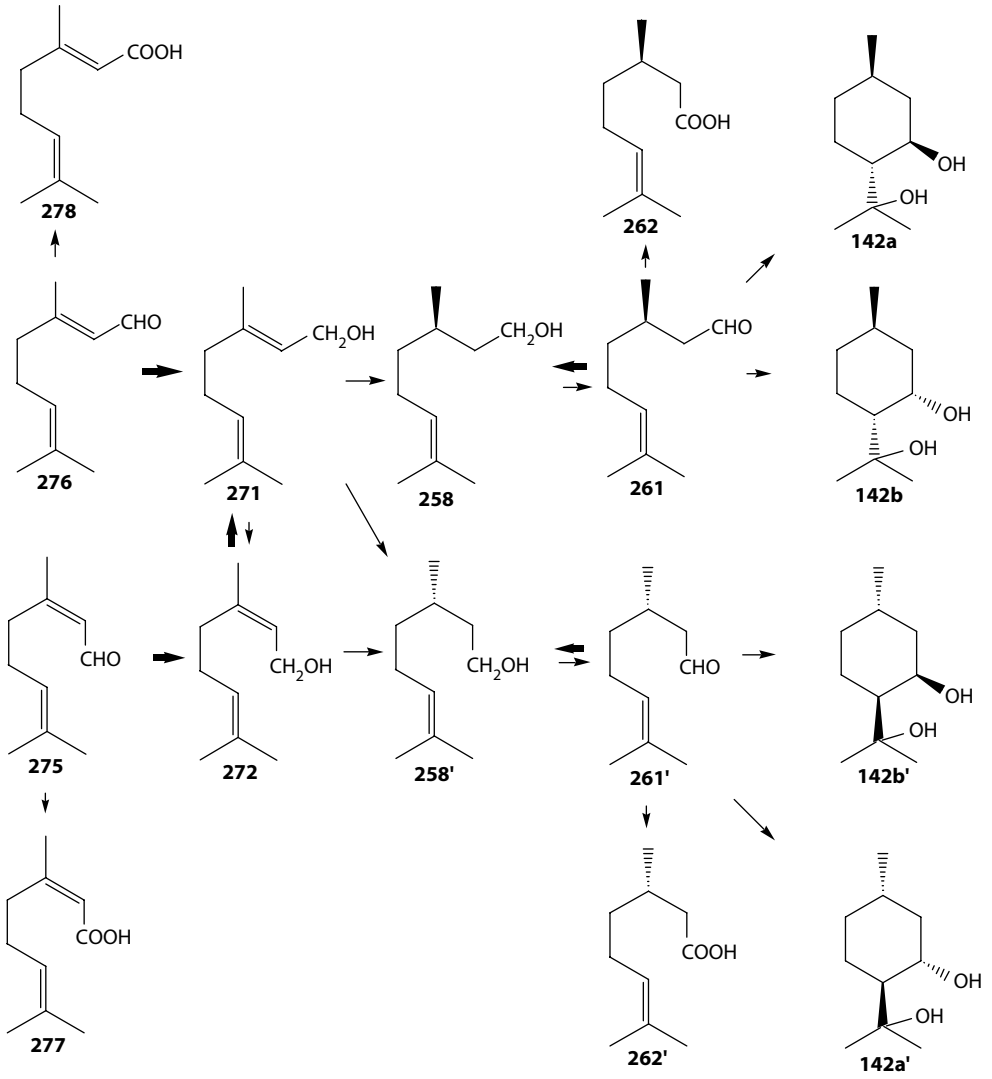


FIGURE 14.8 Metabolic pathways of citral (**275** and **276**) and its metabolites by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1991a. *Phytochem.*, 30: 1147–1151.)

261 and **261′**) were also transformed to the corresponding (+)-, (−)-, and (±)-citronellol (**258**, **258′**, and **258** and **258′**) as the major products and (+)-, (−)-, and (±)-citronellic acids (**262**, **262′**, and **262** and **262′**) as the minor products, respectively (Noma et al., 1991a) (Figure 14.8).

Dunaliella tertiolecta also reduced citral (geranial (**276**) and neral (**275**) = 56:44), (+)-, (−)-, and (±)-citronellal (**261**, **261′**, and **261** and **261′**) to the corresponding alcohols, namely, geraniol (**271**), nerol (**272**), (+)-, (−)-, and (±)-citronellol (**258**, **258′**, and **258** and **258′**) (Noma et al., 1991b, 1992a).

Citral (a mixture of geranial (**276**) and neral (**275**), 56:44 peak area in GC) is easily transformed to geraniol (**271**) and nerol (**272**), respectively, of which geraniol (**32**) is further hydrogenated to (+)-citronellol (**258**) and (−)-citronellol (**258′**). Geranic acid (**278**) and neric acid (**277**) as the minor products are also formed from **276** and **275**, respectively. On the other hand, when either **271** or **272** is used as a substrate, both compounds are isomerized to each other, and then **271** is transformed to citronellol (**258** or **258′**). These results showed the *Euglena* could distinguish between

the stereoisomers, **271** and **272** and hydrogenated selectively **271** to citronellol (**258** or **258′**). (+)-, (−)-, and (±)-Citronellal (**261**, **261′**, and equal mixture of **261** and **261′**) are also transformed to the corresponding citronellol and *p*-menthane-*trans*- and *cis*-3,8-diols (**142a, b, a′** and **b′**) as the major products, which are well known as mosquito repellents and plant growth regulators (Nishimura et al., 1982; Nishimura and Noma, 1996), and (+)-, (−)-, and (±)-citronellic acids (**262**, **262′**, and equal mixture of **262** and **262′**) as the minor products, respectively.

Streptomyces ikutamanensis, Ya-2–1, also reduced citral (geranial (**276**) and neral (**275**) = 56:44), (+)-, (−)-, and (±)-citronellal (**261**, **261′**, and **261** and **261′**) to the corresponding alcohols, namely, geraniol (**271**), nerol (**272**), (+)-, (−)-, and (±)-citronellol (**258**, **258′**, **258** and **258′**). Compounds **271** and **272** were isomerized to each other. Furthermore, terpene alcohols (**258′**, **272**, and **271**) were epoxidized to give 6,7-epoxygeraniol (**274**), 6,7-epoxynerol (**273**), and 2,3-epoxycitronellol (**268**). On the other hand, (+)- and (±)-citronellol (**258** and **258** and **258′**) were not converted at all (Noma et al., 1986) (Figure 14.9).

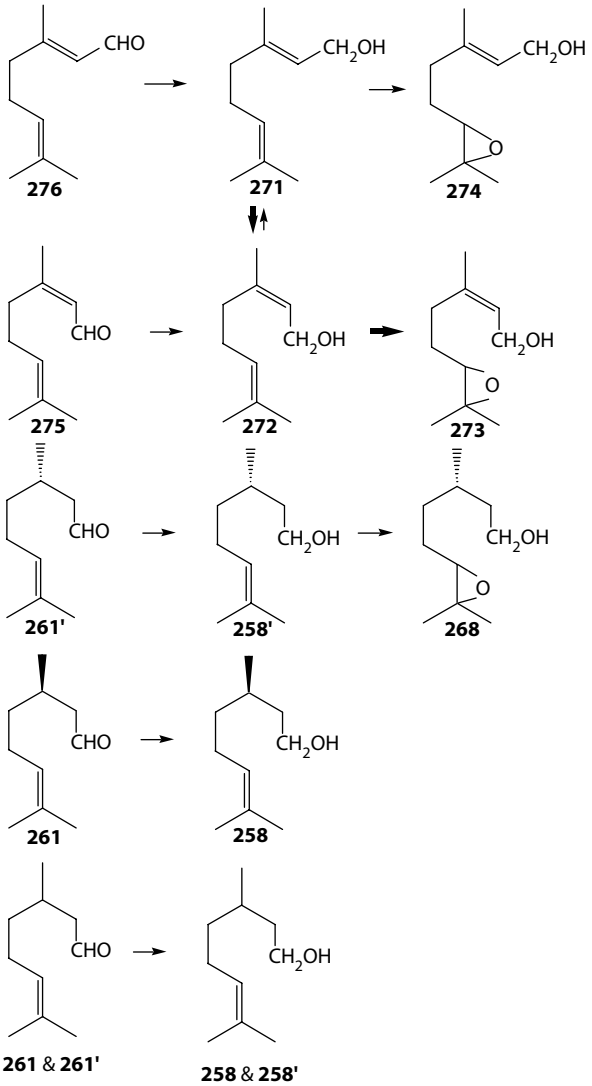


FIGURE 14.9 Reduction of terpene aldehydes and epoxidation of terpene alcohols by *Streptomyces ikutamanensis*, Ya-2-1. (Modified from Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206.)

A strain of *Aspergillus niger*, isolated from garden soil, was able to transform geraniol (**271**), citronellol (**258** and **258′**), and linalool (**206**) to their respective 8-hydroxy derivatives. This reaction was called "ω-hydroxylation" (Madyastha and Krishna Murthy, 1988a, 1988b).

Fermentation of citronellyl acetate with *Aspergillus niger* resulted in the formation of a major metabolite, 8-hydroxycitronellol, accounting for approximately 60% of the total transformation products, accompanied by 38% citronellol. Fermentation of geranyl acetate with *Aspergillus niger* gave geraniol and 8-hydroxygeraniol (50% and 40%, respectively, of the total transformation products).

One of the most important examples of fungal bioconversion of monoterpene alcohols is the biotransformation of citral by *Botrytis cinerea*. *Botrytis cinerea* is a fungus of high interest in wine-making (Rapp and Mandery, 1988). In an unripe state of maturation the infection of grapes by *Botrytis cinerea* is very much feared, as the grapes become mouldy ("gray rot"). With fully ripe grapes, however, the growth of *Botrytis cinerea* is desirable; the fungus is then called "noble rot" and the infected grapes deliver famous sweet wines, such as, for example, Sauternes of France or Tokay Aszu of Hungary (Brunerie et al., 1988).

One of the first reports in this area dealt with the biotransformation of citronellol (**258**) by *Botrytis cinerea* (Brunerie et al., 1987a, 1988). The substrate was mainly metabolized by ω-hydroxylation. The same group also investigated the bioconversion of citral (**275** and **276**) (Brunerie et al., 1987b). A comparison was made between grape must and a synthetic medium. When using grape must, no volatile bioconversion products were found. With a synthetic medium, biotransformation of citral (**275** and **276**) was observed yielding predominantly nerol (**272**) and geraniol (**271**) as reduction products and some ω-hydroxylation products as minor compounds. Finally, the bioconversion of geraniol (**271**) and nerol (**272**) was described by the same group (Bock et al., 1988). When using grape must, a complete bioconversion of geraniol (**271**) was observed mainly yielding ω-hydroxylation products.

The most important metabolites from geraniol (**271**), nerol (**272**), and citronellol (**258**) are summarized in Figure 14.9. In the same year the biotransformation of these monoterpenes by *Botrytis cinerea* in model solutions was described by another group (Rapp and Mandery, 1988). Although the major metabolites found were ω-hydroxylation compounds, it is important to note that some new compounds that were not described by the previous group were detected (Figure 14.9). Geraniol (**271**) was mainly transformed to (2*E*,5*E*)-3,7-dimethyl-2,5-octadiene-1,7-diol (**318**), (*E*)-3,7-dimethyl-2,7-octadiene-1,6-diol (**319**), and (2*E*,6*E*)-2,6-dimethyl-2,6-octadiene-1,8-diol (**300**); nerol (**272**) to (2*Z*,5*E*)-3,7-dimethyl-2,5-octadiene-1,7-diol (**314**), (*Z*)-3,7-dimethyl-2,7-octadiene-1,6-diol (**315**), and (2*E*,6*Z*)-2,6-dimethyl-2,6-octadiene-1,8-diol (**316**). Furthermore, a cyclization product (**318**) that was not previously described was formed. Finally, citronellol (**258**) was converted to *trans*- (**312**) and *cis*-rose oxide (**313**) (a cyclization product not identified by the other group), (*E*)-3,7-dimethyl-5-octene-1,7-diol (**311**), 3,7-dimethyl-7-octene-1,6-diol (**260**), and (*E*)-2,6-dimethyl-2-octene-1,8-diol (**265**) (Miyazawa et al., 1996a) (Figure 14.10).

One of the latest reports in this area described the biotransformation of citronellol by the plant pathogenic fungus *Glomerella cingulata* to 3,7-dimethyl-1,6,7-octanetriol (Miyazawa et al., 1996a).

The ability of fungal spores of *Penicillium digitatum* to biotransform monoterpene alcohols, such as geraniol (**271**) and nerol (**272**) and a mixture of the aldehydes, that is, citral (**276** and **275**), has only been discovered very recently by Demyttenaera and coworkers (Demyttenaera et al., 1996, 2000; Demyttenaera and De Pooter, 1996, 1998). Spores of *Penicillium digitatum* were inoculated on solid media. After a short incubation period, the spores germinated and a mycelial mat was formed. After 2 weeks, the culture had completely sporulated and bioconversion reactions were started. Geraniol (**271**), nerol (**272**), or citral (**276** and **275**) were sprayed onto the sporulated surface culture. After 1 or 2 days, the period during which transformation took place, the cultures were extracted. Geraniol and nerol were transformed into 6-methyl-5-hepten-2-one by sporulated surface cultures of *Pencillium digitatum*. The spores retained their activity for at least 2 months. An overall yield of up to 99% could be achieved.

The bioconversion of geraniol (**271**) and nerol (**272**) was also performed with sporulated surface cultures of *Aspergillus niger*. Geraniol (**271**) was converted to linalool (**206**), α-terpineol (**34**), and

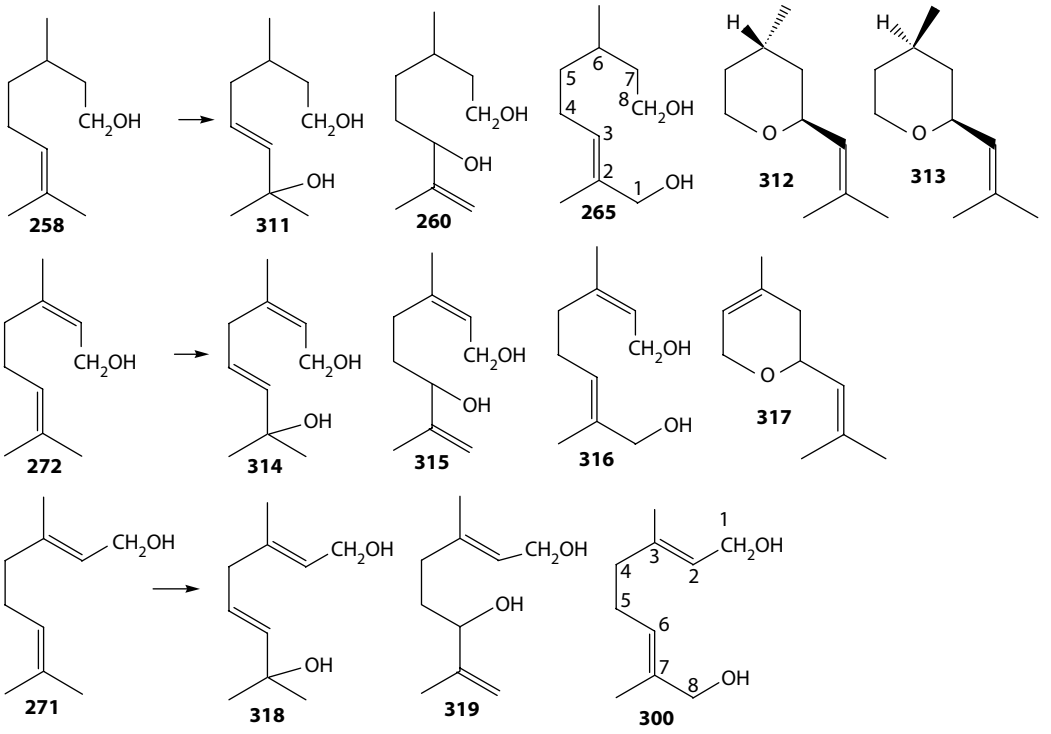


FIGURE 14.10 Biotransformation of geraniol (**271**), nerol (**272**), and citronellol (**258**) by *Botrytis cinerea*. (Modified from Miyazawa, M. et al., 1996a. *Nat. Prod. Lett.*, 8: 303–305.)

limonene (**68**), and nerol (**272**) was converted mainly to linalool (**206**) and α-terpineol (**34**) (Demyttenaera et al., 2000).

The biotransformation of geraniol (**271**) and nerol (**272**) by *Catharanthus roseus* suspension cells was carried out. It was found that the allylic positions of geraniol (**271**) and nerol (**272**) were hydroxylated and reduced to double bond and ketones (Figure 14.11). Geraniol (**271**) and nerol (**272**) were isomerized to each other. Geraniol (**271**) and nerol (**272**) were hydroxylated at C10 to

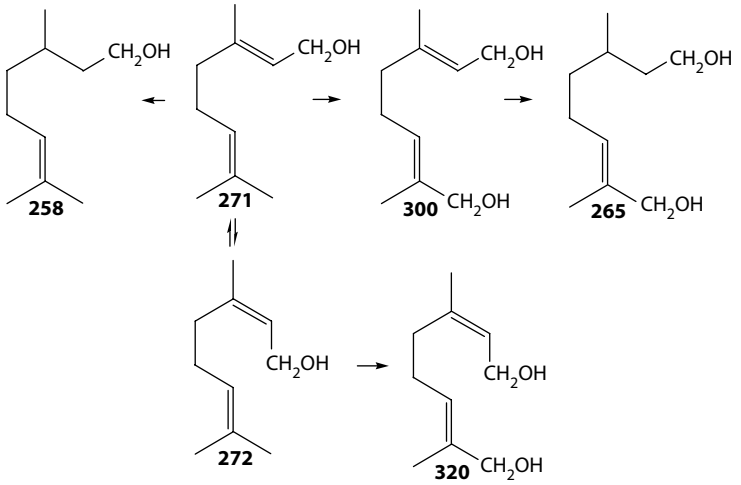


FIGURE 14.11 The biotransformation of geraniol (**271**) and nerol (**272**) by *Catharanthus roseus*. (Modified from Hamada, H. and H. Yasumune, 1995. *Proc. 39th TEAC*, pp. 375–377.)

8-hydroxygeraniol (**300**) and 8-hydroxynerol (**320**), respectively. 8-Hydroxygeraniol (**300**) was hydrogenated to 10-hydroxycitronellol (**265**). Geraniol (**271**) was hydrogenated to citronellol (**258**) (Hamada and Yasumune, 1995).

Cyanobacterium converted geraniol (**271**) to geranic acid (**278**) via geranial (**276**), followed by hydrogenation to give citronellic acid (**262**) via citronellal (**261**). Furthermore, the substrate **271** was isomerized to nerol (**272**), followed by oxidation, reduction, and further oxidation to afford neral (**275**), citronellal (**261**), citronellic acid (**262**), and nerolic acid (**277**) (Kaji et al., 2002; Hamada et al., 2004) (Figure 14.12).

Plant suspension cells of *Catharanthus roseus* converted geraniol (**271**) to 8-hydroxygeraniol (**300**). The same cells converted citronellol (**258**) to 8- (**265**) and 10-hydroxycitronellol (**264**) (Hamada et al., 2004) (Figure 14.13).

Nerol (**272**) was converted by the insect lavae *Spodoptera litura* to give 8-hydroxynerol (**320**), 10-hydroxynerol (**321**), 1-hydroxy-3,7-dimethyl-(2*E*,6*E*)-octadienal (**322**), and 1-hydroxy-3,7-dimethyl-(2*E*,6*E*)-octadienoic acid (**323**) (Takeuchi and Miyazawa, 2004) (Figure 14.14).

14.2.2.2 Linalool and Linalyl Acetate

(+)-Linalool (**206**) [(*S*)-3,7-dimethyl-1,6-octadiene-3-ol] and its enantiomer (**206′**) ((*R*)-3,7-dimethyl-1,6-octadiene-3-ol) occur in many essential oils, where they are often the main component. (*S*)-(+)-Linalool (**206**) makes up 60–70% of coriander oil. (*R*)-(−)-linalool (**206′**), for example, occurs at a concentration of 80–85% in Ho oils from *Cinnamomum camphora*; rosewood oil contains ca. 80% (Bauer et al., 1990).

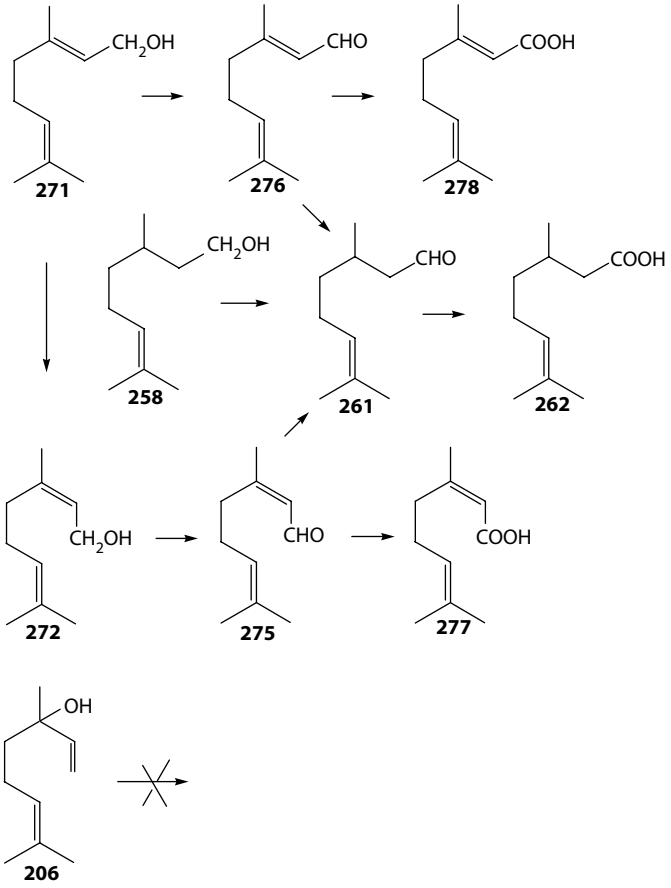


FIGURE 14.12 Biotransformation of geraniol (**271**) and citronellol (**258**) by *Cyanobacterium*.

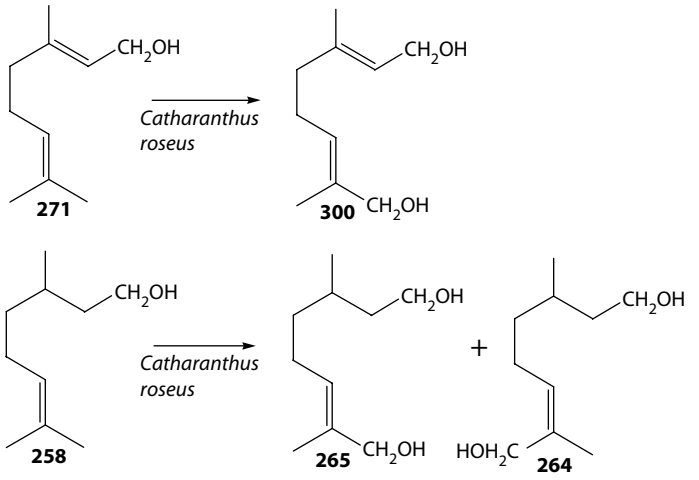


FIGURE 14.13 Biotransformation of geraniol (**271**), citronellol (**258**), and linalool (**206**) by plant suspension cells of *Catharanthus roseus*. (Modified from Hamada, H. et al., 2004. *Proc. 48th TEAC*, pp. 393–395.)

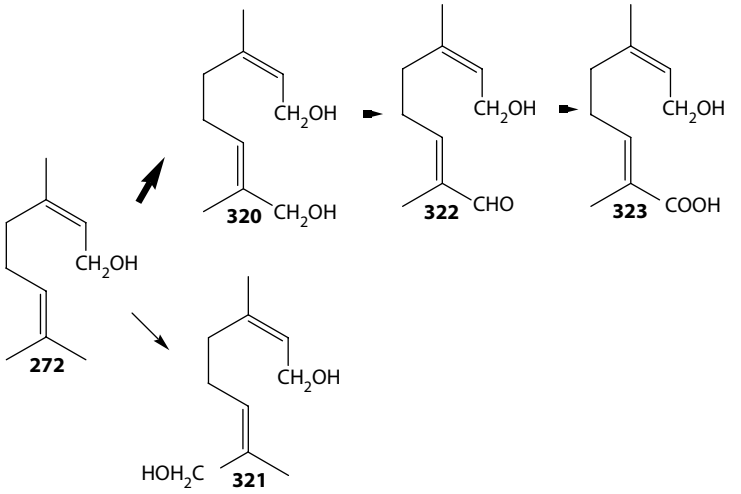


FIGURE 14.14 Biotransformation of nerol (**272**) by *Spodoptera litura*. (Modified from Takeuchi, H. and M. Miyazawa, 2004. *Proc. 48th TEAC*, pp. 399–400.)

Catharanthus roseus converted (+)-linalool (**206**) to 8-hydroxylinalool (**219**) (Hamada et al., 2004) (Figure 14.15).

The biodegradation of (+)-linalool (**206**) by *Pseudomonas pseudomallei* (strain A), which grows on linalool as the sole carbon source, was described in 1973 (Murakami et al., 1973) (Figure 14.16).

Madyastha et al. (1977) isolated a soil Pseudomonad, *Pseudomonas incognita*, by the enrichment culture technique with linalool as the sole carbon source. This microorganism, the "linalool strain" as it was called, was also capable of utilizing limonene (**68**), citronellol (**258**), and geraniol (**271**) but failed to grow on citral (**275** and **276**), citronellal (**261**), and 1,8-cineole (**122**). Fermentation was carried out with shake cultures containing 1% linalool (**206**) as the sole carbon source. It was suggested by the authors that linalool (**206**) was metabolized by at least three different pathways of biodegradation (Figure 14.19). One of the pathways appeared to be initiated by the specific oxygenation of C-8 methyl group of linalool (**206**), leading to 8-hydroxylinalool (**219**), which was further oxidized to linalool-8-carboxylic acid (**220**). The presence of furanoid linalool oxide (**215**) and 2-methyl-2-vinyltetrahydrofuran-5-one (**216**) as the unsaturated lactone in the fermentation medium

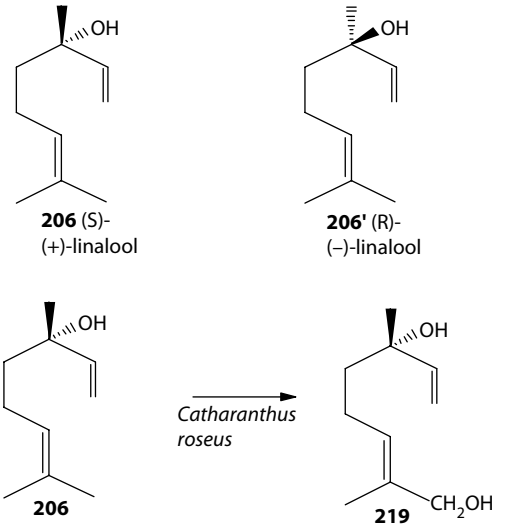


FIGURE 14.15 Biotransformation of linalool (**206**) by plant suspension cells of *Catharanthus roseus*. (Modified from Hamada, H. et al., 2004. *Proc. 48th TEAC*, pp. 393–395.)

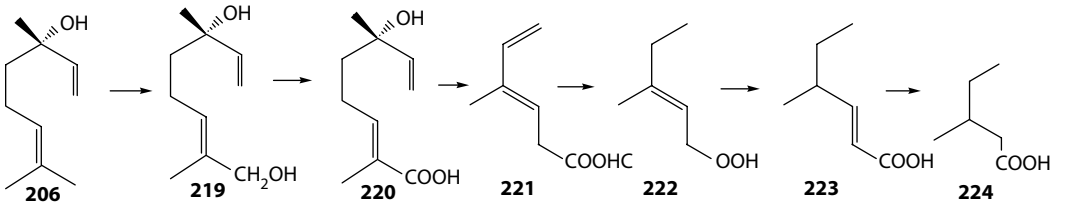


FIGURE 14.16 Degradative metabolic pathway of (+)-linalool (**206**) by *Pseudomonas pseudomallei*. (Modified from Murakami, T. et al., 1973. *Nippon Nogei Kagaku Kaishi*, 47: 699–703.)

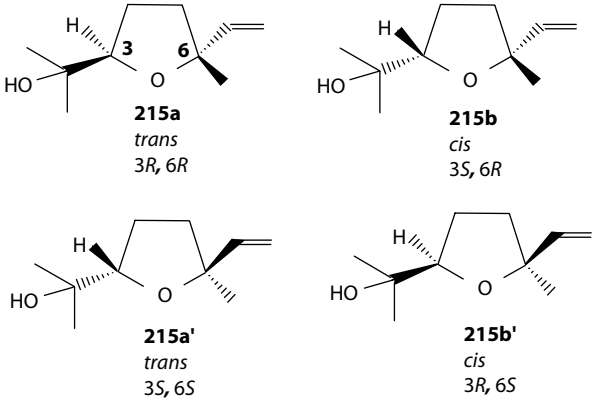


FIGURE 14.17 Four stereoisomers of furanoid linalool oxides. (Modified from Noma, Y. et al., *Proc. 30th TEAC*, pp. 204–206.)

suggested another mode of utilization of linalool (**206**). The formation of these compounds was believed to proceed through the epoxidation of the 6,7-double bond giving rise to 6,7-epoxylinalool (**214**), which upon further oxidation yielded furanoid linalool oxide (**215**) and 2-methyl-2-vinyl-tetrahydrofuran-5-one (**216**) (Figure 14.19).

The presence of oleuropeic acid (**204**) in the fermentation broth suggested a third pathway. Two possibilities were proposed: (3a) water elimination giving rise to a monocyclic cation (**33**), yielding

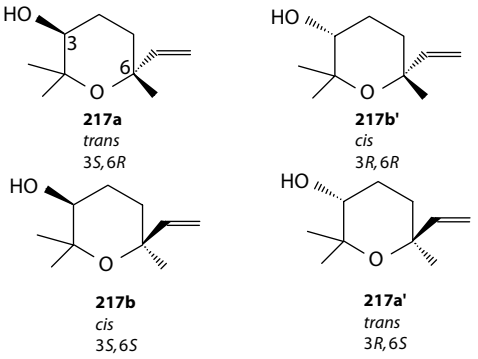


FIGURE 14.18 Four stereoisomers of pyranoid linalool oxides.

α-terpineol (**34**), which upon oxidation gave oleuropeic acid (**204**); (3b) oxidation of the C-10 methyl group of linalool (**206**) before cyclization, giving rise to oleuropeic acid (**204**). This last pathway was also called the "prototropic cyclization" (Madyastha, 1984).

Racemic linalool (**206** and **206′**) is cyclized into *cis*- and *trans*-linalool oxides by various microorganisms such as *Streptomyces albus* NRRL B1865, *Streptomyces hygroscopicus* NRRL B3444, *Streptomyces cinnamonnensis* ATCC 15413, *Streptomyces griseus* ATCC 10137, and *Beauveria sulfurescens* ATACC 7159 (David and Veschambre, 1984) (Figure 14.19).

Aspergillus niger isolated from garden soil biotransformed linalool and its acetates to give linalool (**206**), 2,6-dimethyl-2,7-octadiene-1,6-diol (8-hydroxylinalool (**219a**), α-terpineol (**34**), geraniol (**271**), and some unidentified products in trace amounts (Madyastha and Krishna Murthy, 1988a, 1988b).

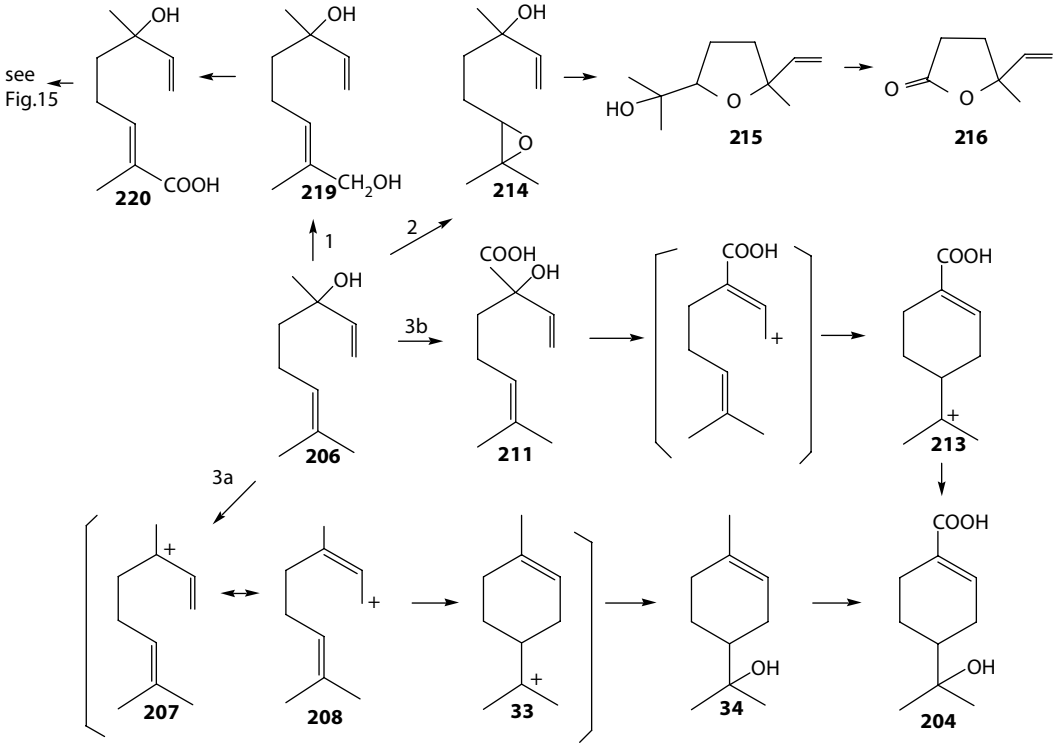


FIGURE 14.19 Biotransformation of linalool (**206**) by *Pseudomonas incognita* (Madyastha et al., 1977) and *Streptomyces albus* NRRL B1865. (Modified from David, L. and H. Veschambre, 1984. *Tetrahadron Lett.*, 25: 543–546.)

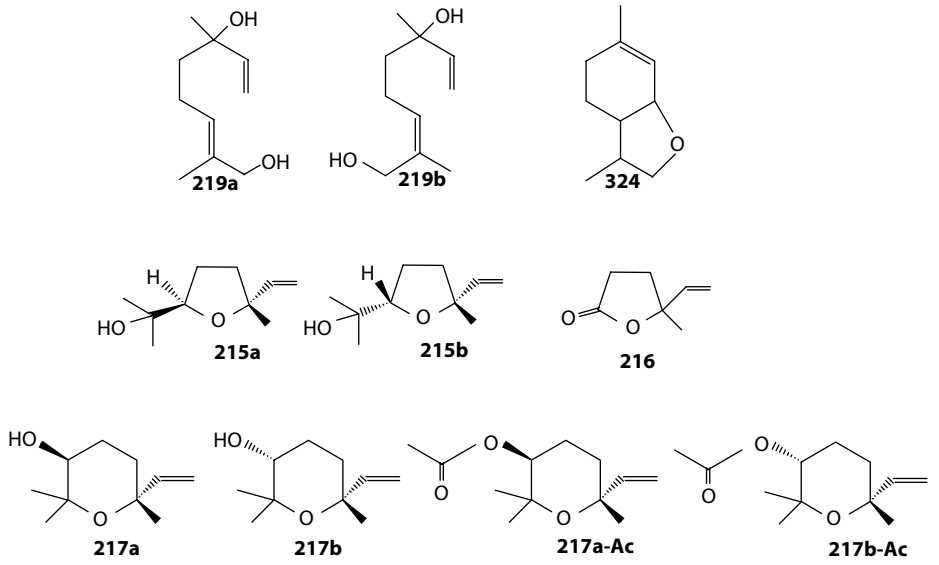


FIGURE 14.20 Biotransformation products of linalool (**206**) by *Botrytis cinerea*. (Modified from Bock, G. et al., 1986. *J. Food Sci.*, 51: 659–662.)

The biotransformation of linalool (**206**) by *Botrytis cinerea* was carried out and identified transformation products such as (*E*)-(**219a**) and (*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol (**219b**), *trans*-(**215a**) and *cis*-furanoid linalool oxide (**215b**), *trans*- (**217a**) and *cis*-pyranoid linalool oxide (**217b**) (Figure 14.18) and their acetates (**217a-Ac**, **217b-Ac**), 3,9-epoxy-*p*-menth-1-ene (**324**) and 2-methyl-2-vinyl-tetrahydrofuran-5-one (**216**) (unsaturated lactone) (Bock et al., 1986) (Figure 14.20). Quantitative analysis, however, showed that linalool (**206**) was predominantly (90%) metabolized to (*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol (**219a**) by *Botrytis cinerea*. The other compounds were only found as by-products in minor concentrations.

The bioconversion of (*S*)-(+)-linalool (**206**) and (*R*)-(−)-linalool (**206′**) was investigated with *Diplodia gossypina* ATCC 10936 (Abraham et al., 1990). The biotransformation of (±)-linalool (**206** and **206′**) by *Aspergillus niger* ATCC 9142 with submerged shaking culture yielded a mixture of *cis*- (**215b**) and *trans*-furanoid linalool oxide (**215a**) (yield 15–24%) and *cis*- (**217b**) and *trans*-pyranoid linalool oxide (**217a**) (yield 5–9%) (Demyttenaere and Willemen, 1998). The biotransformation of (*R*)-(−)-linalool (**206a**) with *Aspergillus niger* ATCC 9142 yielded almost pure *trans*-furanoid linalool oxide (**215a**) and *trans*-pyranoid linalool oxide (**217a**) (ee > 95) (Figure 14.21). These conversions were purely biocatalytic, since in acidified water (pH < 3.5) almost 50% linalool (**206**) was recovered unchanged, the rest was evaporated. The biotransformation was also carried out with growing surface cultures.

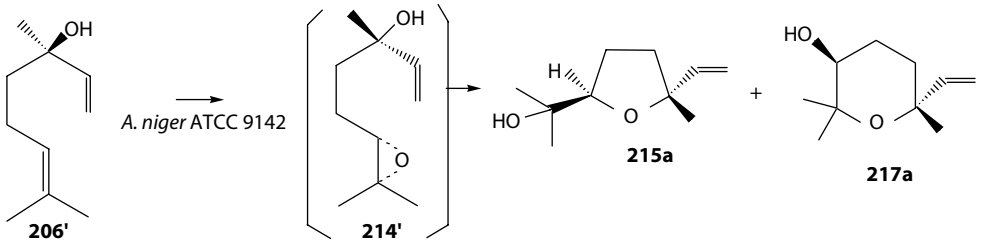


FIGURE 14.21 Biotransformation of (*R*)-(−)-linalool (**206′**) by *Aspergillus niger* ATCC 9142. (Modified from Demyttenaere, J.C.R. and H.M. Willemen, 1998. *Phytochemistry*, 47: 1029–1036.)

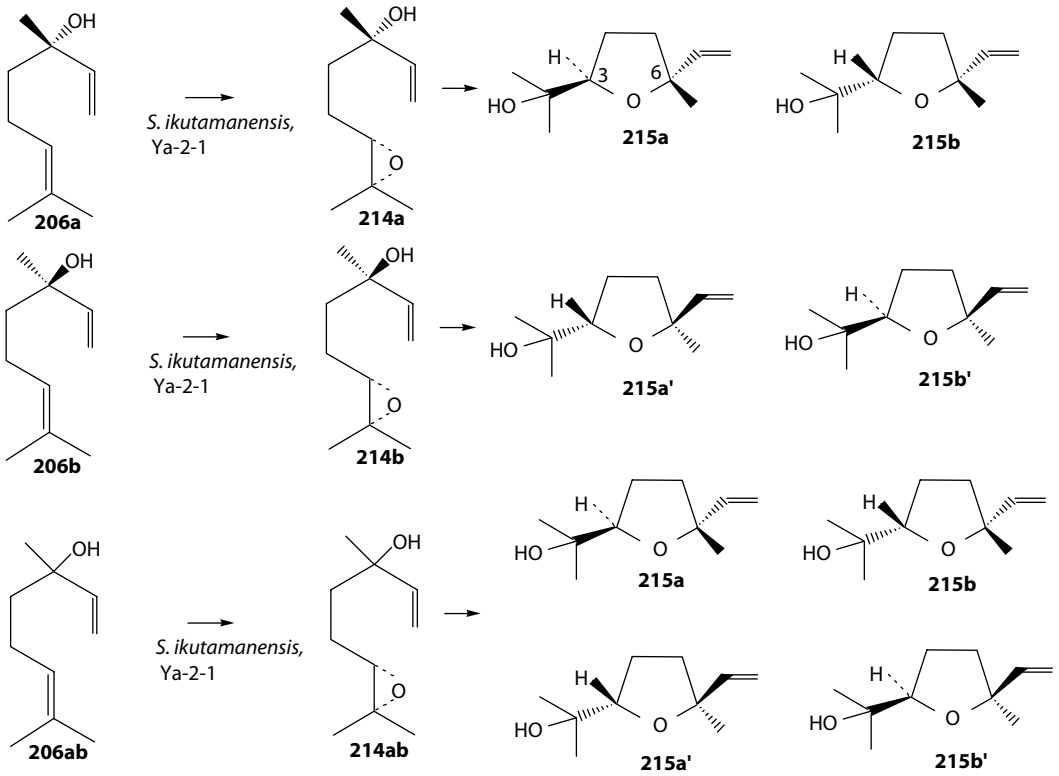


FIGURE 14.22 Metabolic pathway of (+)- (**206**), (–)- (**206′**), and racemic linalool (**206** and **206′**) by *Streptomyces ikutamanensis*, Ya-2-1. (Modified from Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206.)

Streptomyces ikutamanensis, Ya-2-1 also converted (+)- (**206**), (–)- (**206′**), and racemic linalool (**206** and **206′**) via corresponding 2,3-epoxides (**214** and **214′**) to *trans*- and *cis*-furanoid linalool oxides (**215a**, **b**, **a′** and **b′**) (Noma et al., 1986) (Figure 14.22). The absolute configuration at C-3 and C-6 of *trans*- and *cis*-linalool oxides are shown in Figure 14.17.

Biotransformation of racemic *trans*-pyranoid linalool oxide (**217a** and **a′**) and racemic *cis*-linalool-pyranoid (**217b** and **b′**) has been carried out using fungus *Glomerella cingulata* (Miyazawa et al., 1994a). *trans* and *cis*-Pyranoid linalool oxide (**217a** and **217b**) were transformed to *trans*- (**217a′-1**) and *cis*-linalool oxide-3-malonate (**217b′-1**), respectively. In the biotransformation of racemic *cis*-linalool oxide-pyranoid, (+)-(3*R*,6*R*)-*cis*-pyranoid linalool oxide (**217a** and **a′**) was converted to (3*R*,6*R*)-pyranoid-*cis*-linalool oxide-3-malonate (**217a′-1**). (–)-(3*S*, 6*S*)-*cis*-Pyranoid linalool oxide-pyranoid (**217a′**) was not metabolized. On the other hand, in the biotransformation of racemic *trans*-pyranoid linalool oxide (**217b** and **b′**), (–)-(3*R*,6*S*)-*trans*-linalool oxide (**217b′**) was transformed to (3*R*,6*S*)-*trans*-linalool oxide-3-malonate (**217b′-1**) (Figure 14.23). (+)-(3*S*,6*S*)-*trans*-Pyranoid-linalool oxide (**217b**) was not metabolized. These facts showed that *Glomerella cingulata* recognized absolute configuration of the secondary hydroxyl group at C-3. On the basis of this result, it has become apparent the optical resolution of racemic pyranoid linalool oxide proceeded in the biotransformation with *Glomerella cingulata* (Miyazawa et al., 1994a).

Linalool (**206**) and tetrahydrolinalool (**325**) were converted by suspension cells of *Catharanthus roseus* to give 1-hydroxylinalool (**219**) from linalool (**206**) and 3,7-dimethyloctane-3,5-diol (**326**), 3,7-dimethyloctane-3,7-diol (**327**), and 3,7-dimethyloctane-3,8-diol (**328**) from tetrahydrolinalool (**325**) (Hamada and Furuya, 2000; Hamada et al., 2004) (Figure 14.24).

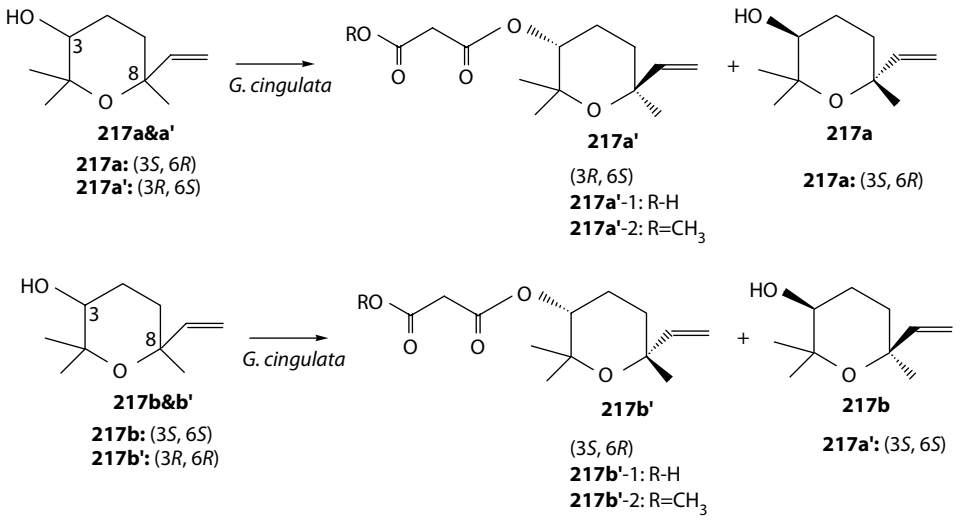


FIGURE 14.23 Biotransformation of racemic *trans*-linalool oxide-pyranoid (**217a** and **a′**) and racemic *cis*-linalool-pyranoid (**217b** and **b′**) by *Glomerella cingulata*. (Modified from Miyazawa, M. et al., 1994a. *Proc. 38th TEAC*, pp. 101–102.)

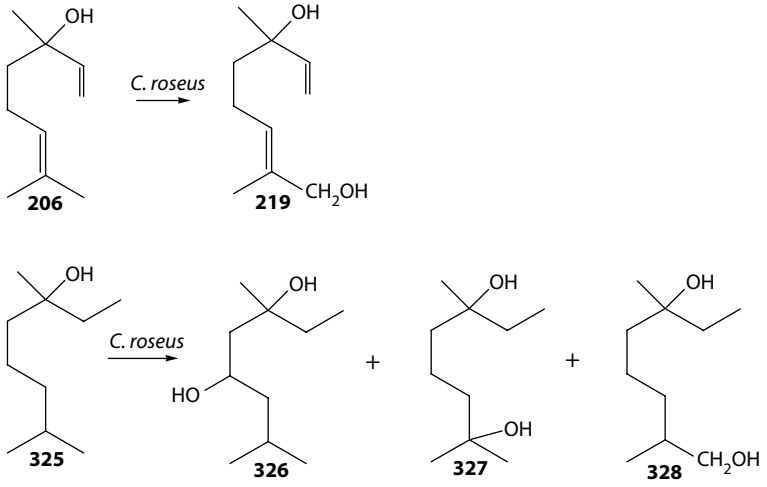


FIGURE 14.24 Biotransformation of linalool (**206**) and tetrahydrolinalool (**325**) by *Catharanthus roseus*. (Modified from Hamada, H. and T. Furuya, 2000. *Proc. 44th TEAC*, pp. 167–168; Hamada, H. et al., 2004. *Proc. 48th TEAC*, pp. 393–395.)

(±)-Linalyl acetate (**206-Ac**) was hydrolyzed to (+)-(*S*)-linalool (**206**) and (±)-linallyl acetate (**206-Ac**) by *Bacillus subtilis*, *Trichoderma S*, *Absidia glauca*, and *Gibberella fujikuroi* as shown in Figure 14.25. But, (±)-dihydrolinallyl acetate (**469-Ac**) was not hydrolyzed by the above microorganisms (Oritani and Yamashita, 1973a).

14.2.2.3 Dihydromycenol

Dihydromyrcenol (**329**) was fed by *Spodptera litura* to give 1,2-epoxydihydro-myrcenol (**330**) as a main products and 3β-hydroxydidyromyrcerol (**331**) as a minor product. Dihydromyrcenyl acetate (**332**) was converted to 1,2-dihydroxydihydromyrcenyl acetate (**333**) (Murata and Miyazawa, 1999) (Figure 14.26).

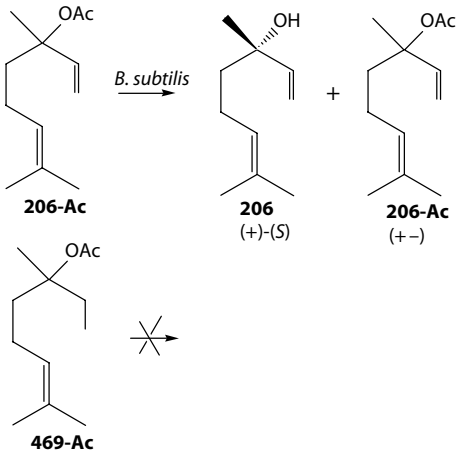


FIGURE 14.25 Hydrolysis of (±)-linalyl acetate (**206-Ac**) by microorganisms. (Modified from Oritani, T. and K. Yamashita, 1973a. *Agric. Biol. Chem*., 37: 1923–1928.)

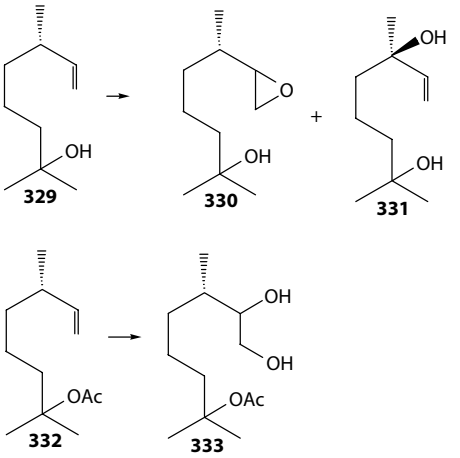
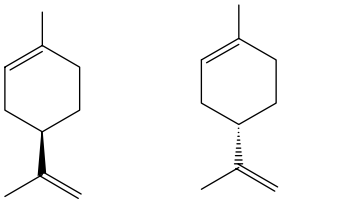


FIGURE 14.26 Biotransformation of dihydromyrcenol (**329**) and dihydromyrcenyl acetate (**332**) by *Spodptera litura*. (Modified from Murata, T. and M. Miyazawa, 1999. *Proc. 43rd TEAC*, pp. 393–394.)

14.3 METABOLIC PATHWAYS OF CYCLIC MONOTERPENOIDS

14.3.1 Monocyclic Monoterpene Hydrocarbon

14.3.1.1 Limonene



68
(*R*)-limonene

68'
(*S*)-limonene

Limonene is the most widely distributed terpene in nature after α-pinene (**4**) (Krasnobajew, 1984). (4*R*)-(+)-Limonene (**68**) is present in *Citrus* peel oils at a concentration of over 90%; a low concentration of the (4*S*)-(–)-limonene (**68′**) is found in oils from the *Mentha* species and conifers

(Bauer et al., 1990). The first microbial biotransformation on limonene was carried out by using a soil Pseudomonad. The microorganism was isolated by the enrichment culture technique on limonene as the sole source of carbon (Dhavalikar and Bhattacharyya, 1966). The microorganism was also capable of growing on α-pinene (**4**), β-pinene (**1**), 1-*p*-menthene (**62**), and *p*-cymene (**178**). The optimal level of limonene for growth was 0.3–0.6% (v/v) although no toxicity was observed at 2% levels. Fermentation of limonene (**68**) by this bacterium in a mineral-salts medium resulted in the formation of a large number of neutral and acidic products such as dihydrocarvone (**64**), carvone (**61**), carveol (**60**), 8-*p*-menthene-1,2-*cis*-diol (**65b**), 8-*p*-menthen-1-ol-2-one (**66**), 8-*p*-menthene-1,2-*trans*-diol (**65a**), and 1-*p*-menthene-6,9-diol (**62**). Perillic acid (**69**), β-isopropenyl pimeric acid (**72**), 2-hydroxy-8-*p*-menthen-7-oic acid (**70**), and 4,9-dihydroxy-1-*p*-menthen-7-oic acid (**73**) were isolated and identified as acidic compounds. Based on these data three distinct pathways for the catabolism of limonene (**68**) by the soil Pseudomonad were proposed by Dhavalikar et al. (1966), involving allylic oxygenation (pathway1), oxygenation of the 1,2-double bond (pathway 2), and progressive oxidation of the 7-methyl group to perillic acid (**82**) (pathway 3) (Figure 14.27) (Krasnobajew, 1984). Pathway

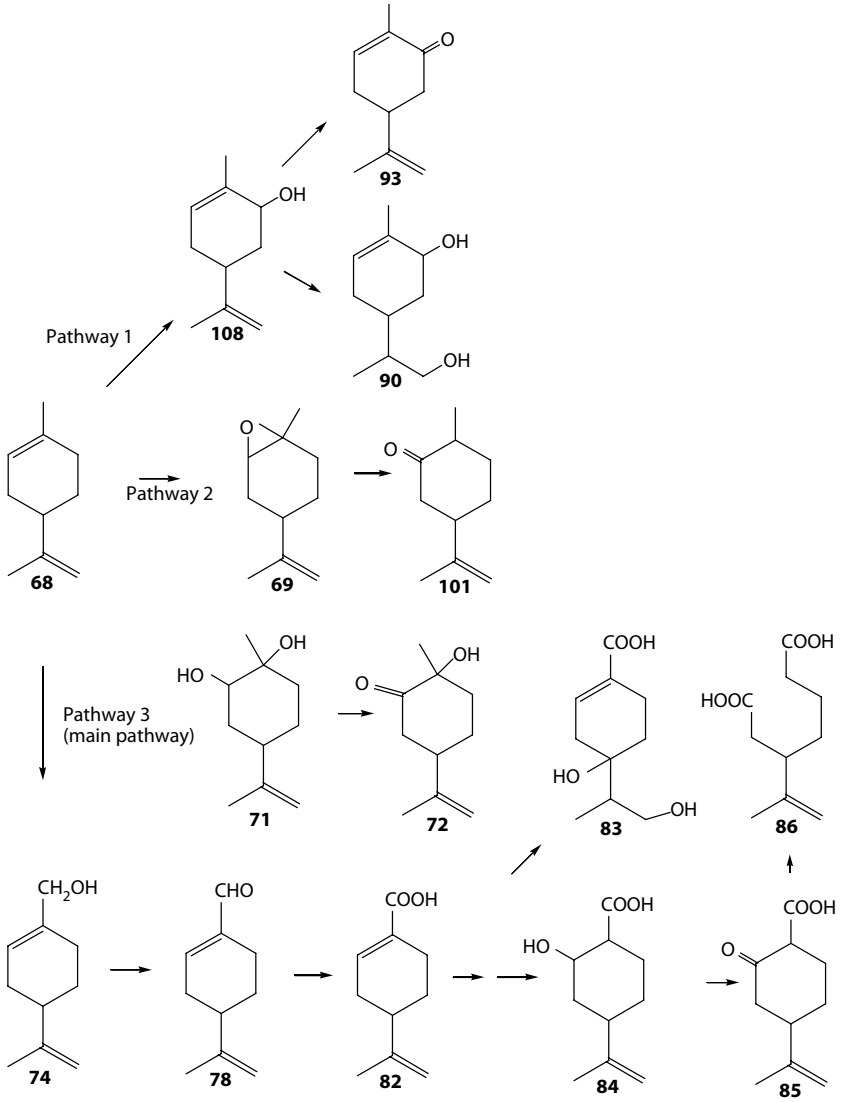


FIGURE 14.27 Pathways for the degradation of limonene (**68**) by a soil Pseudomonad sp. strain (L). (Modified from Krasnobajew, V., 1984. In: *Biotechnology*, K. Kieslich, ed., Vol. 6a, pp. 97–125. Weinheim: Verlag Chemie.)

2 yields (+)-dihydrocarvone (**101**) via intermediate limonene epoxide (**69**) and 8-*p*-menthen-1-ol-2-one (**72**) as oxidation product of limonene-1,2-diol (**71**). The third and main pathway leads to perillyl alcohol (**74**), perillaldehyde (**78**), perillic acid (**82**), constituents of various essential oils and used in the flavour and fragrance industry (Fenaroli, 1975), 2-oxo-8-*p*-menthen-7-oic acid (**85**), β-isopropenyl pimeric acid (**86**), and 4,9-dihydroxy-1-*p*-menthene-7-oic acid (**83**).

(+)-Limonene (**68**) was biotransformed via limonene-1,2-epoxide (**69**) to 8-*p*-menthene 1,2-*trans*-diol (**71b**). On the other hand, (+)-carvone (**93**) was biotransformed via (−)-isodihydrocarvone (**101b**) and 1α-hydroxydihydrocarvone (**72**) to (+)-8-*p*-menthene-1,2-*trans*-diol (**71a**) (Noma et al., 1985a, 1985b) (Figure 14.28). A soil Pseudomonad formed 1-hydroxydihydrocarvone (**72**), 8-*p*-menthene-1,2-*trans*-diol (**71b**) from (+)-limonene (**68**). Dhavalikar and Bhattacharyya (1966) considered that the formation of 1-hydroxy-dihydrocarvone (**66**) is from dihydrocarvone (**64**).

Pseudomonas gladioli was isolated by an enrichment culture technique from pine bark and sap using a mineral salts broth with limonene as the sole carbon source (Cadwallander et al., 1989; Cadwallander and Braddock, 1992). Fermentation was performed during 4–10 days in shake flasks at 25°C using a pH 6.5 mineral salts medium and 1.0% (+)-limonene (**68**). Major products were identified as (+)-α-terpineol (**34**) and (+)-perillic acid (**82**). This was the first report of the microbial conversion of limonene to (+)-α-terpineol (**34**).

The first data on fungal bioconversion of limonene (**68**) date back to the late 1960s (Kraidman et al., 1969; Noma, 2007). Three soil microorganisms were isolated on and grew rapidly in mineral salts media containing appropriate terpene substrates as sole carbon sources. The microorganisms belonged to the class Fungi Imperfecti, and they had been tentatively identified as *Cladosporium*

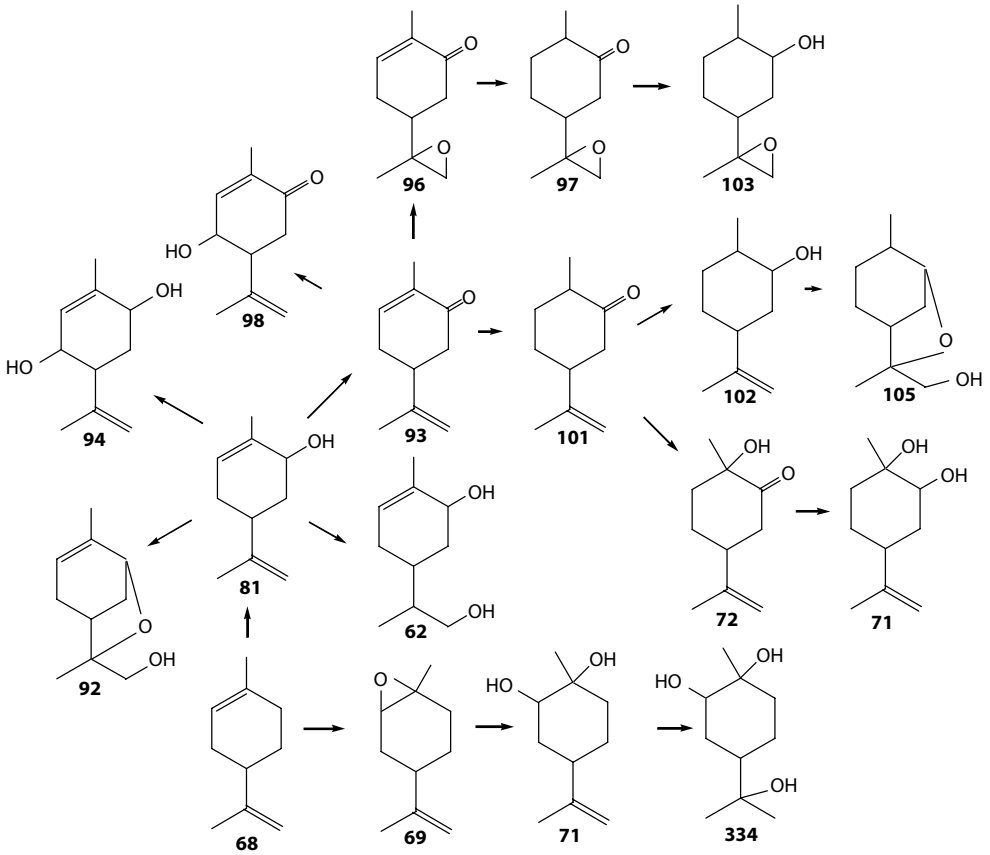


FIGURE 14.28 Formation of (+)-8-*p*-menthene-1,2-*trans*-diol (**71b**) in the biotransformation of (+)-limonene (**68**) and (+)-carvone (**93**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 1985a. *Annual Meeting of Agricultural and Biological Chemistry*, Sapporo, p. 68; Noma, Y. et al., *Proc. 29th TEAC*, pp. 235–237.)

species. One of these strains, designated as *Cladosporium* sp. T_7 was isolated on (+)-limonene (**68a**). The growth medium of this strain contained 1.5 g/L of *trans*-limonene-1,2-diol (**71a**). Minor quantities of the corresponding *cis*-1,2-diol (**71b**) were also isolated. The same group isolated a fourth microorganism from a terpene-soaked soil on mineral salts media containing (+)-limonene as the sole carbon source (Kraidman et al., 1969). The strain, *Cladosporium*, designated T_{12}, was capable of converting (+)-limonene (**68a**) into an optically active isomer of α-terpineol (**34**) in yields of approximately 1.0 g/L.

α-Terpineol (**34**) was obtained from (+)-limonene (**68**) by fungi such as *Penicillium digitatum*, *Pencillium italicum*, and *Cladosporium* and several bacteria (Figure 14.29). (+)-*cis*-Carveol (**81b**), (+)-carvone (**93**) [an important constituent of caraway seed and dill-seed oils (Fenaroli, 1975; Bouwmester et al., 1995), and 1-*p*-menthene-6,9-diol (**90**) were also obtained by *Pencillium digitatum* and *Pencillium italicum*. (+)-(*S*)-Carvone (**93**) is a natural potato sprout inhibiting, fungistatic, and bacteriostatic compound (Oosterhaven et al., 1995a, 1995b). It is important to note that (−)-carvone (**93′**, the "spearmint flavour"] was not yet described in microbial transformation (Krasnobajew, 1984). However, the biotransformation of limonene to (−)-carvone (**93′**) was patented by a Japanese group (Takagi et al., 1972). *Corynebacterium* species grown on limonene was able to produce about 10 mg/L of 99% pure (−)-carvone (**93′**) in 24–48 h.

Mattison et al. (1971) isolated *Penicillium* sp. cultures from rotting orange rind that utilized limonene (**68**) and converted it rapidly to α-terpineol (**34**). Bowen (1975) isolated two common *Citrus* moulds, *Penicillium italicum* and *Penicillium digitatum*, responsible for the postharvest diseases of *Citrus* fruits. Fermentation of *Penicillium italicum* on limonene (**68**) yielded *cis*- (**81b**) and *trans*-carveol (**81a**) (26%) as the main products, together with *cis*- and *trans*-*p*-mentha-2,8-dien-1-ol (**73**) (18%), (+)-carvone (**93′**) (6%), *p*-mentha-1,8-dien-4-ol (**80**) (4%), perillyl alcohol (**74**) (3%), and 8-*p*-menthene-1,2-diol (**71**) (3%). Conversion of **68** by *Penicillium digitatum* yielded the same products in lower yields (Figure 14.29).

The biotransformation of limonene (**68**) by *Aspergillus niger* is a very important example of fungal bioconversion. Screening for fungi capable of metabolizing the bicyclic hydrocarbon terpene α-pinene (**4**) yielded a strain of *Aspergillus niger* NCIM 612 that was also able to transform limonene (**68**) (Rama Devi and Bhattacharyya, 1978). This fungus was able to carry out three types of oxygenative rearrangements α-terpineol (**34**), carveol (**81**), and *p*-mentha-2,8-dien-1-ol (**73**) (Rama Devi and Bhattacharyya, 1978) (Figure 14.30). In 1985, Abraham et al. (1985) investigated the biotransformation of (*R*)-(+)-limonene (**68a**) by the fungus *Penicillium digitatum*. A complete transformation for the substrate to α-terpineol (**34**) by *Penicillium digitatum* DSM 62840 was obtained with 46% yield of pure product.

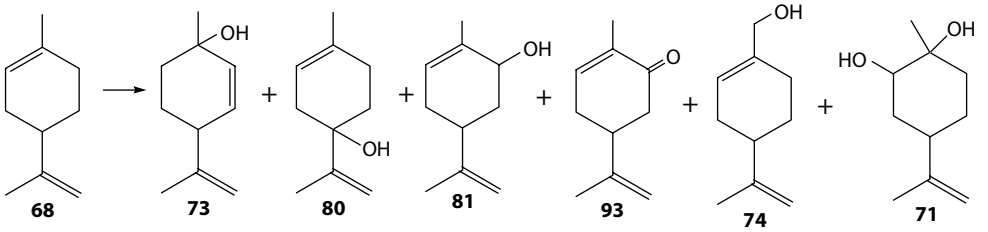


FIGURE 14.29 Biotransformation products of limonene (**68**) by *Penicillium digitatum* and *Penicillium italicum*. (Modified from Bowen, E.R., 1975. *Proc. Fla. State Hortic. Soc.*, 88: 304–308.)

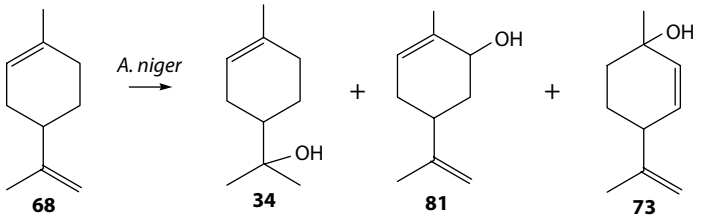


FIGURE 14.30 Biotransformation of limonene (**68**) by *Aspergillus niger* NCIM 612. (Modified from Rama Devi, J. and P.K. Bhattacharyya, 1978. *J. Indian Chem. Soc.*, 55: 1131–1137.)

The production of glycols from limonene (**68**) and other terpenes with a 1-menthene skeleton was reported by *Corynespora cassiicola* DSM 62475 and *Diplodia gossypina* ATCC 10936 (Abraham et al., 1984). Accumulation of glycols during fermentation was observed. An extensive overview on the microbial transformations of terpenoids with a 1-*p*-menthene skeleton was published by Abraham et al. (1986).

The biotransformation of (+)-limonene (**68**) was carried out by using *Aspergillus cellulosae* M-77 (Noma et al., 1992b) (Figure 14.32). It is important to note that (+)-limonene (**68a**) was mainly

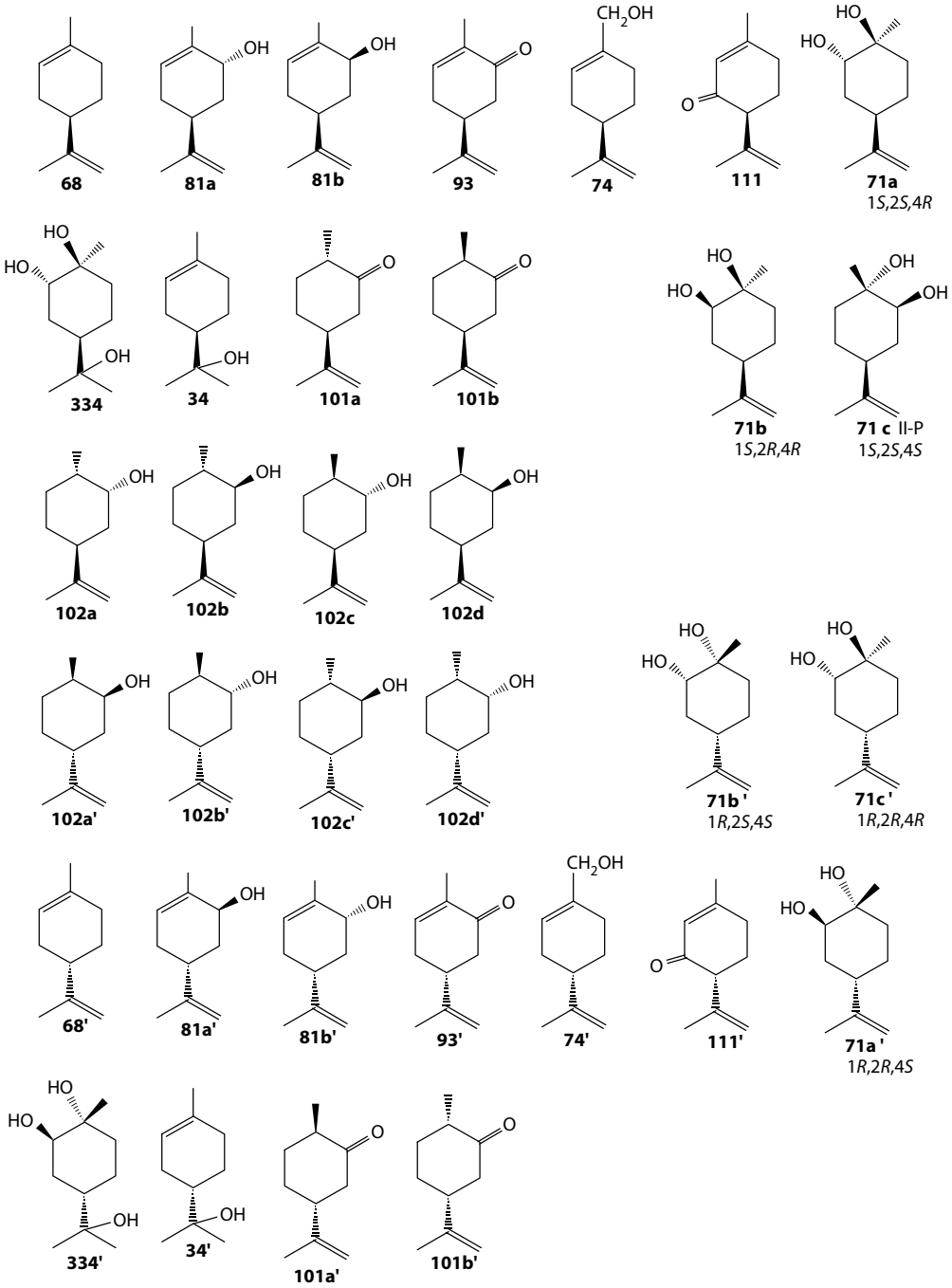


FIGURE 14.31 (+)- and (−)-limonenes (**68** and **68′**) and related compounds.

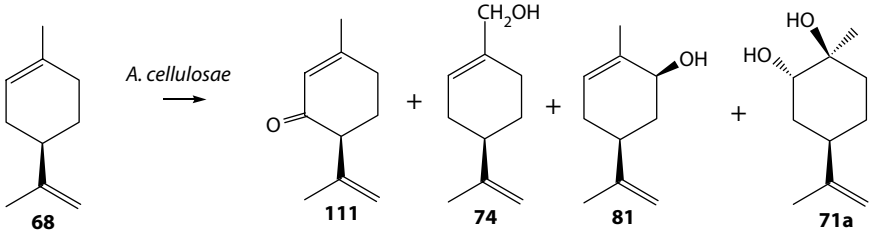


FIGURE 14.32 Biotransformation of (+)-limonene (**68**) by *Aspergillus cellulosae* IFO4040. (Modified from Noma, Y. et al., 1992b. *Phytochemistry*, 31: 2725–2727.)

converted to (+)-isopiperitenone (**111**) (19%) as new metabolite, (1*S*,2*S*,4*R*)-(+)-limonene-1,2-*trans*-diol (**71a**) (21%), (+)-*cis*-carveol (**81b**) (5%), and (+)-perillyl alcohol (**74**) (12%) (Figure 14.32).

(+)-Limonene (**68**) was biotransformed by a kind of *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN. to isopiperitenone (**111**, 7% GC ratio), 2α-hydroxy-1,8-cineole (**125b**, 7%), (+)-limonene-1,2-*trans*-diol (**71a**, 6%), and (+)-*p*-menthane-1β,2α,8-triol (**334**, 45%) as main products and (+)-*trans*-sobrerol (**95a**, 2%), (+)-*trans*-carveol (**81a**), (+)-carvone (**93**), (−)-isodihydrocarvone (**101b**), and (+)-*trans*-isopiperitenol (**110a**) as minor products (Noma and Asakawa, 2006a, 2007a) (Figure 14.33). The metabolic pathways of (+)-limonene by *Penicillium digitatum* is shown in Figure 14.34.

On the other hand, (−)-limonene (**68′**) was also biotransformed by a kind of *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN. to give isopiperitenone (**111′**), 2α-hydroxy-1,8-cineole (**125b′**), (−)-limonene-1,2-*trans*-diol (**71′**), and *p*-menthane-1,2,8-triol (**334′**) as main products together with (+)-*trans*-sobrerol (**80′**), (+)-*trans*-carveol (**81a′**), (−)-carvone (**93′**), (−)-dihydrocarvone (**101a′**), and (+)-isopiperitenol (**110a′**) as minor products (Noma and Asakawa, 2007b) (Figure 14.35.)

Newly isolated unidentified red yeast, *Rhodotorula* sp., converted (+)-limonene (**68**) mainly to (+)-limonene-1,2-*trans*-diol (**71a**), (+)-*trans*-carveol (**81a**), (+)-*cis*-carveol (**81b**), and (+)-carvone (**93′**) together with (+)-limonene-1,2-*cis*-diol (**71b**) as minor product (Noma and Asakawa, 2007b) (Figure 14.36).

Cladosporium sp. T_7 was cultivated with (+)-limonene (**68**) as the sole carbon source; it converted **68** to *trans*-*p*-menthane-1,2-diol (**71a**) (Figure 14.36) (Mukherjee et al., 1973).

On the other hand, the same red yeast converted (−)-limonene (**68′**) mainly to (−)-limonene-1,2-*trans*-diol (**71a′**), (−)-*trans*-carveol (**81a′**), (−)-*cis*-carveol (**81b′**), and (−)-carvone (**93′**) together with (−)-limonene-1,2-*cis*-diol (**71b′**) as minor product (Noma and Asakawa, 2007b) (Figure 14.37).

The biotransformation of (+)- and (−)-limonene (**68** and **68′**), (+)- and (−)-α-terpineol (**34** and **34′**), (+)- and (−)-limonene-1,2-epoxide (**69** and **69′**), and caraway oil was carried out by *Citrus*

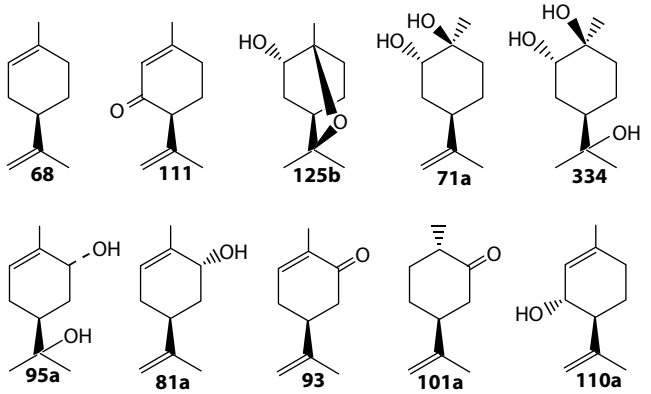


FIGURE 14.33 Metabolites of (+)-limonene (**68**) by a kind of *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN. (Modified from Noma, Y. and Y. Asakawa, 2006a. *Proc. 50th TEAC*, pp. 431–433; Noma, Y. and Y. Asakawa, 2007a. *Book of Abstracts of the 38th ISEO*, p. 7.)

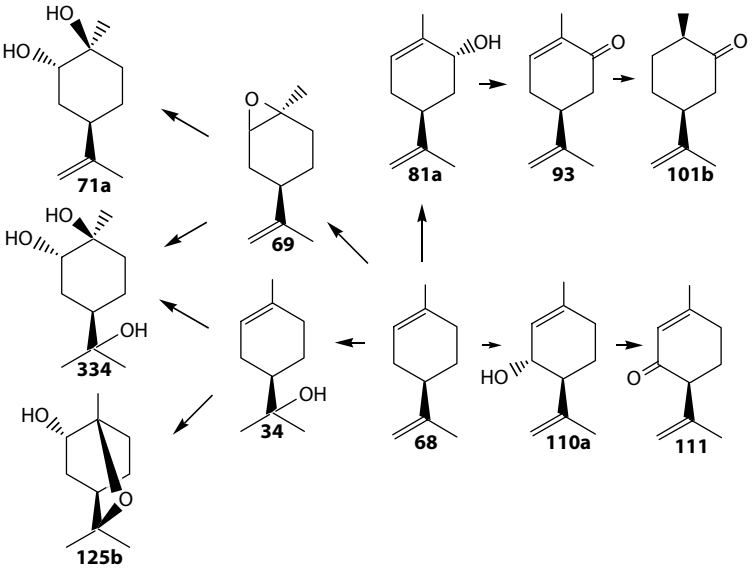


FIGURE 14.34 Biotransformation of (+)-limonene (**68**) by *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN. (Modified from Noma, Y. and Y. Asakawa, 2006a. *Proc. 50th TEAC*, pp. 431–433; Noma, Y. and Y. Asakawa, 2007a. *Book of Abstracts of the 38th ISEO*, p. 7.)

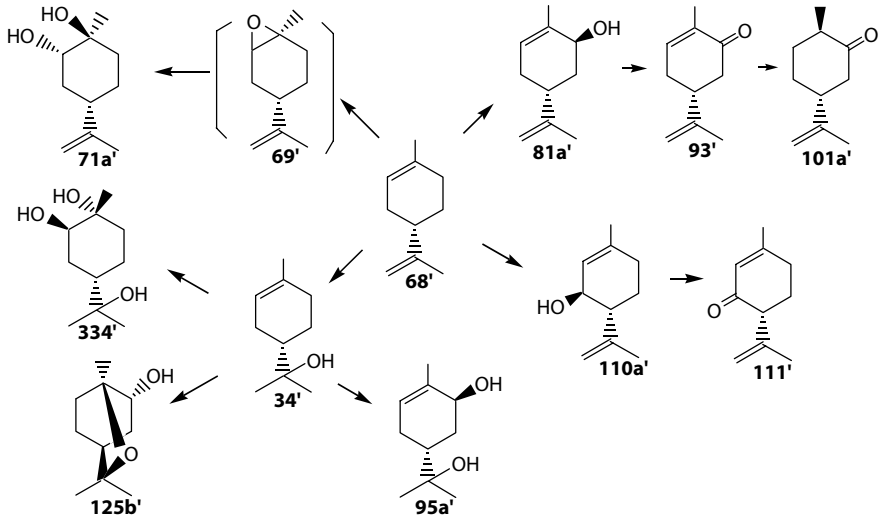


FIGURE 14.35 Biotransformation of (–)-limonene (**68′**) by *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN. (Modified from Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

pathogenic fungi *Penicillium* (Pers.; Fr.) Sacc. KCPYN and newly isolated red yeast, a kind of *Rhodotorula* sp. *Penicillium digitatum* KCPYN converted limonenes (**68** and **68′**) to the corresponding isopiperitone (**111** and **111′**), 1α-hydroxy-1,8-cineole (**125b** and **125b′**), limonene-1,2-*trans*-diol (**71a** and **71a′**), *p*-menthane-1,2,8-triol (**334** and **334′**), and *trans*-sobrerol as main products. (+)- and (–)-α-Terpineol (**34** and **34′**) were the precursors of 2α-hydroxy-1,8-cineole (**125b** and **b′**) and *p*-menthane-1,2,8-triol (**334**). (+)- and (–)-Limonene-1,2-epoxide (**69** and **69′**) were also the precursor of limonene-1,2-*trans*-diol (**71a**). *Rhodotorula* sp. also biotransformed (+)- and (–)-limonene (**68** and **68′**) to the corresponding *trans*- and *cis*-carveols (**81a** and **b**) as main products. This microbe also converted caraway oil, equal mixture of (+)-limonene (**68**) and (+)-carvone (**93**). (+)-Limonene (**68)** disappeared and (+)-carvone (**93)** was produced and accumulated in the cultured broth (Noma and Asakawa, 2007b).

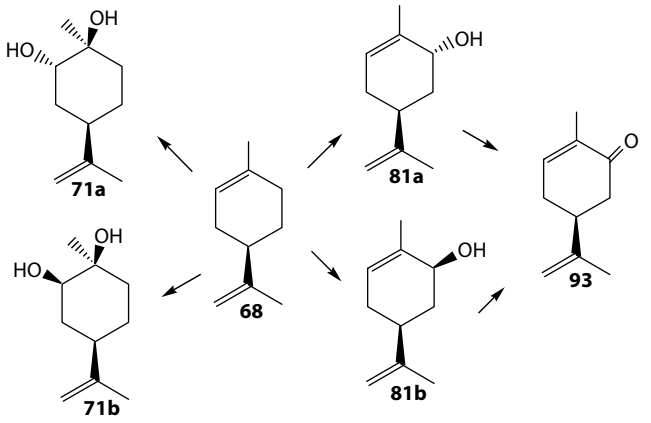


FIGURE 14.36 Biotransformation of (+)-limonene (**68**) by red yeast, *Rhodotorula* sp. and *Cladosporium* sp. T_7. (Modified from Mukherjee, B.B. et al., 1973. *Appl. Microbiol.*, 25: 447–453; Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

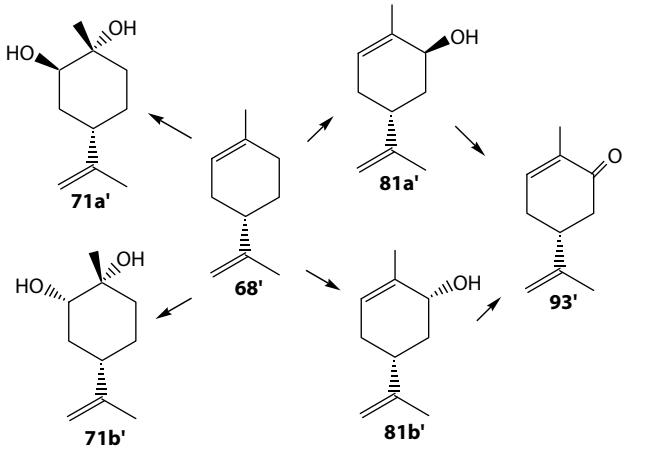


FIGURE 14.37 Biotransformation of (–)-limonene (**68′**) by a kind of *Rhodotorula* sp. (Modified from Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

(4*S*)-(–)- (**68′**) and (4*R*)-(+)-Limonene (**68**) and their epoxides (**69** and **69′**) were incubated by *Cyanobacterium*. It was found that the transformation was enantio- and regioselective. *Cyanobacterium* biotransformed only (4*S*)-limonene (**68′**) to (–)-*cis*- (**81b′**, 11.1%) and (–)-*trans*-carveol (**81a′**, 5%) in low yield. On the other hand, (4*R*)-limonene oxide (**69**) was converted to limonene-1,2-*trans*-diol (**71a′**) and 1-hydroxy-(+)-dihydrocarvone (**72a′**). However, (4*R*)-(+)-limonene (**68**) and (4*S*)-limonene oxide (**69′**) were not converted at all (Figure 14.38) (Hamada et al., 2003).

(+)-Limonene (**68**) was fed by *Spodptera litura* to give (+)-limonene-7-oic acid (**82**), (+)-limonene-9-oic acid (**70**), and (+)-limonene-8,9-diol (**79**); (–)-limonene (**68′**) was converted to (–)-linonene-7-oic acid (**82′**), (–)-limonene-9-oic acid (**70′**), and (–)-limonene-8,9-diol (**79′**) (Figure 14.39) (Miyazawa et al., 1995a).

Kieslich et al. (1985) found a nearly complete microbial resolution of a racemate in the biotransformation of (±)-limonene by *Penicillium digitatum* (DSM 62840). The (*R*)-(+)-limonene (**68**) is converted to the optically active (+)-α-terpineol, $[\alpha]_D = +99°$, while the (*S*)-(–)-limonene (**68′**) is presumably adsorbed onto the mycelium or degraded via unknown pathways (Kieslich et al., 1985) (Figure 14.40).

(4*S*)- and (4*R*)-Limonene epoxides (**69a′** and **a**) were biotransformed by *Cyanobacterium* to give 8-*p*-menthene-1α,2β-ol (**71a**, 68.4%) and 1α-hydroxy-8-*p*-menthen-2-one (**72**, 31.6%) (Hamada et al., 2003) (Figure 14.41).

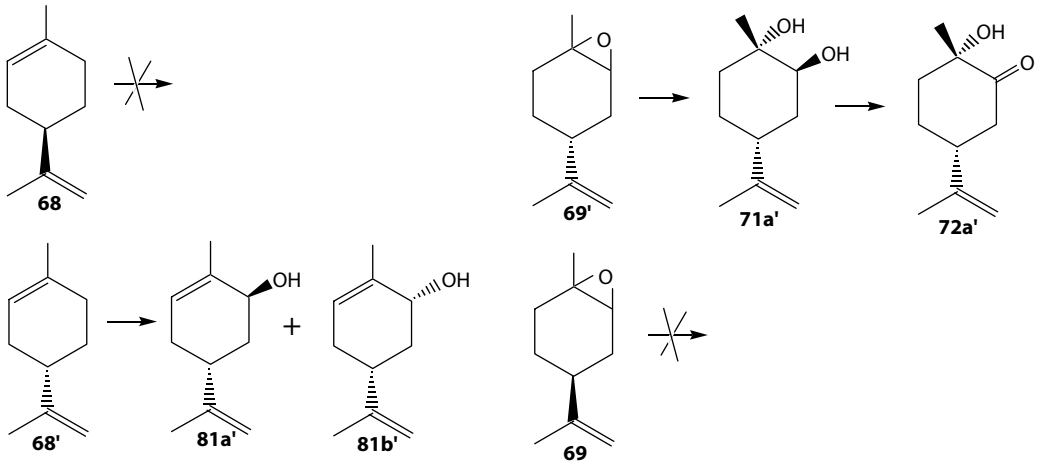


FIGURE 14.38 Biotransformation of (+)- and (−)-limonene (**68** and **68′**) and limonene epoxide (**69** and **69′**) by *Cyanobacterium*. (Modified from Hamada, H. et al., 2003. *Proc. 47th TEAC*, pp. 162–163.)

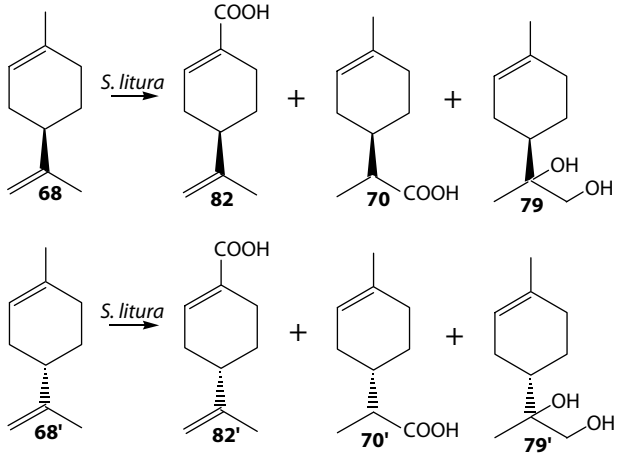


FIGURE 14.39 Biotransformation of (+)-limonene (**68**) and (−)-limonene (**68′**) by *Spodptera litura*. (Modified from Miyazawa, M. et al., 1995a. *Proc. 39th TEAC*, pp. 362–363.)

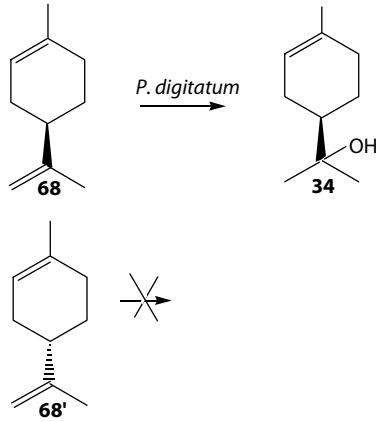


FIGURE 14.40 Microbial resolution of racemic limonene (**68** and **68′**) and the formation of optically active α-terpineol by *Penicillium digitatum*. (Modified from Kieslich, K. et al., 1985. In: *Topics in flavor research*, R.G. Berger, S. Nitz, and P. Schreier, eds, pp. 405–427. Marzling Hangenham: Eichborn.)

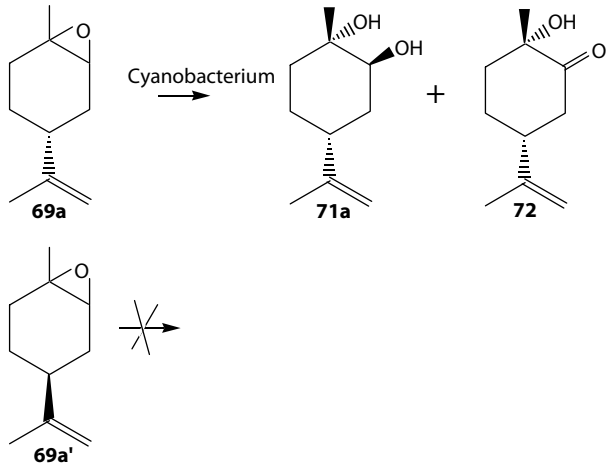
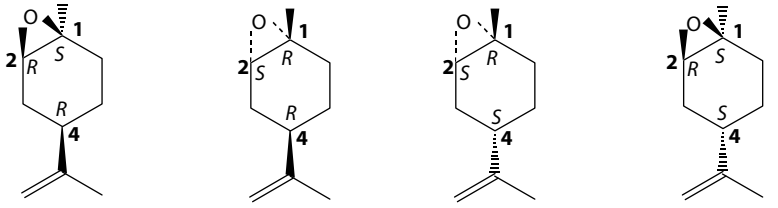


FIGURE 14.41 Enantioselective biotransformation of (4*S*)- (**69a′**) and (4*R*)-limonene epoxides (**69a**) by *Cyanobacterium*. (Modified from Hamada, H. et al., 2003. *Proc. 47th TEAC*, pp. 162–163.)



The mixture of (+)-*trans*- (**69a**) and *cis*- (**69b**), and the mixture of (–)-*trans*- (**69a′**) and *cis*-limonene-1,2-epoxide (**69b′**) were biotransformed by *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN to give (1*R*,2*R*,4*R*)- (–)-*trans*-(**71a**) and (1*S*,2*S*,4*S*)-(+)-8-*p*-menthene-1,2-*trans*-diol (**71a′**) and (–)-*p*-menthane-1,2,8-triols (**334a** and **334a′**) (Noma and Asakawa, 2007b) (Figure 14.42).

Biotransformation of 1,8-cineole (**122**) by *Aspergillus niger* gave racemic 2α-hydroxy-1,8-cineole (**125b** and **b′**) (Nishimura et al., 1982). When racemic 2α-hydroxy-1,8-cineole (**125b** and **b′**) was biotransformed by *Glomerella cingulata*, only (–)-2α-hydroxy-1,8-cineole (**125b′**) was selectively esterified with malonic acid to give its malonate (**125b′**-Mal). The malonate was hydrolyzed to give optical pure **125b′** (Miyazawa et al., 1995b). On the other hand, Citrus pathogenic fungi, *Penicillium digitatum*, biotransformed limonene (**68**) to give optical pure **125b** (Noma and Asakawa, 2007b) (Figure 14.43).

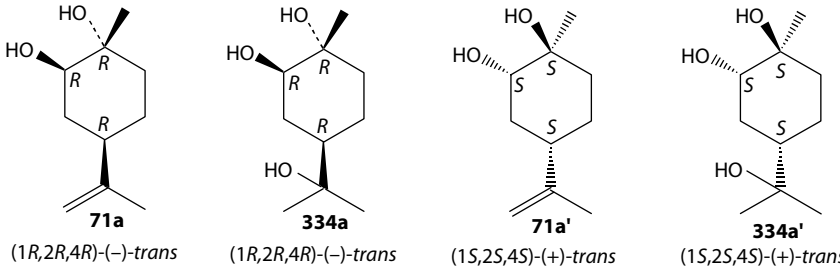


FIGURE 14.42 Biotransformation of (+)-*trans*- (**69a**) and *cis*- (**69b**), and (–)-*trans*- (**69a′**) and *cis*-limonene-1,2-epoxide (**69b′**) by *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN and their metabolites. (Modified from Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

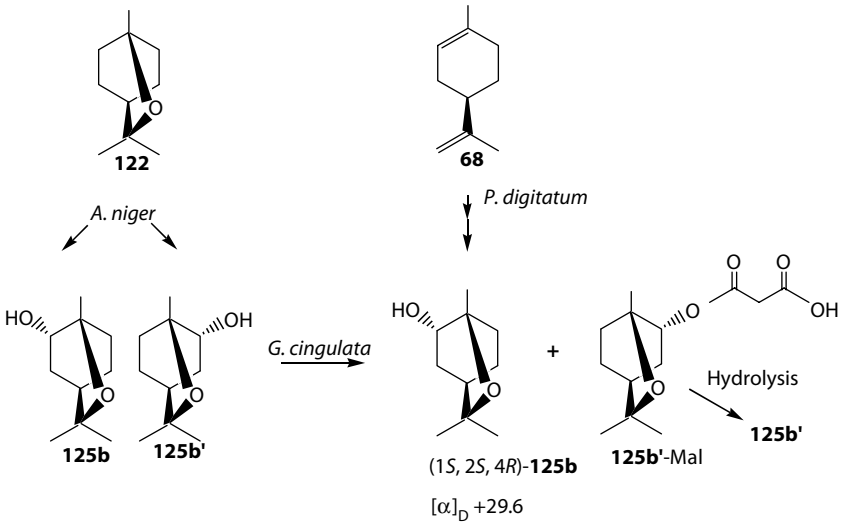


FIGURE 14.43 Formation of optical pure (+)- and (−)-2α-hydroxy-1,8-cineole (**125b** and **b′**) from the biotransformation of 1,8-cineole (**122**) and (+)-limonene (**68**) by *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN and *Aspergillus niger* TBUYN-2. (Modified from Nishimura, H. et al., 1982. *Agric. Biol. Chem.*, 46: 2601–2604; Miyazawa, M. et al., 1995b. *Proc. 39th TEAC*, pp. 352–353; Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

When monoterpenes, such as limonene (**68**), α-pinene (**4**), and 3-carene (**336**), were administered to the cultured cells of *Nicotiana tabacum*, they were converted to the corresponding epoxides enantio- and stereoselectively. The enzyme (p38) concerning with the epoxidation reaction was purified from the cultured cells by cation exchanged chromatography. The enzyme had not only epoxidation activity but also peroxidase activity. Amino acid sequence of p38 showed 89% homology in their 9 amino acid overlap with horseradish peroxidase (Yawata et al., 1998) (Figure 14.44). It was found that limonene and carene were converted to the corresponding epoxides in the presence of hydrogen

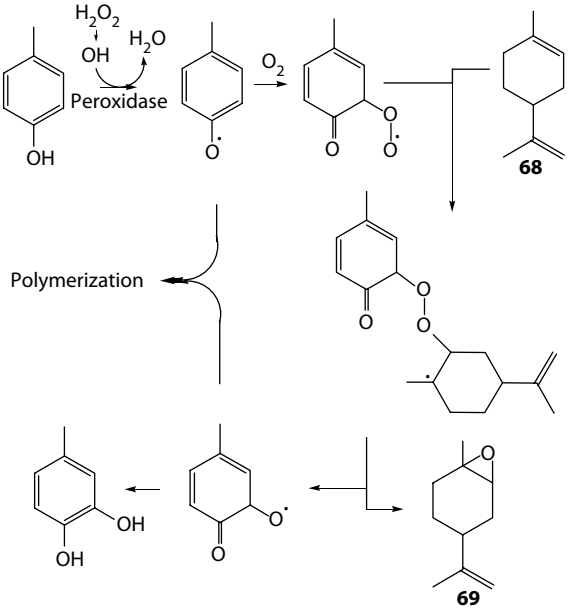


FIGURE 14.44 Proposed mechanism for the epoxidation of (+)-limonene (**68**) with p38 from the cultured cells of *Nicotiana tabacum*. (Modified from Yawata, T. et al., 1998. *Proc. 42nd TEAC*, pp. 142–144.)

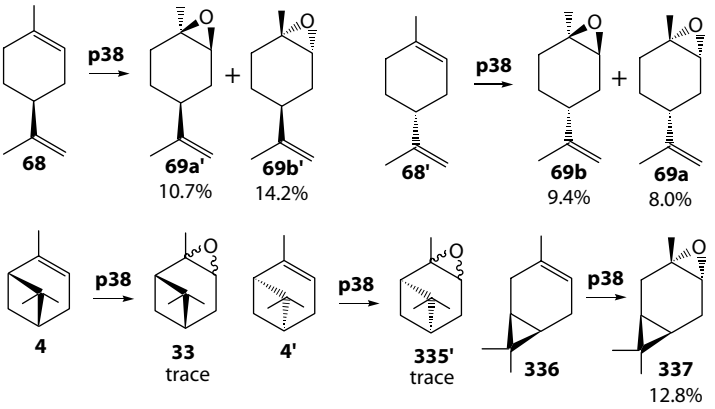


FIGURE 14.45 Epoxidation of limonene (**68**), α-pinene (**4**) and 3-carene (**336**) with p38 from the cultured cells of *Nicotiana tabacum*. (Modified from Yawata, T. et al., 1998. *Proc. 42nd TEAC*, pp. 142–144.)

peroxide and *p*-cresol by a radical mechanism with the peroxidase. (*R*)-limonene (**68**), (*S*)-limonene (**68′**), (1*S*,5*R*)-α-pinene (**4**), (1*R*,5*R*)-α-pinene (**4**), and (1*R*,6*R*)-3-carene (**336**) were oxidized by cultured cells of *Nicotiana tabacum* to give corresponding epoxides enantio- and stereoselectively (Yawata et al., 1998) (Figure 14.45).

14.3.1.2 Isolimonene

Spodptera litura converted (1*R*)-*trans*-isolimonene (**338**) to (1*R*,4*R*)-*p*-menth-2-ene-8,9-diol (**339**) (Miyazawa et al., 1996b) (Figure 14.46).

14.3.1.3 *p*-Menthane

Hydroxylation of *trans*- and *cis*-*p*-menthane (**252a** and **b**) by microorganisms is also very interesting from the viewpoint of the formation of the important perfumes such as (−)-menthol (**137b′**), (−)-carvomenthol (**49b′**), etc., plant growth regulators, and mosquito repellents such as *p*-menthane-*trans*-3,8-diol (**142a**), *p*-menthane-*cis*-3,8-diol (**142b**) (Nishimura and Noma, 1996), and *p*-menthane-2,8-diol (**93**) (Noma, 2007). *Pseudomonas mendocina* strain SF biotransformed **252b** stereoselectively to *p*-*cis*-menthan-1-ol (**253**) (Tsukamoto et al., 1975) (Figure 14.47).

On the other hand, the biotransformation of the mixture of *p*-*trans*- (**252a**) and *cis*-menthane (**252b**) (45:55, peak area in GC) by *Aspergillus niger* gave *p*-*cis*-menthane-1,9-diol (**254**) via *p*-*cis*-menthan-1-ol (**253**). No metabolite was obtained from **252a** at all (Noma et al., 1990) (Figure 14.47).

14.3.1.4 1-*p*-Menthene

Concentrated cell suspension of *Pseudomonas* sp. strain (PL) was inoculated to the medium containing 1-*p*-menthene (**62**) as the sole carbon source. It was degraded to give β-isopropyl pimelic acid (**248**) and methylisopropyl ketone (**251**) (Hungund et al., 1970) (Figure 14.48).

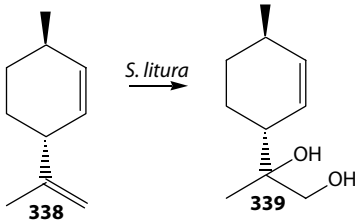


FIGURE 14.46 Biotransformation of (1*R*)-*trans*-isolimonene (**338**) by *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1996b. *Proc. 40th TEAC*, pp. 80–81.)

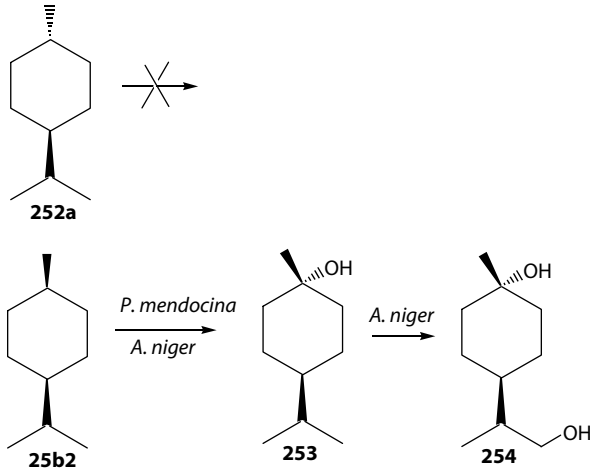


FIGURE 14.47 Biotransformation of the mixture of *trans*- (**252a**) and *cis*-*p*-menthane (**252b**) by *Pseudomonas mendocina* SF and *Aspergillus niger* TBUYN-2. (Modified from Tsukamoto, Y. et al., 1974. *Proc. 18th TEAC*, pp. 24–26; Tsukamoto, Y. et al., 1975. *Agric. Biol. Chem*., 39: 617–620; Noma, Y., 2007. *Aromatic Plants from Asia their Chemistry and Application in Food and Therapy*, L. Jiarovetz, N.X. Dung, and V.K. Varshney, pp. 169–186. Dehradun: Har Krishan Bhalla & Sons.)

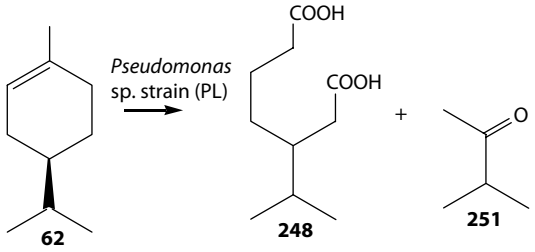


FIGURE 14.48 Biodegradation of (4*R*)-1-*p*-menthene (**62**) by *Pseudomonas* sp. *strain* (PL). (Modified from Hungund, B.L. et al., 1970. *Indian J. Biochem*., 7: 80–81.)

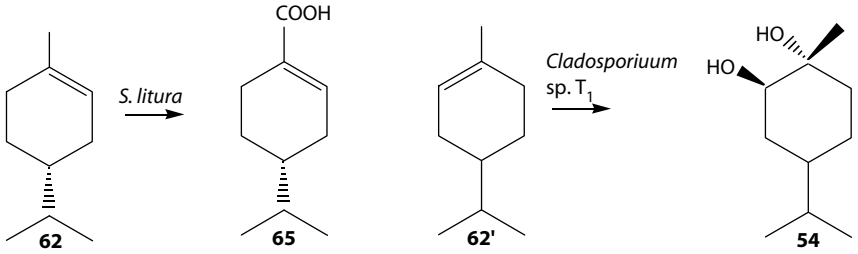


FIGURE 14.49 Biotransformation of (4*R*)-*p*-menth-1-ene (**62**) by *Spodoptera litura* and *Cladosporium* sp. T_1. (Modified from Miyazawa, M. et al., 1996b. *Proc. 40th TEAC*, pp. 80–81; Mukherjee, B.B. et al., 1973. *Appl. Microbiol*., 25: 447–453.)

As shown in Figure 14.49, *Spodoptera litura* converted (4*R*)-*p*-menth-1-ene (**62**) at C-7 position to (4*R*)-phellandric acid (**65**) (Miyazawa et al., 1996b). On the other hand, when *Cladosporium* sp. T_1 was cultivated with (+)-limonene (**68**) as the sole carbon source, it converted **62′** to *trans*-*p*-menthane-1,2-diol (**54**) (Mukherjee et al., 1973).

14.3.1.5 3-*p*-Menthene

When *Cladosporium* sp. T_8 was cultivated with 3-*p*-menthene (**147**) as the sole carbon source, it was converted to *trans*-*p*-menthane-3,4-diol (**141**) as shown in Figure 14.50 (Mukherjee et al., 1973).

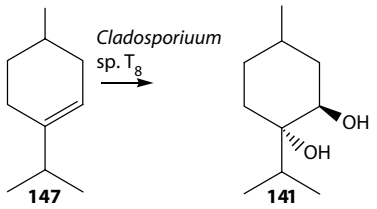


FIGURE 14.50 Biotransformation of *p*-Menth-3-ene (**147**) by *Cladosporium* sp. T_8. (Modified from Mukherjee, B.B. et al., 1973. *Appl. Microbiol.*, 25: 447–453.)

14.3.1.6 α-Terpinene

α-Terpinene (**340**) was converted by *Spodoptera litura* to give α-terpinene-7-oic acid (**341**) and *p*-cymene-7-oic acid (**194**, cuminic acid) (Miyazawa et al., 1995a) (Figure 14.51).

A soil Pseudomonad has been found to grow with *p*-mentha-1,3-dien-7-al (**463**) as the sole carbon source and to produce α-terpinene-7-oic acid (**341**) in a mineral salt medium (Kayahara et al., 1973) (Figure 14.51).

14.3.1.7 γ-Terpinene

γ-Terpinene (**344**) was converted by *Spodoptera litura* to give γ-terpinene-7-oic acid (**345**) and *p*-cymene-7-oic acid (**194**, cuminic acid) (Miyazawa et al., 1995a) (Figure 14.52).

14.3.1.8 Terpinolene

Terpinolene (**346**) was converted by *Aspergillus niger* to give (1*R*)-8-hydroxy-3-*p*-menthen-2-one (**347**), (1*R*)-1,8-dihydroxy-3-*p*-menthen-2-one (**348**), and 5β-hydroxyfenchol (**350b′**). In case of *Corynespora cassiicola* it was converted to terpinolene-1,2-*trans*-diol (**351**) and terpinolene-4,8-diol (**352**). Furthermore, in case of rabbit terpinolene-9-ol (**353**) and terpinolene-10-ol (**354**) were formed from **346** (Asakawa et al., 1983). *Spodoptera litura* also converted **346** to give 1-*p*-menthene-4,8-diol (**352**), cuminic acid (**194**, 29% main product), and terpinolene-7-oic acid (**357**) (Figure 14.53).

14.3.1.9 α-Phellandrene

α-Phellandrene (**355**) was converted by *Spodoptera litura* to give α-phellandrene-7-oic acid (**356**) and *p*-cymene-7-oic acid (**194**, cuminic acid) (Miyazawa et al., 1995a) (Figure 14.54).

14.3.1.10 *p*-Cymene

Pseudomonas sp. strain (PL) was cultivated with *p*-cymene (**178**) as the sole carbon source to give cumyl alcohol (**192**), cumic acid (**194**), 3-hydroxycumic acid (**196**), 2,3-dihydroxycumic acid (**197**),

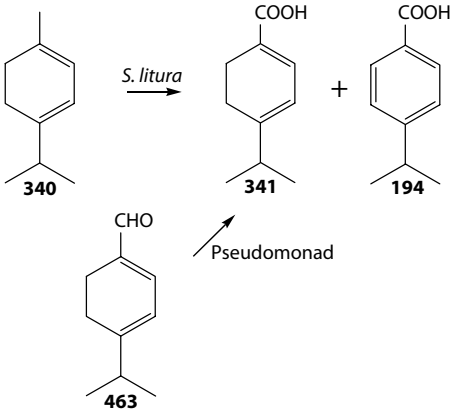


FIGURE 14.51 Biotransformation of α-terpinene (**340**) by *Spodoptera litura* and *p*-mentha-1,3-dien-7-al (**463**) by a soil Pseudomonad. (Modified from Kayahara, H. et al., 1973. *J. Ferment. Technol.*, 51: 254–259; Miyazawa, M. et al., 1995a. *Proc. 39th TEAC*, pp. 362–363.)

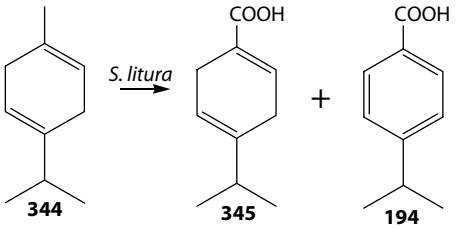


FIGURE 14.52 Biotransformation of γ−terpinene (**344**) by *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1995a. *Proc. 39th TEAC*, pp. 362–363.)

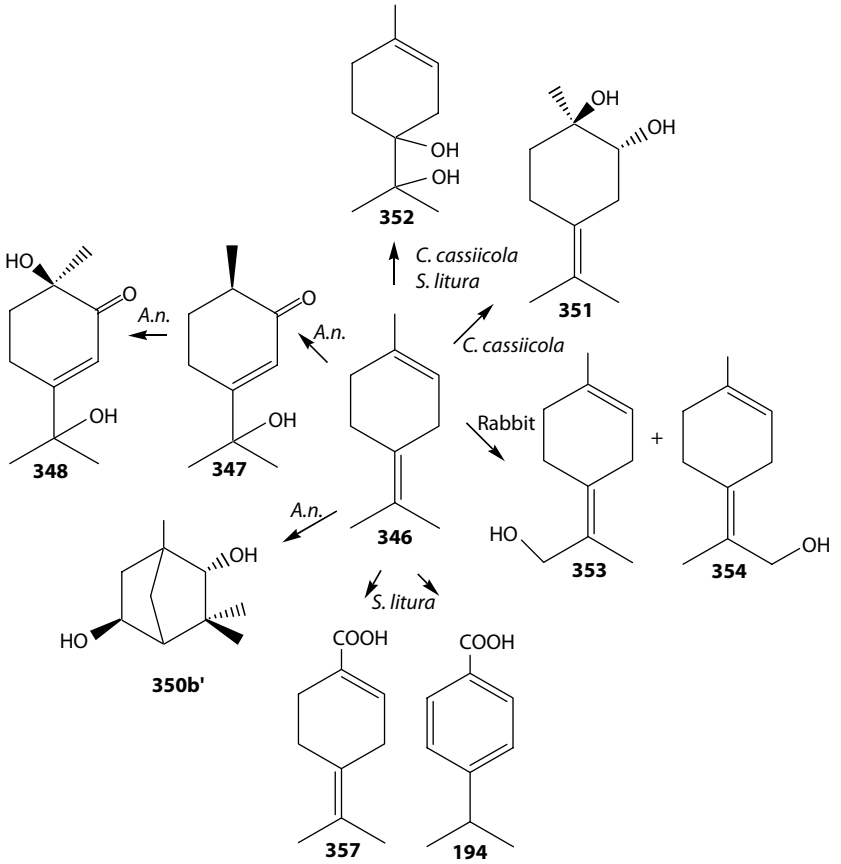


FIGURE 14.53 Biotransformation of terpinolene (**346**) by *Aspergillus niger* (Asakawa et al., 1991), *Corynespora cassiicola* (Abraham et al., 1985), rabbit (Asakawa et al., 1983), and *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1995a. *Proc. 39th TEAC*, pp. 362–363.)

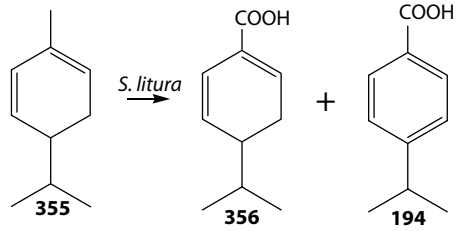


FIGURE 14.54 Biotransformation of α-phellandrene (**355**) by *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1995a. *Proc. 39th TEAC*, pp. 362–363.)

2-oxo-4-methylpentanoic acid (**201**), 9-hydroxy-*p*-cymene (**189**), and *p*-cymene-9-oic acid (**190**) as shown in Figure 14.55 (Madyastha and Bhattacharyya, 1968). On the other hand, *p*-cymene (**178**) was converted regiospecifically to cumic acid (**194**) by *Pseudomonas* sp., *Pseudomonas desmolytica*, and *Nocardia salmonicolor* (Madyastha and Bhattacharyya, 1968) (Figure 14.56).

p-Cymene (**178**) is converted to thymoquinone (**358**) and analogues, **179** and **180**, by various kinds of microorganisms (Demirci et al., 2007) (Figure 14.57).

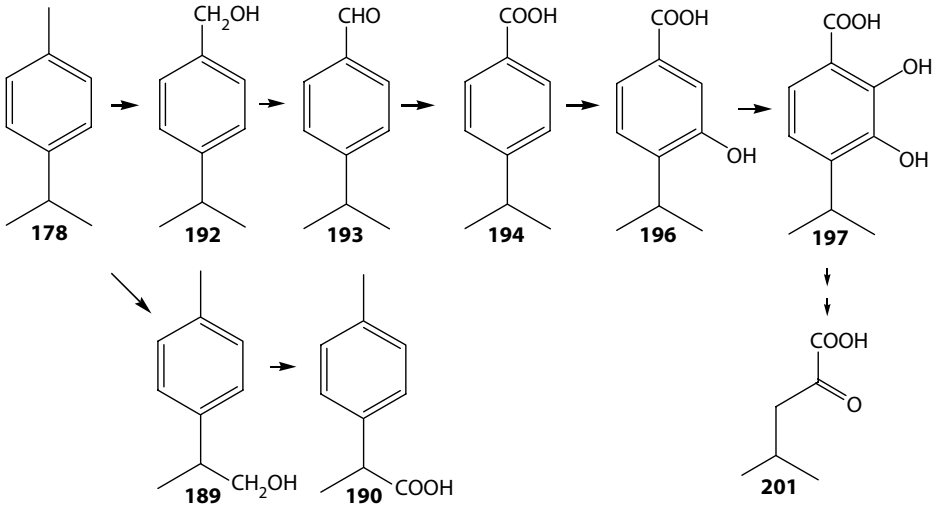


FIGURE 14.55 Biotransformation of *p*-cymene (**178**) by *Pseudomonas* sp. strain (PL). (Modified from Madyastha, K.M. and P.K. Bhattacharyya, 1968. *Indian J. Biochem.*, 5: 161–167.)

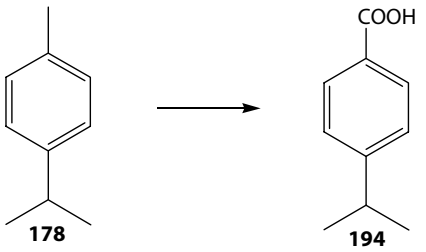


FIGURE 14.56 Biotransformation of *p*-cymene (**178**) to cumic acid (**194**) by *Pseudomonas* sp., *Pseudomonas desmolytica* and *Nocardia salmonicolor.* (Modified from Yamada, K. et al., 1965. *Agric. Biol. Chem.*, 29: 943–948; Madyastha, K.M. and P.K. Bhattacharyya, 1968. *Indian J. Biochem.*, 5: 161–167; Noma, Y., 2000. unpublished data.)

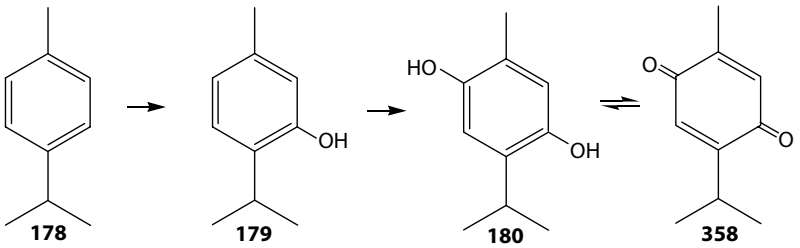
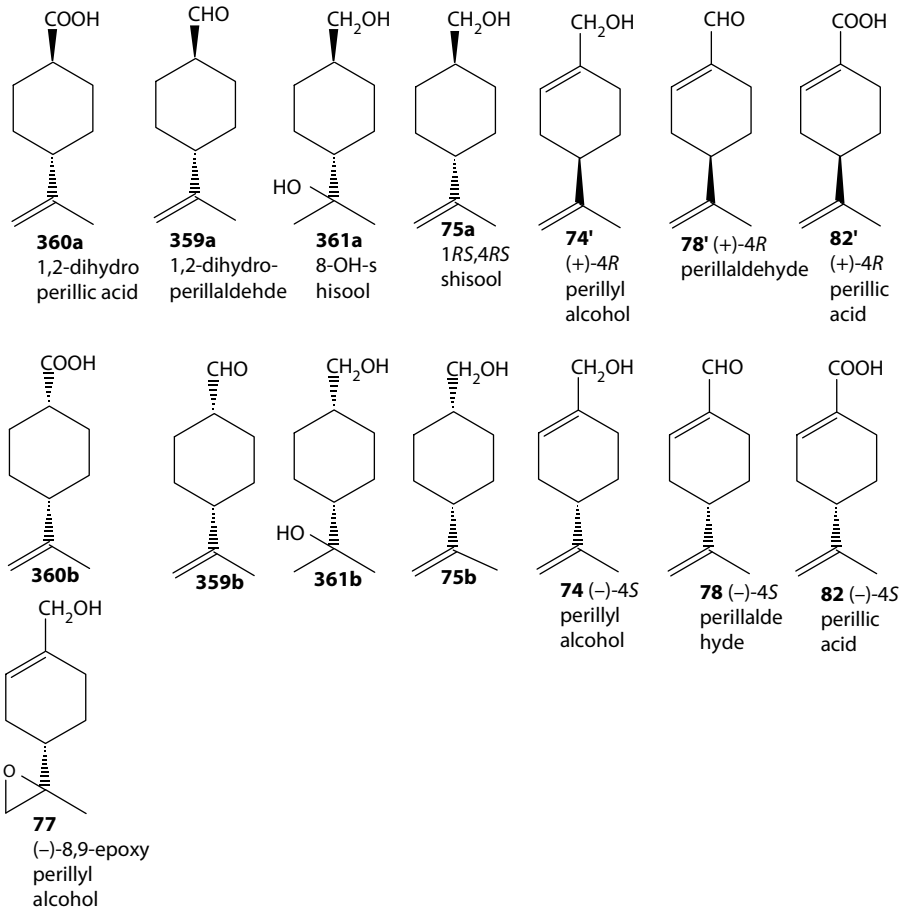


FIGURE 14.57 Biotransformation of *p*-cymene (**178**) to thymoquinone (**358**) and analogues by microorganisms. (Modified from Demirci, F. et al., 2007. *Book of Abstracts of the 38th ISEO*, SL-1, p. 6.)

14.3.2 Monocyclic Monoterpene Aldehyde



14.3.2.1 Perillaldehyde

Biotransformation of (–)-perillaldehyde (**78**), (+)-perillaldehyde (**78′**), (–)-perillyl alcohol (**74**), *trans*-1,2-dihydroperillaldehyde (**359a**) and *cis*-1,2-dihydroperillaldehyde (**359b**), and *trans*-shisoic acid (**360a**) and *cis*-shisoic acid (**360b**) was carried out by *Euglena gracilis* Z. (Noma et al., 1991a), *Dunaliella tertiolecta* (Noma et al., 1991b, 1992a), *Chlorella ellipsoidea* IAMC-27 (Noma et al., 1997), *Streptomyces ikutamanensis* Ya-2-1 (Noma et al., 1984, 1986), and other microorganisms (Kayahara et al., 1973) (Figure 14.58).

(–)-Perillaldehyde (**78**) is easily transformed to give (–)-perillyl alcohol (**74**) and *trans*-shisool (**75a**), which is well known as a fragrance, as the major product, and (–)-perillic acid (**82**) as the minor product. (–)-Perillyl alcohol (**74**) is also transformed to *trans*-shisool (**75a**) as the major product with *cis*-shisool (**75b**) and 8-hydroxy-*cis*-shisool (**361b**). Furthermore, *trans*-shisool (**75a**) and *cis*-shisool (**75b**) are hydroxylated to 8-hydroxy-*trans*-shisool (**361a**) and 8-hydroxy-*cis*-shisool (**361b**), respectively. *trans*-1,2-Dihydroperillaldehyde (**359a**) and *cis*-1,2-dihydroperillaldehyde (**359b**) are also transformed to **75a** and **75b** as the major products and *trans*-shisoic acid (**360a**) and *cis*-shisoic acid (**360b**) as the minor products, respectively. Compound **360a** was also formed from **75a**. In the biotransformation of (±)-perillaldehyde (**74** and **74′**), the same results were obtained as described in the case of **74**. In the case of *Streptomyces ikutamanensis* Ya-2-1, (–)-perillaldehyde (**78**) was converted to (–)-perillic acid (**82**), (–)-perillyl alcohol (**74**), and (–)-perillyl alcohol-8,9-epoxide (**77**) which was the major product.

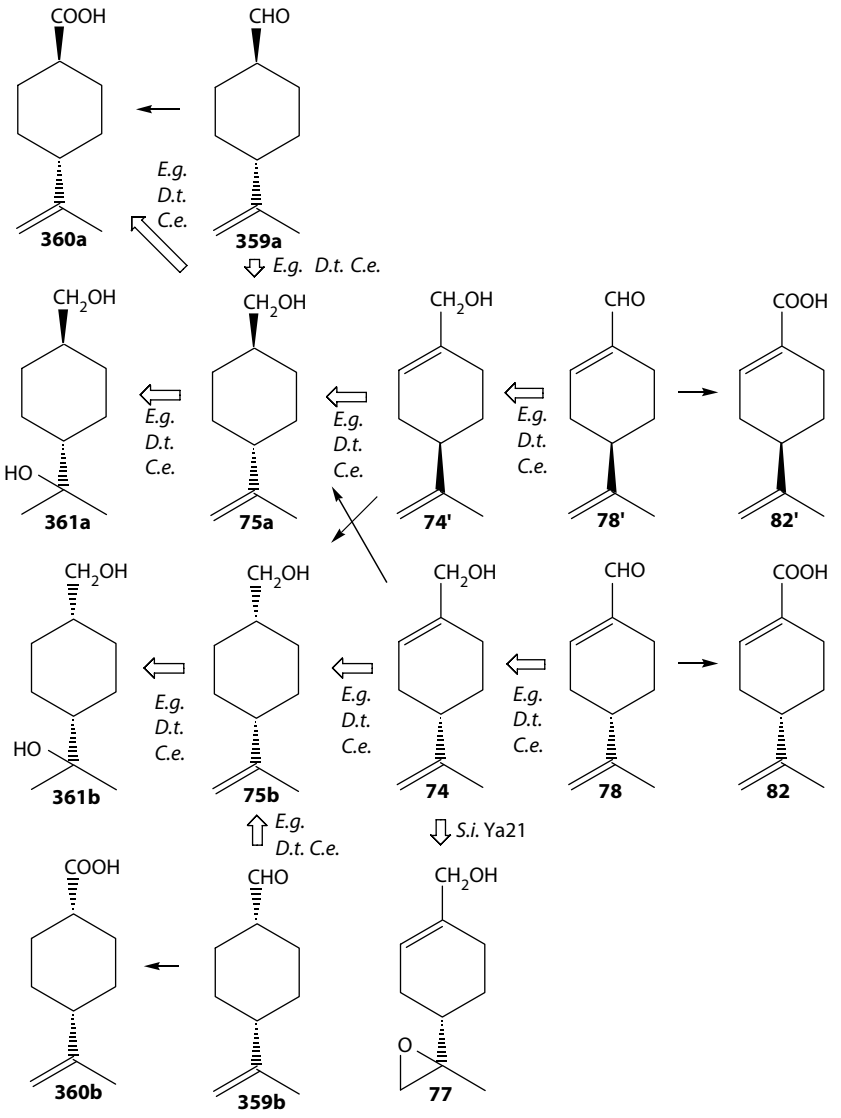


FIGURE 14.58 Metabolic pathways of perillaldehyde (**78** and **78′**) by *Euglena gracilis* Z (Noma et al., 1991a), *Dunaliella tertiolecta* (Noma et al., 1991b; 1992a), *Chlorella ellipsoidea* IAMC-27 (Noma et al., 1997), *Streptomyces ikutamanensis* Ya-2-1 (Noma et al., 1984, 1986), a soil Pseudomonad (Kayahara et al., 1973), and rabbit (Ishida et al., 1981a).

A soil Pseudomonad has been found to grow with (–)-perillaldehyde (**78**) as the sole carbon source and to produce (–)-perillic acid (**82**) in a mineral salt medium (Kayahara et al., 1973).

On the other hand, rabbit metabolized (–)-perillaldehyde (**78**) to (–)-perillic acid (**82**) along with minor shisool (**75a**) (Ishida et al., 1981a).

14.3.2.2 Phellandral and 1,2-Dihydrophellandral

Biotransformation of (–)-phellandral (**64**), *trans*-tetrahydroperillaldehyde (**362a**), and *cis*-tetrahydroperillaldehyde (**362b**) was carried out by microorganisms (Noma et al., 1986, 1991a, 1991b, 1997). (–)-Phellandral (**64**) was metabolized mainly via (–)-phellandrol (**63**) to *trans*-tetrahydroperillyl alcohol (**66a**). *trans*-Tetrahydroperillaldehyde (**362a**) and *cis*-tetrahydroperillaldehyde (**362b**) were also transformed to *trans*-tetrahydroperillyl alcohol (**66a**) and *cis*-tetrahydroperillyl alcohol (**66b**)

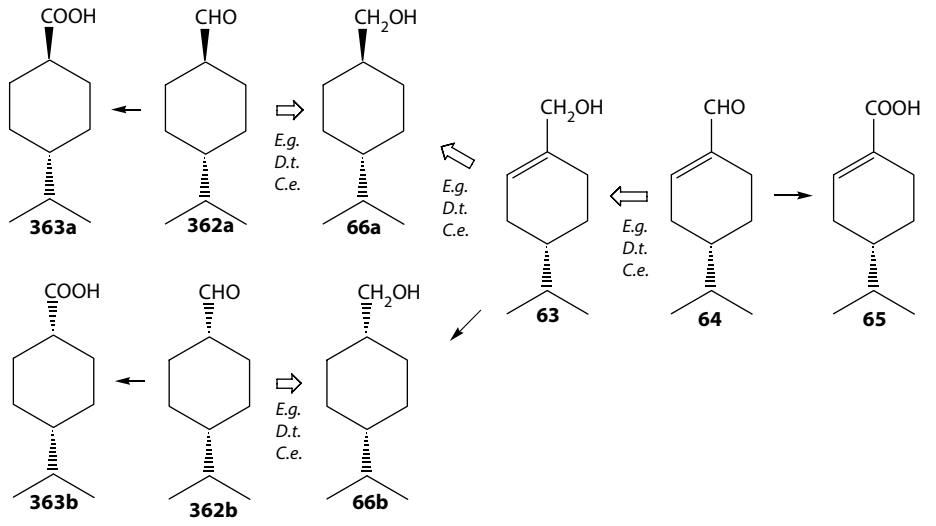


FIGURE 14.59 Metabolic pathways of (–)-phellandral (**64**) by microorganisms. (Modified from Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206; Noma, Y. et al., 1991a. *Phytochem.*, 30: 1147–1151; Noma, Y. et al., 1991b. *Proc. 35th TEAC*, pp. 112–114; Noma, Y. et al., 1997. *Proc. 41st TEAC*, pp. 227–229.)

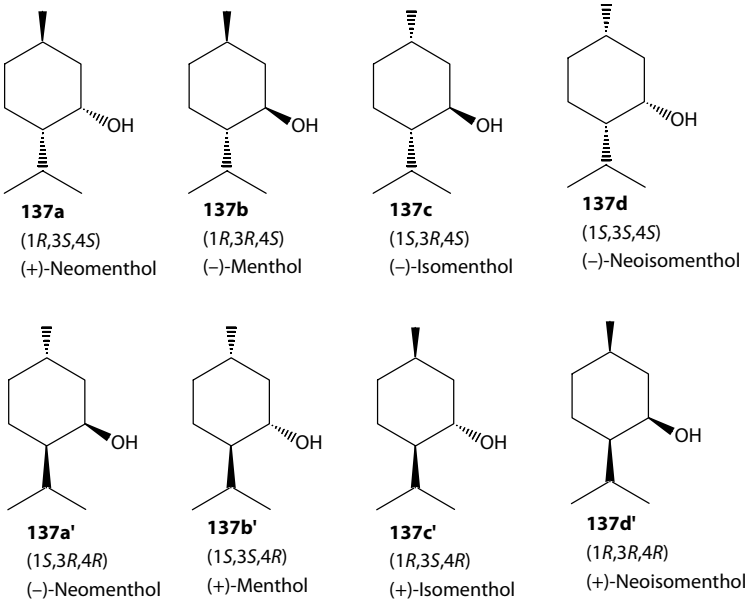
as the major products and *trans*-tetrahydroperillic acid (**363a**) and *cis*-tetrahydroperillic acid (**363b**) as the minor products, respectively (Figure 14.59).

14.3.2.3 Cuminaldehyde

Cumin aldehyde (**193**) is transformed by *Euglena* (Noma et al., 1991a), *Dunaliella* (Noma et al., 1991b), and *Streptomyces ikutamanensis* (Noma et al., 1986) to give cumin alcohol (**192**) as the major product and cuminic acid (**194**) as the minor product (Figure 14.60).

14.3.3 Monocyclic Monoterpene Alcohol

14.3.3.1 Menthol



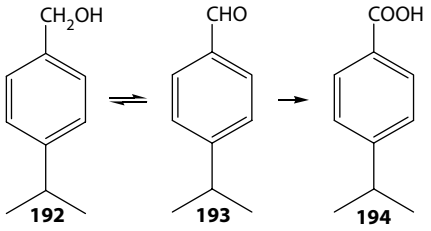


FIGURE 14.60 Metabolic pathway of cumin aldehyde (**193**) by microorganism. (Modified from Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206; Noma, Y. et al., 1991a. *Phytochem.*, 30: 1147–1151; Noma, Y. et al., 1991b. *Proc. 35th TEAC*, pp. 112–114.)

Menthol (**137**) is one of the rare naturally occurring monocyclic monoterpene alcohols that have not only various physiological properties, such as sedative, anesthetic, antiseptic, gastric, and antipruritic, but also characteristic fragrance (Bauer et al., 1990). There are in fact eight isomers with a menthol (*p*-menthan-3-ol) skeleton; (−)-menthol (**137b**) is the most important one, because of its cooling and refreshing effect. It is the main component of peppermint and cornmint oils obtained from the *Mentha piperita* and *Mentha arvensis* species. Many attempts have been made to produce (−)-menthol (**137b**) from inexpensive terpenoid sources, but these sources also unavoidably yielded the (±)-isomers (**137b** and **137b′**): isomenthol (**137c**), neomenthol (**137a**), and neoisomenthol (**137d**) (Krasnobajew, 1984). Japanese researchers have been active in this field, maybe because of the large demand for (−)-menthol (**137b**) in Japan itself, namely 500 t/year (Janssens et al., 1992). Indeed, most literature deals with the enantiomeric hydrolysis of (±)-menthol (**137b** and **137b′**) esters to optically pure *l*-menthol (**137b**). The asymmetric hydrolysis of (±)-menthyl chloroacetate by an esterase of *Arginomonas non-fermentans* FERM-P-1924 has been patented by the Japanese Nippon Terpene Chemical Co. (Watanabe and Inagaki, 1977a, 1977b). Investigators from the Takasago Perfumery Co. Ltd. claim that certain selected species of *Absidia*, *Penicillium*, *Rhizopus*, *Trichoderma*, *Bacillus*, *Pseudomonas*, and others asymmetrically hydrolyze esters of (±)-menthol isomers such as formates, acetates, propanoates, caproates, and esters of higher fatty acids (Moroe et al., 1971; Yamaguchi et al., 1977) (Figure 14.61).

Numerous investigations into the resolution of the enantiomers by selective hydrolysis with microorganisms or enzymes were carried out. Good results were described by Yamaguchi et al. (1977) with the asymmetric hydrolysis of (±)-methyl acetate by a mutant of *Rhodotorula mucilaginosa*, yielding 44 g of (−)-menthol (**137b**) form a 30% (±)-menthyl acetate mixture per liter of cultured medium for 24 h. The latest development is the use of immobilized cells of *Rhodotorula minuta* in aqueous saturated organic solvents (Omata et al., 1981) (Figure 14.62).

Besides the hydrolysis of menthyl esters, the biotransformation of menthol and its enantiomers has also been published (Shukla et al., 1987; Asakawa et al., 1991). The fungal biotransformation of (−)-(**137b**) and (+)-menthols (**137b′**) by *Aspergillus niger* and *Aspergillus cellulosae* was described

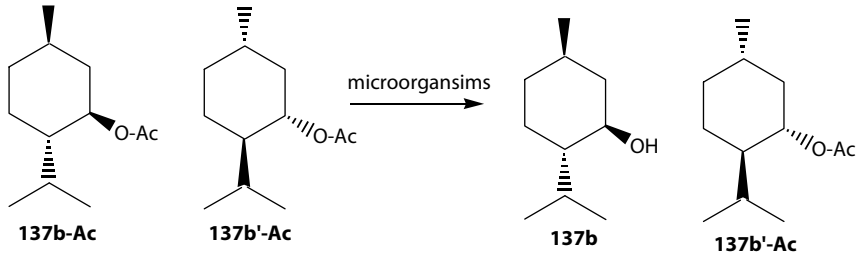


FIGURE 14.61 Asymmetric hydrolysis of racemic menthyl acetate (**137b-Ac** and **137b′-Ac**) to obtain pure (−)-menthol (**137b**). (Modified from Watanabe, Y. and T. Inagaki, 1977a. Japanese Patent 77.12.989. No. 187696x; Watanabe, Y. and T. Inagaki, 1977b. Japanese Patent 77.122.690. No. 87656g; Moroe, T. et al., 1971. Japanese Patent, 2.036. 875. no. 98195t; Oritani, T. and Yamashita, K. 1973b. *Agric. Biol. Chem.*, 37: 1695–1700.)

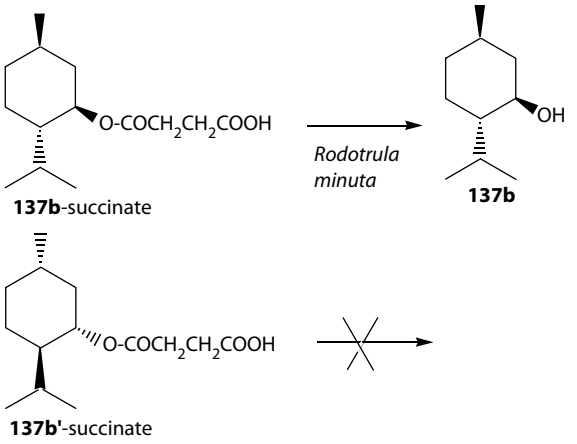


FIGURE 14.62 Asymmetric hydrolysis of racemic menthyl succinate (**137b-** and **137b′-succinates**) to obtain pure (–)-menthol (**137b**). (Modified from Yamaguchi, Y. et al., 1977. *J. Agric. Chem. Soc. Jpn.*, 51: 411–416,)

(Asakawa et al., 1991). *Aspergillus niger* converted (–)-menthol (**137b**) to 1- (**138b**), 2- (**140b**), 6- (**139b**), 7- (**143b**), 9-hydroxymenthols (**144b**), and the mosquito repellent-active 8-hydroxymenthol (**142b**), whereas (+)-menthol (**137b′**) was smoothly biotransformed by the same microorganism to 7-hydroxymenthol (**143b**). The bioconversion of (+)- (**137a′**) and (–)-neomenthol (**137a**) and (+)-isomenthol (**137c′**) by *Aspergillus niger* was studied later by Takahashi et al. (1994), mainly giving hydroxylated products. Noma and Asakawa (1995) reviewed the schematic menthol hydroxylation in detail.

Incubation of (–)-menthol (**137b**) with *Cephalosporium aphidicola* for 12 days yielded 10-acetoxymenthol (**144bb-Ac**), 1α-hydroxymenthol (**138b**), 6α-hydroxy- menthol (**139bb**), 7-hydroxymenthol (**143b**), 9-hydroxymenthol (**144ba**), and 10-hydroxymenthol (**144bb**) (Atta-ur-Rahman et al., 1998) (Figure 14.63).

Aspergillus niger TBUYN-2 converted (–)-menthol (**137b**) to 1α- (**138b**), 2α- (**140b**), 4β- (**141b**), 6α- (**139bb**), 7- (**143b**)-, 9-hydroxymenthols (**144ba**), and the mosquito repellent-active 8-hydroxymenthol (**142b**) (Figure 14.64). *Aspergillus cellulosae* M-77 biotransformed (–)-menthol (**137b**) to 4β-hydroxymenthol (**141b**) predominantly. The formation of **141b** is also observed in *Aspergillus cellulosae* IFO 4040 and *Aspergillus terreus* IFO 6123, but its yield is much less than that obtained from **137b** by *Aspergillus cellulosae* M-77 (Asakawa et al., 1991) (Table 14.1).

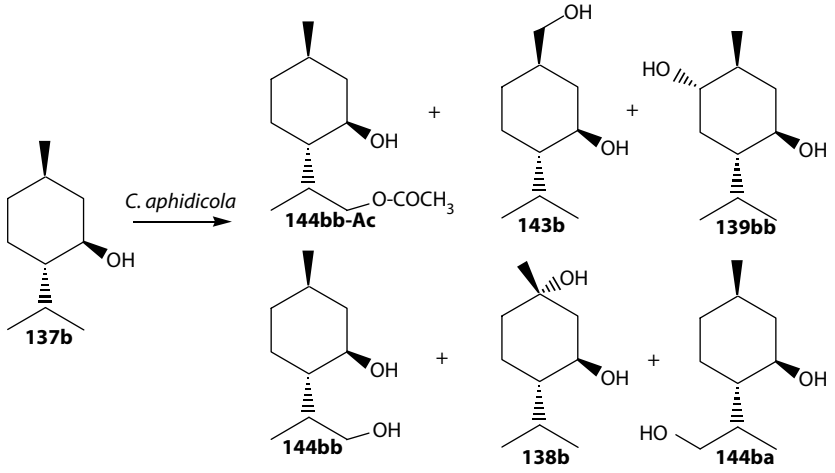


FIGURE 14.63 Biotransformation of (–)-menthol (**137b**) by *Cephalosporium aphidicola*. (Modified from Atta-ur-Rahman, M. et al., 1998. *J. Nat. Prod.*, 61: 1340–1342.)

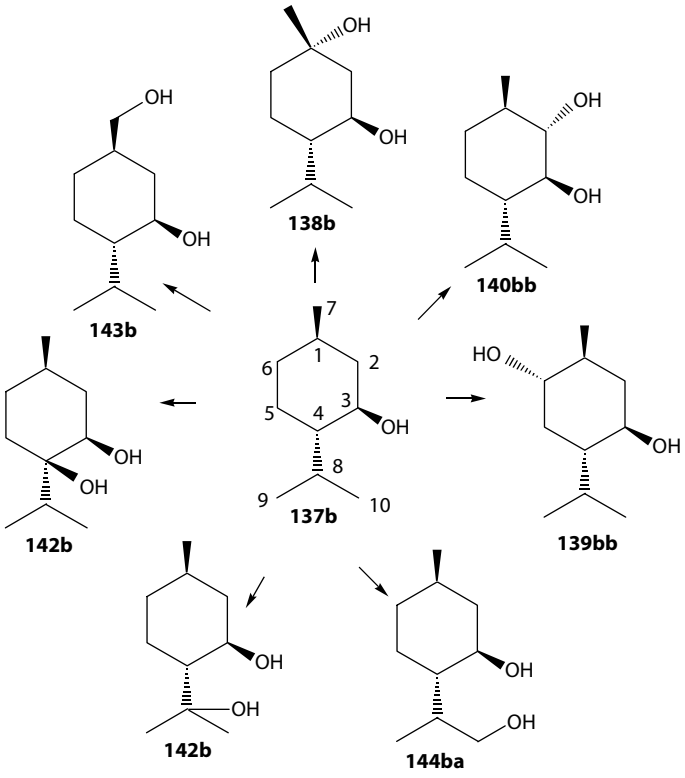


FIGURE 14.64 Metabolic pathways of (–)-menthol (**137b**) by *Aspergillus niger*. (Modified from Asakawa, Y. et al., 1991. *Phytochemistry*, 30: 3981–3987.)

On the other hand, (+)-menthol (**137b′**) was smoothly biotransformed by *Aspergillus niger* to give 1β-hydroxymenthol (**138b′**), 6β-hydroxymenthol (**139ba′**), 2β-hydroxymenthol (**140ba′**), 4α-hydroxymenthol (**141b′**), 7-hydroxymenthol (**143b′**), 8-hydroxymenthol (**142b′**), and 9-hydroxymenthol (**144ba′**) (Figure 14.65) (Table 14.2).

Spodoptera litura converted (–)- and (+)-menthols (**137b** and **137b′**) gave the corresponding 10-hydroxy products (**143b** and **143b′**) (Miyazawa et al., 1997a) (Figure 14.66).

TABLE 14.1
Metabolites of (–)-Menthol (137b) by Various *Aspergillus* spp. (Static Culture)

Microorganisms	138b	142b	139bb	143b	139bb	144ba	141b
A. awamori IFO 4033	+[a]	++	–	+	++	+++	–
A. fumigatus IFO 4400	–	+	–	+	+	+	–
A. sojae IFO 4389	++	+	+	–	–	++++	–
A. usami IFO 4338	–	–	–	+	–	+++	–
A. cellulosae M-77	+	–	–	+	–	++	++++
A. cellulosae IFO 4040	–	+	–	–	–	++	++
A. terreus IFO 6123	+	+	+	–	+	+	–
A. niger IFO 4049	–	+	–	+	–	+++	–
A. niger IFO 4040	–	+	–	+++	–	+++	–
A. niger TBUYN-2	+	++	+	+	++	++	–

[a] Symbols +, ++, +++, etc. are relative concentrations estimated by GC-MS.

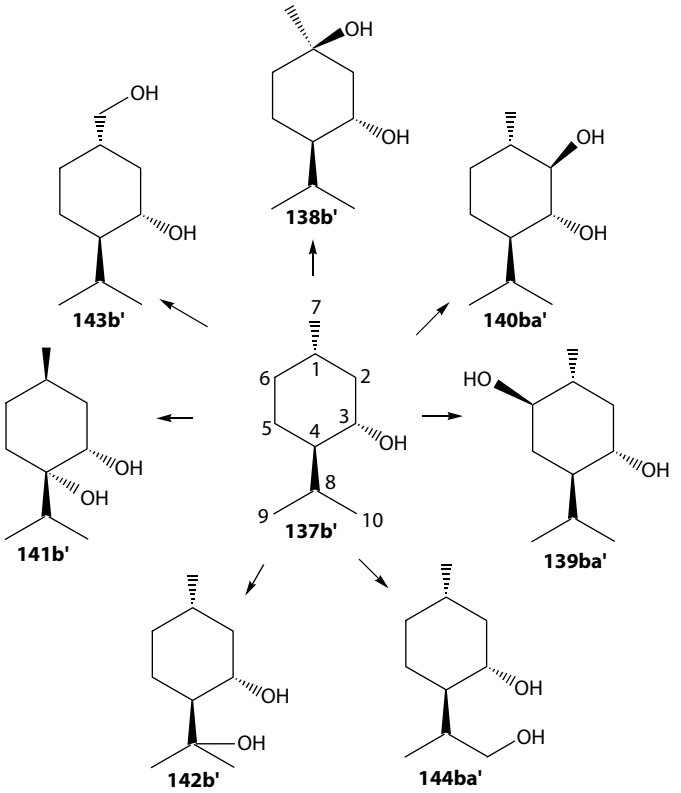


FIGURE 14.65 Metabolic pathways of (+)-menthol (**137b′**) by *Aspergillus niger*. (Modified from Noma, Y. et al., 1989. *Proc. 33rd TEAC*, pp. 124–126; Asakawa, Y. et al., 1991. *Phytochemistry*, 30: 3981–3987.)

(–)-Menthol (**137b**) was glycosylated by *Eucalyptus perriniana* suspension cells to (–)-menthol diglucoside (**364**, 26.6%) and another menthol glycoside. On the other hand, (+)-menthol (**137b′**) was glycosylated by *Eucalyptus perriniana* suspension cells to (+)-menthol di- (**364′**, 44.0%) and triglucosides (**365**, 6.8%) (Hamada et al., 2002) (Figure 14.67).

TABLE 14.2
Metabolites of (+)-Menthol (137b′) by Various *Aspergillus* spp. (Static Culture)

Microorganisms	138b′	142b′	140ba′	143b′	139ba′	144ba′	141b′
A. awamori IFO 4033	+[a]	++	–	+++	–	+++	–
A. fumigatus IFO 4400	+	++	–	+	–	++	–
A. sojae IFO 4389	+	++	–	–	–	+++	–
A. usami IFO 4338	+	–	–	+	–	+++	–
A. cellulosae M-77	–	+	–	–	–	++	++++
A. cellulosae IFO 4040	+	+	–	–	++	+	+
A. terreus IFO 6123	+	+++	+	+	+	++	–
A. niger IFO 4049	+	–	–	–	+	+++	–
A. niger IFO 4040	+	++	–	+	–	++	–
A. niger TBUYN-2	++	+	–	+++++	+	+	–

[a] Symbols +, ++, +++, etc. are relative concentrations estimated by GC-MS.

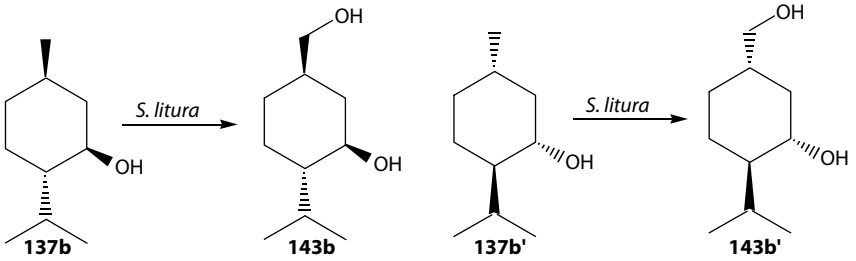


FIGURE 14.66 Biotransformation of (–)- (**137b**) and (+)-menthol (**137b′**) by *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1997a. *Proc. 41st TEAC*, pp. 391–392.)

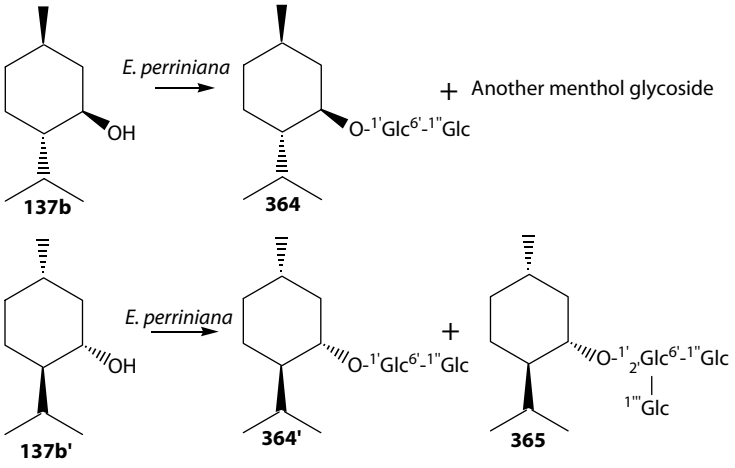


FIGURE 14.67 Biotransformation of (–)- (**137b**) and (+)-menthol (**137b′**) by *Eucalyptus perriniana* suspension cells. (Modified from Hamada, H. et al., 2002. *Proc. 46th TEAC*, pp. 321–322.)

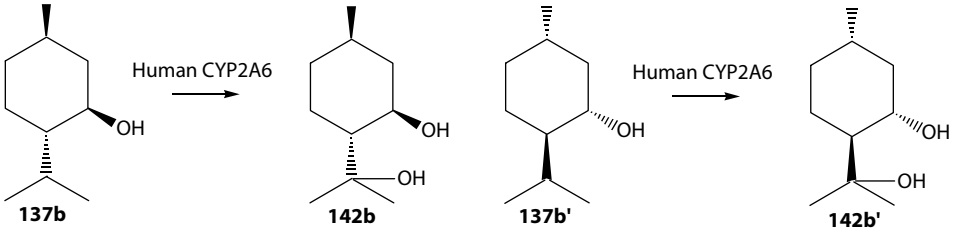


FIGURE 14.68 Biotransformation of (–)-menthol (**137b**) and its enantiomer (**137b′**) by human CYP 2A6. (Modified from Nakanishi, K. and M. Miyazawa, 2005. *Proc. 49th TEAC*, pp. 423–425.)

(–)-Menthol (**137b**) and its enantiomer (**137b′**) were converted to their corresponding 8-hydroxy derivatives (**142b** and **142b′**) by human CYP 2A6 (Nakanishi and Miyazawa, 2005) (Figure 14.68). By various assays, cytochrome P450 molecular species responsible for the metabolism of (–)- (**137b**) and (+)-menthol (**137b′**) was determined to be CYP 2A6 and CYP2B1 in human and rat, respectively. Also, kinetic analysis showed that K_m and V_{max} values for the oxidation of (–)- (**137b**) and (+)-menthol (**137b′**) recombinant CYP2A6 and CYP2B1 were determined to be 28 μM and 10.33 nmol/min/nmol P450 and 27 μM, 5.29 nmol/min/nmol P450, 28 μM and 3.58 nmol/min/nmol P450, and 33 μM and 5.3 nmol/min/nmol P450, respectively (Nakanishi and Miyazawa, 2005) (Figure 14.68).

14.3.3.2 Neomenthol

(+)-Neomenthol (**137a**) is biotransformed by *Aspergillus niger* TBUYN-2 to give five kinds of diols (**138a**, **143a**, **144aa**, **144ab**, and **142a**) and two kinds of triols (**145a** and **146a**) as shown in Figure 14.69 (Takahashi et al., 1994).

(−)-Neomenthol (**137a′**) is biotransformed by *Aspergillus niger* to give six kinds of diols (**140a′**, **139a′**, **143a′**, **144aa′**, **144ab′**, and **142a′**) and a triol (**146a′**) as shown in Figure 14.70 (Takahashi et al., 1994).

14.3.3.3 (+)-Isomenthol

(+)-Isomenthol (**137c**) is biotransformed to give two kinds of diols such as 1β-hydroxy- (**138c**) and 6β-hydroxyisomenthol (**139c**) by *Aspergillus niger* (Takahashi et al., 1994) (Figure 14.71).

(±)-Isomenthyl acetate (**137c-Ac** and **137c′-Ac**) was asymmetrically hydrolyzed to (−)-isomenthol (**137c**) with (+)-isomenthol acetate (**137c′-Ac**) by many microorganisms and esterases (Oritani and Yamashita, 1973b) (Figure 14.72).

14.3.3.4 Isopulegol

(−)-Isopulegol (**366**) was biotransformed by *Spodoptera litura* larvae to give 7-hydroxy-(−)-isopulegol (**367**), 9-hydroxy-(−)-menthol (**144ba**) and 10-hydroxy-(−)-isopulegol (**368**). On the other hand, (+)-isopulegol (**366′**) was biotransformed by the same larvae in the same manner to give 7-hydroxy-(+)-isopulegol (**367′**), 9-hydroxy-(+)-menthol (**144ba′**), and 10-hydroxy-(+)-isopulegol (**368′**) (Ohsawa and Miyazawa, 2001) (Figure 14.73).

Microbial resolution of (±)-isopulegyl acetate (**366-Ac** and **366′-Ac**) was studied by microorganisms. (±)-Isopulegyl acetate (**366-Ac** and **366′-Ac**) was hydrolyzed asymmetrically to give a mixture of (−)-isopulegol (**366**) and (+)-isopulegyl acetate (**366′-Ac**) (Oritani and Yamashita, 1973c) (Figure 14.74).

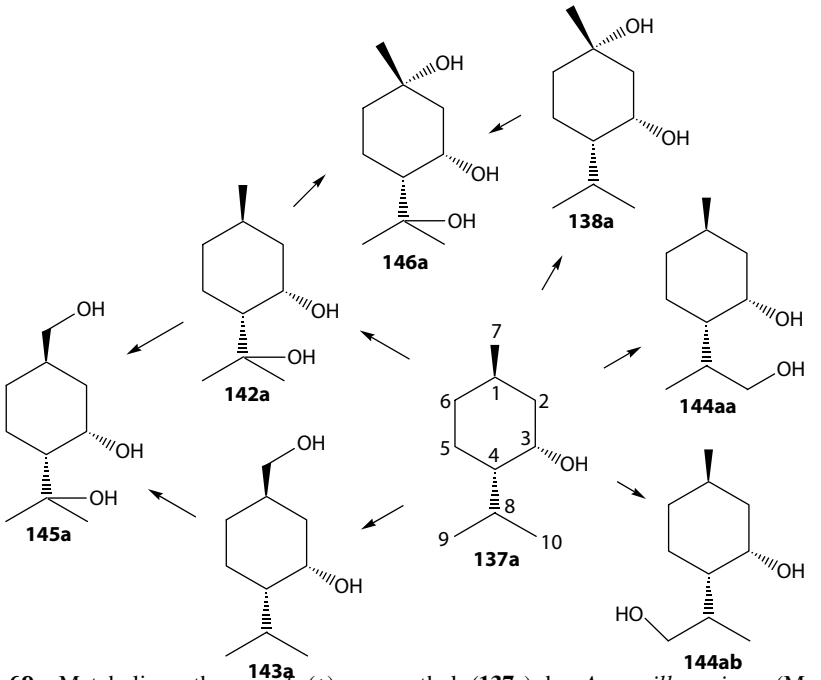


FIGURE 14.69 Metabolic pathways of (+)-neomenthol (**137a**) by *Aspergillus niger*. (Modified from Takahashi, H. et al., 1994. *Phytochemistry*, 35: 1465–1467.)

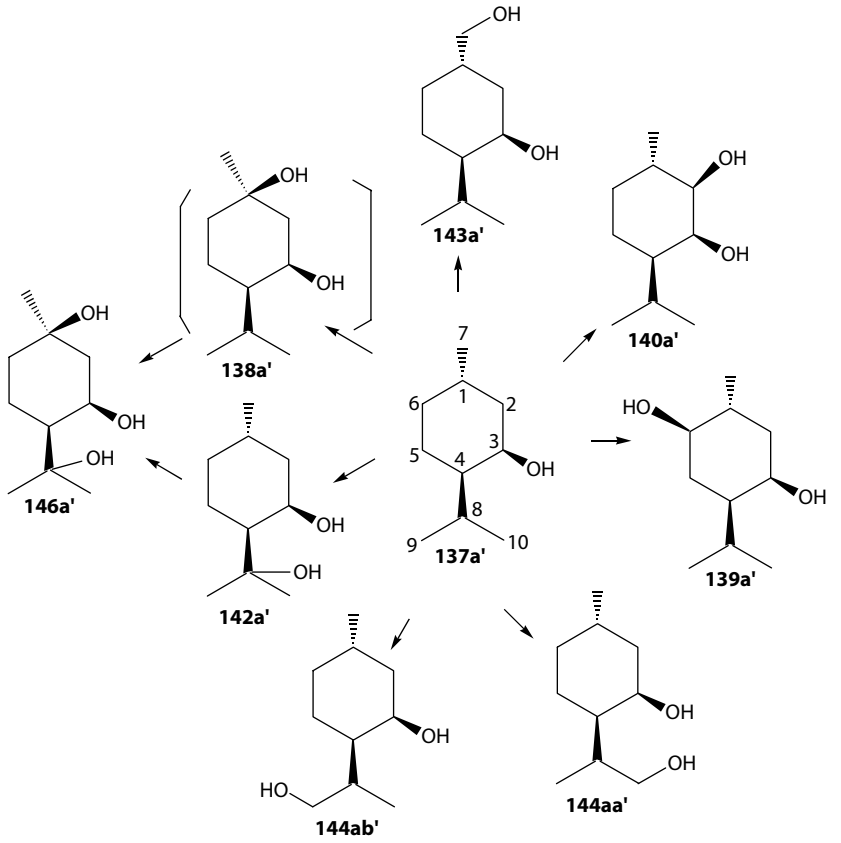


FIGURE 14.70 Metabolic pathways of (–)-neomenthol (**137a′**) by *Aspergillus niger.* (Modified from Takahashi, H. et al., 1994. *Phytochemistry*, 35: 1465–1467.)

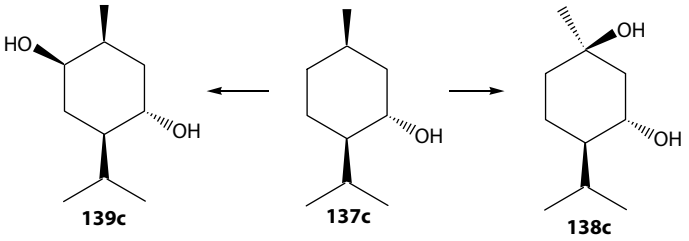


FIGURE 14.71 Metabolic pathways of (+)-isomenthol (**137c**) by *Aspergillus niger.* (Modified from Takahashi, H. et al., 1994. *Phytochemistry*, 35: 1465–1467.)

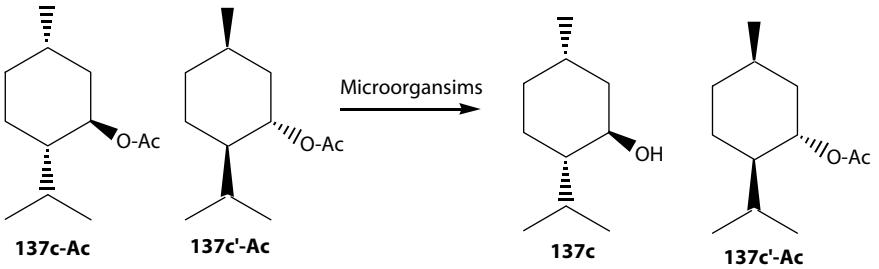


FIGURE 14.72 Microbial resolution of (±)-isomenthyl acetate (**137c-Ac** and **137c′-Ac**) by microbial esterase. (Modified from Oritani, T. and Yamashita, K. 1973b. *Agric. Biol. Chem*., 37: 1695–1700.)

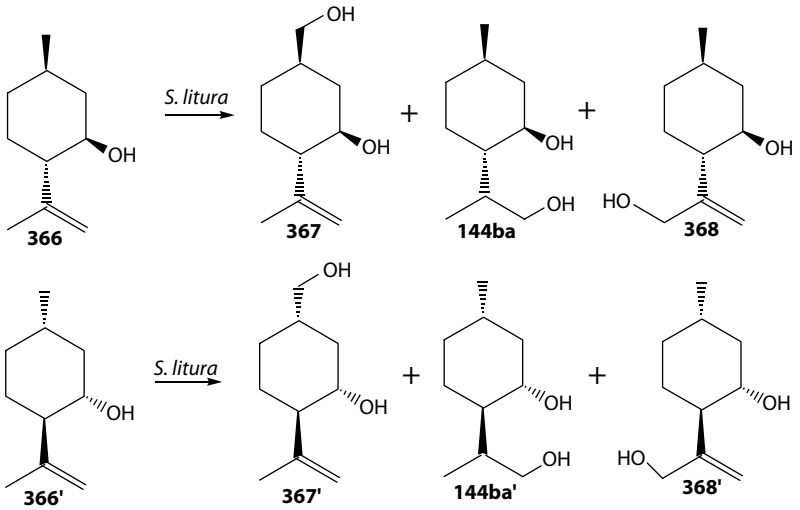


FIGURE 14.73 Biotransformation of (–)- (**366**) and (+)-isopulegol (**366′**) by *Spodoptera litura*. (Modified from Ohsawa, M. and Miyazawa, M. 2001. *Proc. 45th TEAC*, pp. 375–376.)

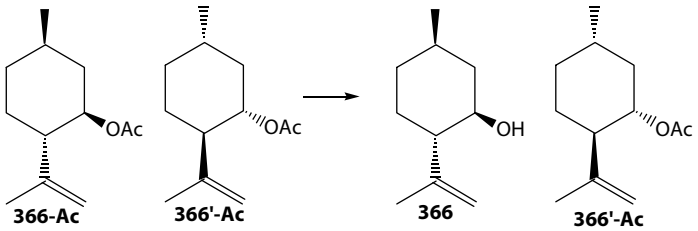


FIGURE 14.74 Microbial resolution of (±)-isopulegyl acetate (**366-Ac** and **366′-Ac**) by microorganisms. (Modified from Oritani, T. and K. Yamashita, 1973c. *Agric. Biol. Chem*., 37: 1687–1689.)

14.3.3.5 α-Terpineol

Pseudomonas pseudomonalli strain T was cultivated with α-terpineol (**34**) as the sole carbon source to give 8,9-epoxy-*p*-menthan-1-ol (**58**) via epoxide (**369**) and diepoxide (**57**) as intermediates (Hayashi et al., 1972) (Figure 14.75).

(+)-α-Terpineol (**34**) was formed from (+)-limonene (**34**) by *Citrus* pathogenic *Pencillium digitatum* (Pers.; Fr.) Sacc. KCPYN, which was further biotransformed to *p*-menthane-1β,2α,8-triol (**334**), 2α-hydroxy-1,8-cineole (**125b**), and (+)-*trans*-sobrerol (**95a**) (Noma and Asakawa 2006a, 2007a) (Figure 14.76). *Penicillium* sp. YuzuYN also biotransformed **34** to **334**. Furthermore, *Aspergillus niger* Tiegh, CBAYN and *Catharanthus roseus* biotransformed **34** to give **95a** and (+)-oleuropeyl alcohol (**204**), respectively (Hamada et al., 2001; Noma and Asakawa 2006a, 2007a) (Figure 14.76).

Gibberella cyanea DSM 62719 biotransformed (–)-α-terpineol (**34′**) to give *p*-menthane-1-β,2α,8-triol (**334′**), 2α-hydroxy-1,8-cineole (**125b′**), 1,2-epoxy-α-terpineol (**369′**), (–)-oleuropeyl alcohol (**204′**), (–)-*trans*-sobrerol (**95a′**), and *cis*-sobrerol (**95b′**) (Abraham et al., 1986) (Figure 14.76). In cases of *Pencillium digitatum* (Pers. Fr.) Sacc. KCPYN, *Penicillium* sp. YuzuYN, *Aspergillus niger* Tiegh, CBAYN **34′** was biotransformed to give **369′**, **95a′**, and **334′**, respectively (Noma and Asakawa 2006a, 2007a) (Figure 14.77). *Catharanthus roseus* biotransformed **34′** to give **95a′** and **204′** (Hamada et al., 2001) (Figure 14.77).

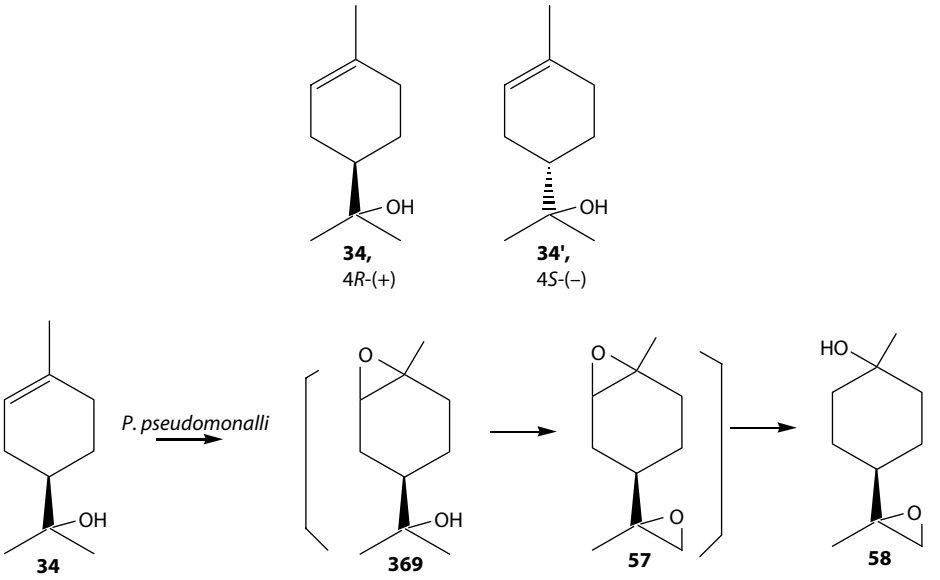


FIGURE 14.75 Biotransformation of (+)-α-terpineol (**34**) to 8,9-epoxy-*p*-menthan-1-ol (**58**) by *Pseudomonas pseudomonalli* strain T. (Modified from Hayashi, T. et al., 1972. *Biol. Chem.*, 36: 690–691.)

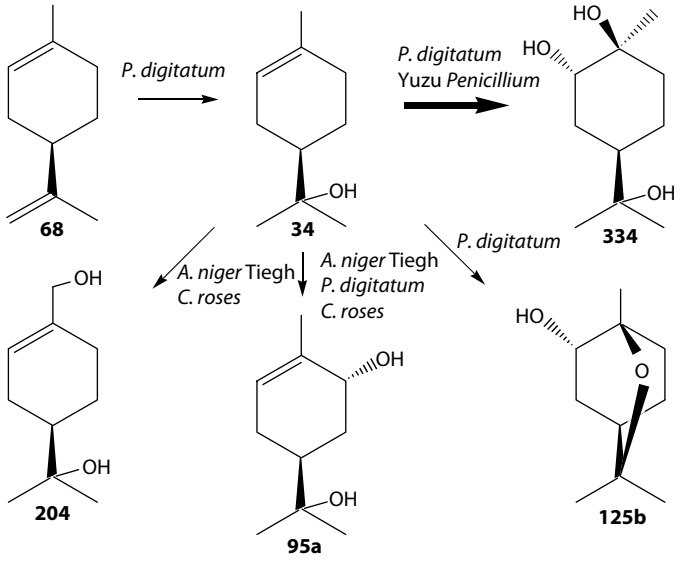


FIGURE 14.76 Biotransformation of (+)-α-terpineol (**34**) by *Citrus* pathogenic fungi, *Pencillium digitatum* (Pers.; Fr.) Sacc. KCPYN, *Penicillium* sp. YuzuYN, *Aspergillus niger* Tiegh, CBAYN. (Modified from Noma, Y. and Y. Asakawa, 2006a. *Proc. 50th TEAC*, pp. 431–433; Noma, Y. and Y. Asakawa, 2007a. *Book of Abstracts of the 38th ISEO*, p. 7.)

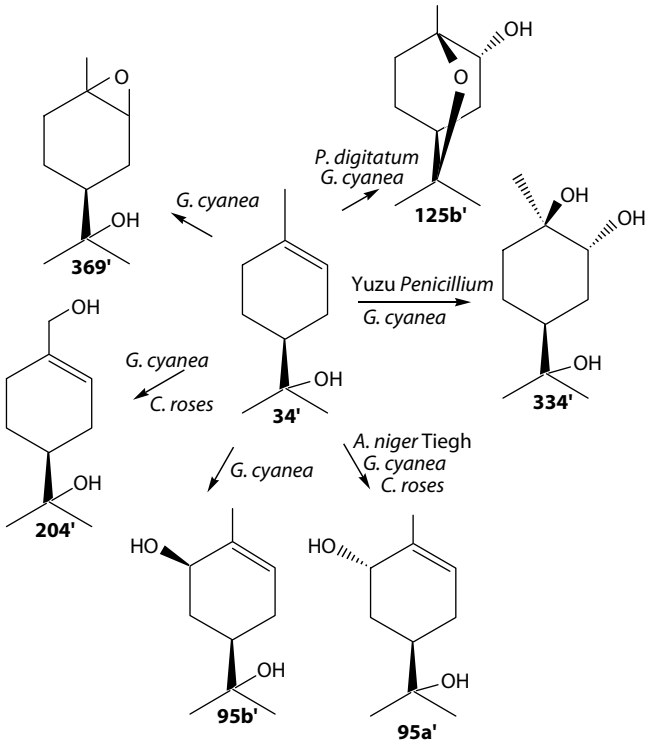
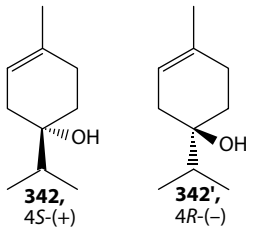


FIGURE 14.77 Biotransformation of (–)-α-terpineol (**34′**) by *Gibberella cyanea* DSM 62719, *Pencillium digitatum* (Pers. Fr.) Sacc. KCPYN, *Penicillium* sp. Yuzu YN, *Aspergillus niger* Tiegh, CBAYN. (Modified from Abraham, W.-R. et al., 1986. *Appl. Microbiol. Biotechnol.*, 24: 24–30; Noma, Y. and Y. Asakawa, 2006a. *Proc. 50th TEAC*, pp. 431–433; Noma, Y. and Y. Asakawa, 2007a. *Book of Abstracts of the 38th ISEO*, p. 7.)

14.3.3.6 (–)-Terpinen-4-ol



Gibberella cyanea DSM 62719 biotransformed (*S*)-(–)-terpinen-4-ol (**342**) (1-*p*-menthen-4-ol) to give 2α-hydroxy-1,4-cineole (**132b**), 1-*p*-menthene-4α,6-diol (**372**), and *p*-menthane-1β,2α,4α-triol (**371**) (Abraham et al., 1986). On the other hand, *Aspergillus niger* TBUYN-2 also biotransformed (–)-terpinen-4-ol (**342**) to give 2α-hydroxy-1,4-cineole (**132b**) and (+)-*p*-menthane-1β,2α,4α-triol (**371**) (Noma and Asakawa 2007b) (Figure 14.78). On the other hand, *Spodoptera litura* biotransformed (*R*)-terpinen-4-ol (**342′**) to (4*R*)-*p*-menth-1-en-4,7-diol (**373′**) (Kumagae and Miyazawa, 1999) (Figure 14.78).

14.3.3.7 Thymol and Thymol Methyl Ether

Thymol (**179**) was converted at the concentration of 14% by *Streptomyces humidus*, Tu-1 to give (1*R*,2*S*)- (**181a**) and (1*R*,2*R*)-2-hydroxy-3-*p*-menthen-5-one (**181b**) as the major products (Noma et al., 1988a) (Figure 14.79). On the other hand, in a *Pseudomonas*, thymol (**179**) was biotransformed to 6-hydroxy- (**180**), 7-hydroxy- (**479**), 9-hydroxy- (**480**), 7,9-dihydroxythymol (**482**), thymol-7-oic acid (**481**), and thymol-9-oic acid (**483**) (Chamberlain and Dagley, 1968) (Figure 14.79).

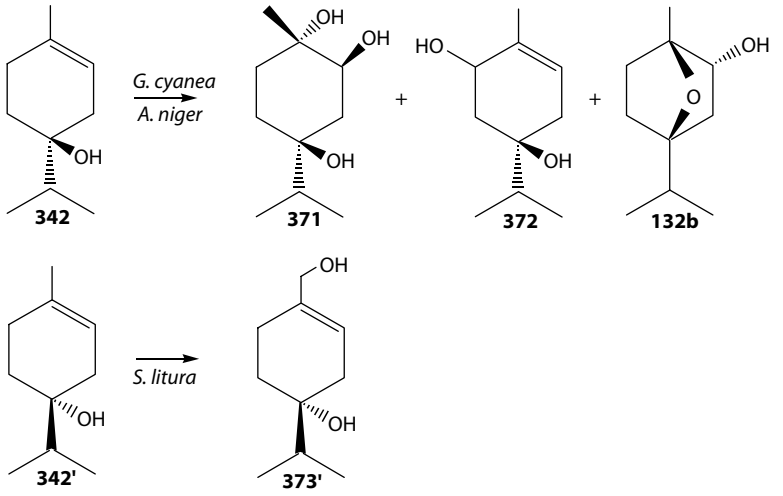


FIGURE 14.78 Biotransformation of (–)-terpinen-4-ol (**342**) by *Gibberella cyanea* DSM 62719, *Aspergillus niger* TBUYN-2, and *Spodoptera litura*. (Modified from Abraham, W.-R. et al., 1986. *Appl. Microbiol. Biotechnol*., 24: 24–30; Kumagae, S. and M. Miyazawa, 1999. *Proc. 43rd TEAC*, pp. 389–390; Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

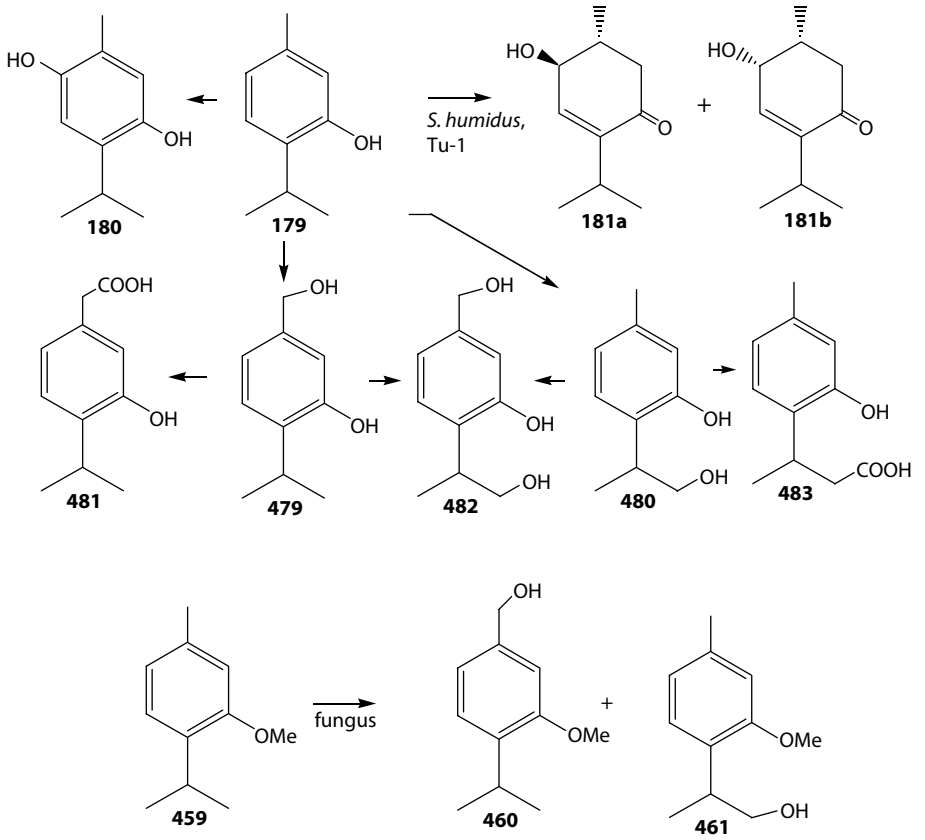


FIGURE 14.79 Biotransformation of thymol (**179**) and thymol methyl ether (**459**) by actinomycetes *Streptomyces humidus*, Tu-1 and fungi *Aspergillus niger*, *Mucor ramannianus*, *Rhizopus arrhizus*, and *Trichothecium roseum*. (Modified from Chamberlain, E.M. and S. Dagley, 1968. *Biochem. J.*, 110: 755–763; Noma, Y. et al., 1988a. *Proc. 28th TEAC*, pp. 177–179; Demirci, F. et al., 2001. *XII Biotechnology Congr.*, Book of abstracts, p. 47.)

Thymol methyl ether (**459**) was converted by fungi, *Aspergillus niger*, *Mucor ramannianus*, *Rhizopus arrhizus*, and *Trichothecium roseum* to give 7-hydroxy- (**460**) and 9-hydroxythymol methyl ether (**461**) (Demirci et al., 2001) (Figure 14.79).

14.3.3.8 Carvacrol and Carvacrol Methyl Ether

When cultivated in a liquid medium with carvacrol (**191**), as a sole carbon source, the bacterial isolated from savory and pine consumed the carvacrol in the range of 19–22% within 5 days of cultivation. The fungal isolates grew much slower and after 13 days of cultivation consumed 7.1–11.4% carvacrol (**191**). Pure strains belonging to the bacterial genera of Bacterium, *Bacillus* and *Pseudomonas* as well as fungal strain from *Aspergillus*, *Botrytis*, and *Geotrichum genera*, were also tested for their ability to grow in medium containing carvacrol (**191**). Among them, only in *Bacterium* sp. and *Pseudomonas* sp. Carvacrol (**191**) uptake was monitored. Both *Pseudomonas* sp. 104 and 107 consumed the substrate in the amount of 19%. These two strains also exhibited the highest cell mass yield and the highest productivity (1.1 and 1.2 g/L per day) (Schwammle et al., 2001).

Carvacrol (**191**) was biotransformed to 3-hydroxy- (**470**), 9-hydroxy (**471**), 7-hydroxy- (**475**), and 8-hydroxycarvacrol (**474**), 8,9-dehydrocarvacrol (**473**), carvacrol-9-oic acid (**472**), carvacrol-7-oic acid (**476**), and 8,9-dihydroxycarvacrol (**477**) by rats (Ausgulen et al., 1987) and microorganisms (Demirci, 2000) including *Trichothecium roseum* and *Cladosporium* sp. (Figure 14.80). Furthermore, carvacrol methyl ether (**191-Me**) was converted by the same fungi to give 7-hydroxy- (**475-Ac**) and

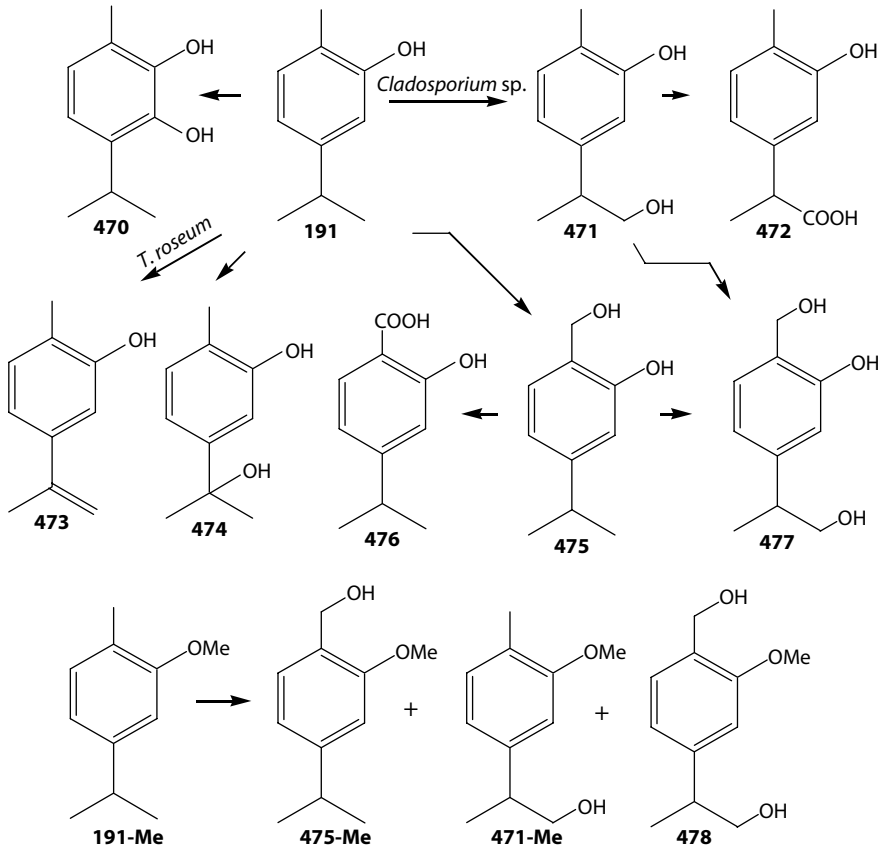
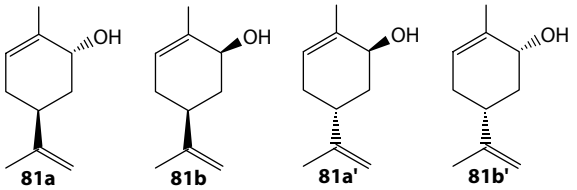


FIGURE 14.80 Biotransformation of carvacrol (**191**) and carvacrol methyl ether (**191-Me**) by rats (Modified from Ausgulen, L.T. et al., 1987. *Pharmacol. Toxicol*., 61: 98–102) and microorganisms (Modified from Demirci, F., 2000. *Microbial transformation of bioactive monoterpenes*. Ph.D. thesis, pp. 1–137. Anadolu University, Eskisehir, Turkey).

9-hydroxycarvacrol methyl ether (**471-Me**) and 7,9-dihydroxycarvacrol methyl ether (**478**) (Demirci, 2000) (Figure 14.80).

14.3.3.9 Carveol



At first, soil Pseudomonad biotransformed (+)-limonene (**68**) to (+)-carvone (**93**) and (+)-1-*p*-menthene-6,9-diol (**90**) via (+)-*cis*-carveol (**81b**) as shown in Figure 14.81 (Dhavalikar and Bhattacharyya, 1966; Dhavalikar et al., 1966).

Secondary, *Pseudomonas ovalis*, strain 6-1 (Noma, 1977) biotransformed the mixture of (–)-*cis*-carveol (**81b′**) and (–)-*trans*-carveol (**81a′**) (94:6, GC ratio) to (–)-carvone (**93'**) (Noma, 1977), which was further metabolized reductively to give (+)-dihydrocarvone (**101a′**), (+)-isodihydrocarvone (**101b′**), (+)-neodihydrocarveol (**102a),** and (–)-dihydrocarveol (**102b**) (Noma et al., 1984). Hydrogenation at C1, 2-position did not occur, but the dehydrogenation at C6-position occured to give (–)-carvone (**93**) (Figure 14.82).

On the other hand, in *Streptomyces*, A-5-1 and *Nocardia*, 1-3-11, which were isolated from soil, (–)-carvone (**93′)** was reduced to give mainly (–)-*trans*-carveol (**81a′**) and (–)-*cis*-carveol (**81b′**), respectively. On the other hand, (–)-*trans*-carveol (**81a′**) and (–)-*cis*-carveol (**81b′**) were dehydrogenated to give **93′** by strain 1-3-11 and other microorganisms (Noma et al., 1986). The reaction between *trans*- and *cis*-carveols (**81a′** and **81b′**) and (–)-carvone (**93′)** is reversible (Noma, 1980) (Figure 14.82).

Thirdly, the investigation for the biotransformation of the mixture of (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) (60:40 in GC ratio) was carried out by using 81 strains of soil actinomycetes. All actinomycetes produced (–)-carvone (**93′**) from the mixture of (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) (60:40 in GC ratio). However, 41 strains of actinomycetes converted (–)-*cis*-carveol (**81b′**) to give (4*R*,6*R*)-(+)-6,8-oxidomenth-1-en-9-ol (**92a′**), which is named as bottrospicatol after the name of the microorganism, *Streptomyces bottropensis* ⌈Bottro⌋, and (–)-*cis*-carveol (**81b′**) containing *Mentha spicata* ⌈spicat⌋ and alcohol ⌈ol⌋ (Nishimura et al., 1983a) (Figure 14.83).

(+)-Bottrospicatol (**92a′**) was prepared by epoxidation of (–)-carvone (**93′**) with *m*CPBA to (–)-carvone-8,9-epoxide (**96′**), followed by stereoselective reduction with $NaBH_4$ to alcohol, which was immediately cyclized with 0.1 N H_2SO_4 to give diastereo mixture of bottrospicatol (**92a′** and **b′**) (Nishimura et al., 1983a) (Figure 14.84).

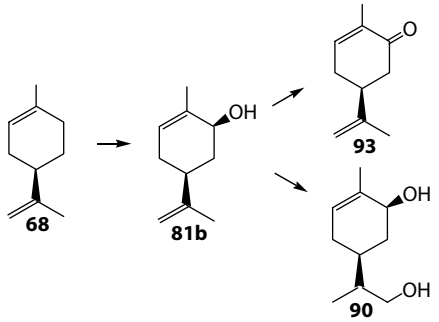


FIGURE 14.81 Proposed metabolic pathway of (+)-limonene (**68**) and (+)-*cis*-carveol (**81b**) by soil Pseudomonad. (Modified from Dhavalikar, R.S. and P.K. Bhattacharyya, 1966. *Indian J. Biochem*., 3: 144–157; Dhavalikar, R.S. et al., 1966. *Indian J. Biochem*., 3: 158–164.)

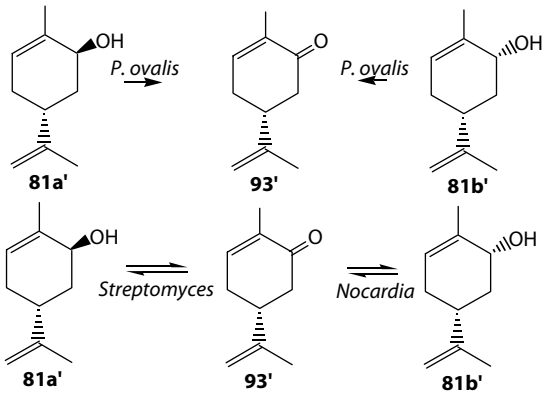


FIGURE 14.82 Biotransformation of (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) (6:94, GC ratio) by *Pseudomonas ovalis*, strain 6-1, *Streptomyces* , A-5-1, and *Nocardia*, 1-3-11. (Modified from Noma, Y., 1977. *Nippon Nogeikagaku Kaishi*, 51: 463–470; Noma, Y., 1980. *Agric. Biol. Chem*., 44: 807–812.)

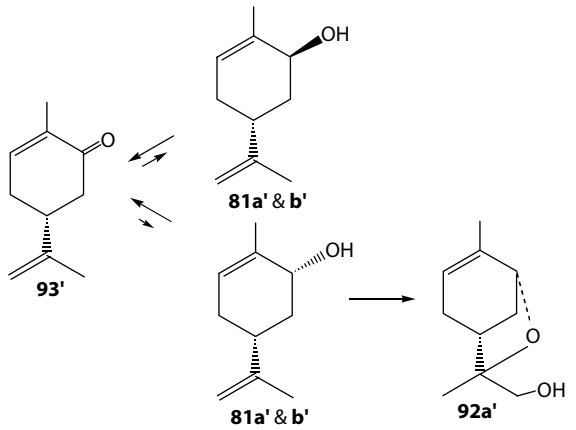


FIGURE 14.83 The Metabolic pathways of *cis*-carveol (**81b′**) by *Pseudomonas ovalis*, strain 6-1 (Modified from Noma, Y., 1977. *Nippon Nogeikagaku Kaishi*, 51: 463–470) and *Streptomyces bottropensis* SY-2-1 and other microorganisms (Modified from Noma, Y. et al., 1982. *Agric. Biol. Chem*., 46: 2871–2872; Nishimura, H. et al., 1983a. *Proc. 27th TEAC*, pp. 107–109).

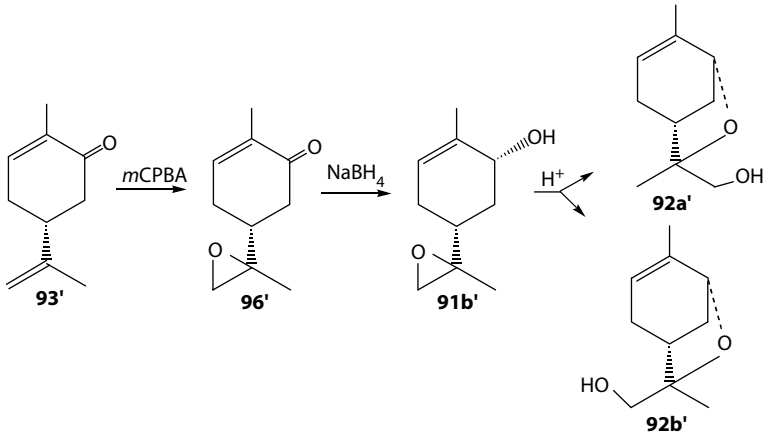


FIGURE 14.84 Preparation of (+)-bottrospicatol (**92a′**) and (+)-isobottrospicatol (**92b′**) from (–)-carvone (**93′**) with *m*CPBA. (Modified from Nishimura, H. and Y. Noma, 1996. *Biotechnology for Improved Foods and Flavors*, G.R. Takeoka, et al., ACS Symp. Ser. 637, pp. 173–187. American Chemical Society, Washington, DC.)

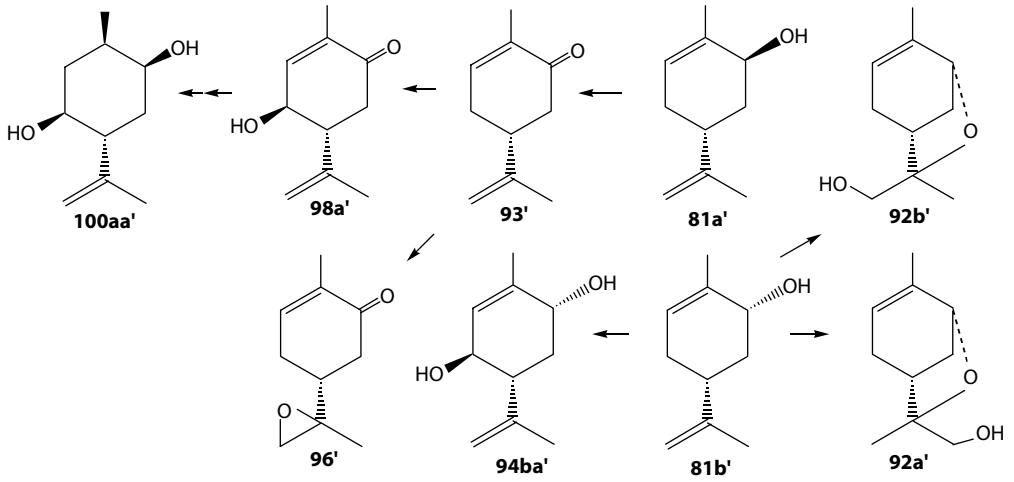


FIGURE 14.85 Biotransformation of (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) by *Streptomyces bottropensis* SY-2-1 and *Streptomyces ikutamanensis* Ya-2-1. (Modified from Noma, Y. et al., 1982. *Agric. Biol. Chem*., 46: 2871–2872; Noma, Y. and H. Nishimura, 1984. *Proc. 28th TEAC*, pp. 171–173; Noma, Y. and H. Nishimura, 1987. *Agric. Biol. Chem*., 51: 1845–1849.)

Further investigation showed *Streptomyces bottropensis* SY-2-1 (Noma and Iwami, 1994) has different metabolic pathways for (–)-*trans*-carveol (**81a′**) and (–)-*cis*-carveol (**81b′**). Namely, *Streptomyces bottropensis* SY-2-1 converted (–)-*trans*-carveol (**81a′**) to (–)-carvone (**93′**), (–)-carvone-8,9-epoxide (**96′**), (–)-5β-hydroxycarvone (**98a′**), and (+)-5β-hydroxyneodihydrocarveol (**100aa′**) (Figure 14.85). On the other hand, *Streptomyces bottropensis* SY-2-1 converted (–)-*cis*-carveol (**81b′**) to give (+)-bottrospicatol (**92a′**) and (–)-5β-hydroxy-*cis*-carveol (**94ba′**) as main products together with (+)-isobottrospicatol (**92b′**) as the minor product as shown in Figure 14.85.

In the metabolism of *cis*-carveol by microorganisms there are four pathways (pathways 1–4) as shown in Figure 14.86. At first, *cis*-carveol (**81**) is metabolized to carvone (**93**) by C2 dehydrogenation (Noma, 1977, 1980) (pathway 1). Secondly, *cis*-carveol (**81b**) is metabolized via epoxide as intermediate to bottrospicatol (**92**) by rearrangement at C2 and C8 (Noma et al., 1982; Nishimura et al., 1983a, 1983b; Noma and Nishimura, 1987) (pathway 2). Thirdly, *cis*-carveol (**81b**) is hydroxylated at C5 position to give 5-hydroxy-*cis*-carveol (**94**) (Noma and Nishimura, 1984) (pathway 3).

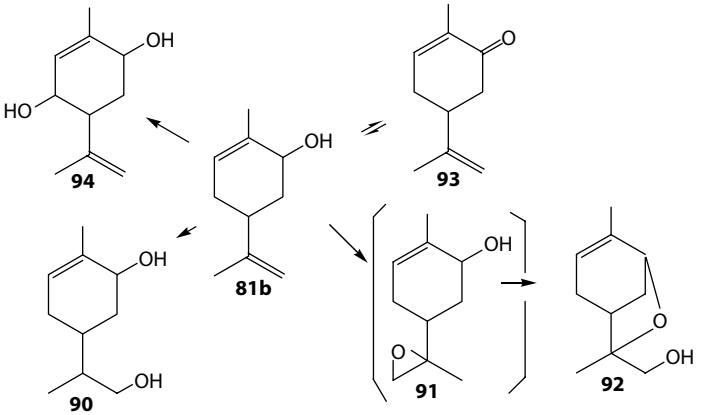


FIGURE 14.86 General metabolic pathways of carveol (**81**) by microorganisms. (Modified from Noma, Y. et al., 1982. *Agric. Biol. Chem*., 46: 2871–2872; Noma, Y. and H. Nishimura, 1984. *Proc. 28th TEAC*, pp. 171–173; Noma, Y. and H. Nishimura, 1987. *Agric. Biol. Chem*., 51: 1845–1849; Nishimura, H. and Y. Noma, 1996. *Biotechnology for Improved Foods and Flavors*, G.R. Takeoka, et al., ACS Symp. Ser. 637, pp.173–187. American Chemical Society, Washington, DC.)

TABLE 14.3
Effects of (–)-*cis*- (81b′) and (–)-*trans*-Carveol (81a′) Conversion Products by *Streptomyces bottropensis* SY-2-1 on the Germination of Lettuce Seeds

	Germination Rate (%)	
Compounds	**24 h**	**48 h**
(–)-Carvone (**93′**)	47	89
(+)-Bottrospicatol (**92′**)	3	48
(–)-Carvone-8,9-epoxide (**96′**)	2	77
5β-Hydroxyneodihydrocarveol (**102aa′**)	86	96
5β-Hydroxycarvone (**98a′**)	91	96
Control	95	96

Note: Concentration of each compound was adjusted at 200 ppm.

Finally, *cis*-carveol (**81b**) is metabolized to 1-*p*-menthene-2,9-diol (**90**) by hydroxylation at C9 position (Dhavalikar and Bhattacharyya, 1966; Dhavalikar et al., 1966) (pathway 4).

Effects of (–)-*cis*- (**81b′**) and (–)-*trans*-carveol (**81a′**) conversion products by *Streptomyces bottropensis* SY-2-1 on the germination of lettuce seeds was examined and the result is shown in Table 14.3. (+)-Bottrospicatol (**92′**) and (–)-carvone-8,9-epoxide (**96′**) showed strong inhibitory activity for the germination of lettuce seeds.

Streptomyces bottropensis SY-2-1 has also different metabolic pathways for (+)-*trans*-carveol (**81a**) and (+)-*cis*-carveol (**81b**) (Noma and Iwami, 1994). Namely, *Streptomyces bottropensis* SY-2-1 converted (+)-*trans*-carveol (**81a**) to (+)-carvone (**93**), (+)-carvone-8,9-epoxide (**96**), and (+)-5α-hydroxycarvone (**98a**) (Noma and Nishimura, 1982, 1984) (Figure 14.87). On the other hand, *Streptomyces bottropensis* SY-2-1 converted (+)-*cis*-carveol (**81b**) to give (–)-isobottrospicatol (**92b**) and (+)-5-hydroxy-*cis*-carveol (**94b**) as the main products and (–)-bottrospicatol (**92a**) as the minor product as shown in Figure 14.88 (Noma et al., 1980, Noma and Nishimura, 1987; Nishimura and Noma, 1996).

Biological activities of (+)-bottrospicatol (**92a′**) and related compounds for plant's seed germination and root elongation were examined towards barnyard grass, wheat, garden cress, radish, green foxtail, and lettuce (Nishimura and Noma, 1996).

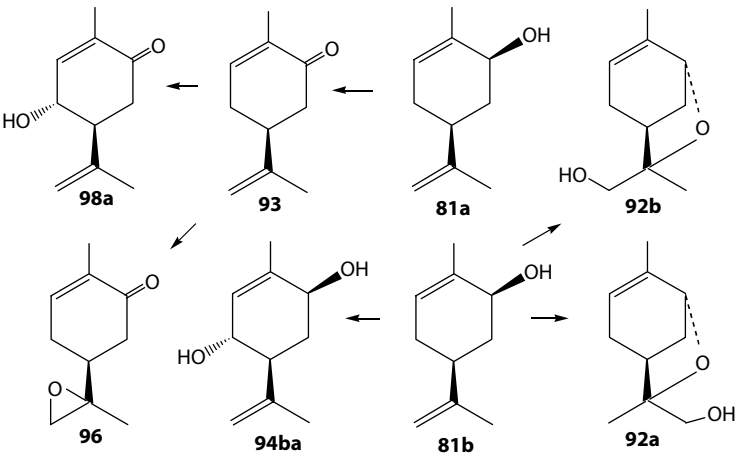


FIGURE 14.87 Metabolic pathways of (+)-*trans*- (**81a**) and (+)-*cis*-carveol (**81b**) by *Streptomyces bottropensis* SY-2-1. (Modified from Noma, Y. and H. Nishimura, 1987. *Agric. Biol. Chem*., 51: 1845–1849; Nishimura, H. and Y. Noma, 1996. *Biotechnology for Improved Foods and Flavors*, G.R. Takeoka, et al., ACS Symp. Ser. 637, pp.173–187. American Chemical Society, Washington, DC.)

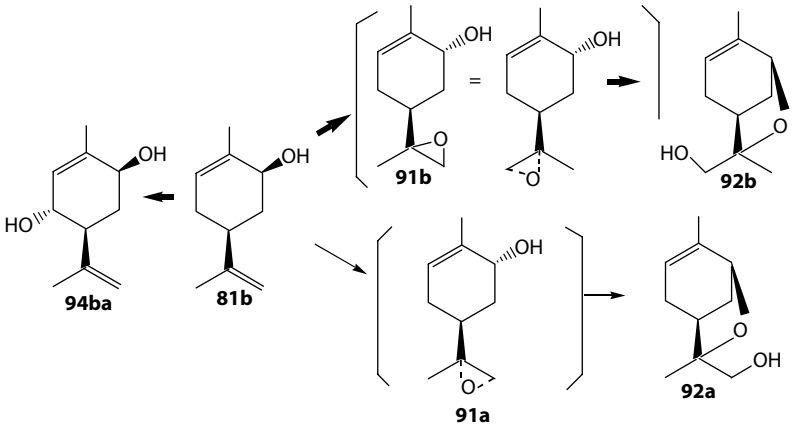


FIGURE 14.88 Metabolic pathways of (+)-*cis*-carveol (**81b**) by *Streptomyces bottropensis* SY-2-1 and *Streptomyces ikutamanensis*, Ya-2-1. (Modified from Noma, Y. and H. Nishimura, 1987. *Agric. Biol. Chem*., 51: 1845–1849; Nishimura, H. and Y. Noma, 1996. *Biotechnology for Improved Foods and Flavors*, G.R. Takeoka, et al., ACS Symp. Ser. 637, pp.173–187. American Chemical Society, Washington, DC.)

Isomers and derivatives of bottrospicatol were prepared by the procedure shown in Figure 14.89. The chemical structure of each compound was confirmed by the interpretation of spectral data. The effects of all isomers and derivatives on the germination of lettuce seeds were compared. The germination inhibitory activity of (+)-bottrospicatol (**92a′**) was the highest of isomers. Interestingly, (−)-isobottrospicatol (**92b**) was not effective even in a concentration of 500 ppm. (+)-Bottrospicatol methyl ether (**92a′**-methyl ether) and esters [**92a′**-methyl (ethyl and *n*-propyl) ester] exhibited weak inhibitory activities. The inhibitory activity of (−)-isodihydrobottrospicatol (**105c′**) was as high as that of (+)-bottrospicatol (**92a′**). Furthermore, an oxidized compound, (+)-bottrospicatal (**374a′**), exhibited higher activity than (+)-bottrospicatol (**92a′**). So, the germination inhibitory activity of (+)-bottrospicatal (**374a′**) against several plant seeds, lettuce, green foxtail, radish, garden cress, wheat, and

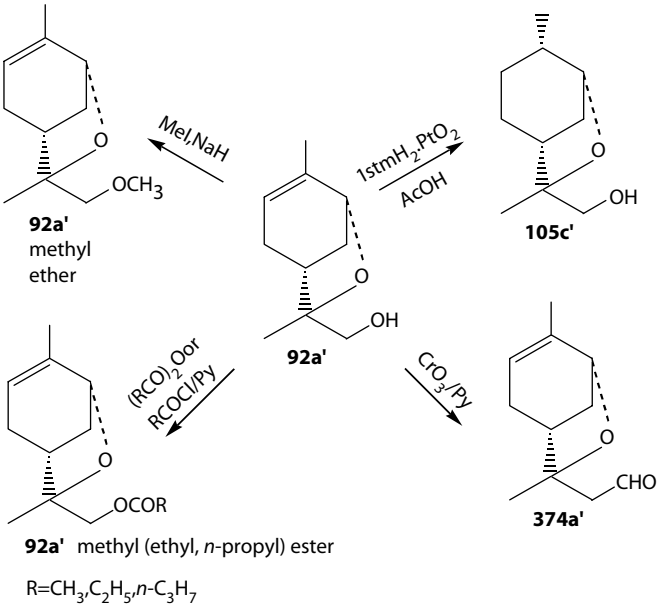


FIGURE 14.89 Preparation of (+)-bottrospicatol (**92a′**) derivatives. (Modified from Nishimura, H. and Y. Noma, 1996. *Biotechnology for Improved Foods and Flavors*, G.R. Takeoka, et al., ACS Symp. Ser. 637, pp. 173–187. American Chemical Society, Washington, DC.)

barnyard grass was examined. The result indicates that (+)-bottrospicatal (**374a′**) is a selective germination inhibitor as follows: lettuce > green foxtail > radish > garden cress > wheat > barnyard grass.

Enantio- and diastereoselective biotransformation of *trans*- (**81a** and **81a′**) and *cis*-carveols by *Euglena gracilis* Z. (Noma and Asakawa 1992) and *Chlorella pyrenoidosa* IAM C-28 was studied (Noma et al., 1997).

In the biotransformation of racemic *trans*-carveol (**81a** and **81a′**), *Chlorella pyrenoidosa* IAM C-28 showed high enantioselectivity for (−)-*trans*-carveol (**81a′**) to give (−)-carvone (**93′**), while (+)-*trans*-carveol (**81a**) was not converted at all. The same *Chlorella pyrenoidosa* IAM C-28 showed high enantioselectivity for (+)-*cis*-carveol (**81b**) to give (+)-carvone (**93**) in the biotransformation of racemic *cis*-carveol (**81b** and **81b′**). (−)-*cis*-Carveol (**81b′**) was not converted at all. The same phenomenon was observed in the biotransformation of mixture of (−)-*trans*- and (−)-*cis*-carveol (**81a′** and **81b′**) and the mixture of (+)-*trans*- and (+)-*cis*-carveol (**81a** and **81b**) as shown in Figure 14.90. The high enantioselectivity and the high diastereoselectivity for the dehydrogenation of (−)-*trans*- and (+)-*cis*-carveols (**81a** and **81b′**) were shown in *Euglena gracilis* Z. (Noma and Asakawa, 1992), *Chlorella pyrenoidosa* IAM C-28 (Noma et al., 1997), *Nicotiana tabacum*, and other *Chlorella* spp.

On the other hand, the high enantioselecivity for **81a′** was observed in the biotransformation of racemic (+)-*trans*-carveol (**81a**) and (−)-*trans*-carveol (**81a′**) by *Chlorella sorokiniana* SAG to give (−)-carvone (**93′**).

It was considered that the formation of (−)-carvone (**93′**) from (−)-*trans*-carveol (**81a′**) by diastereo- and enantioselective dehydrogenation is a very interesting phenomenon in order to produce mosquito repellent (+)-*p*-menthane-2,8-diol (**50a′**) (Noma, 2007).

(4*R*)-*trans*-Carveol (**81a′**) was converted by *Spodptera litura* to give 1-*p*-menthene-6,8,9-triol (**375**) (Miyazawa et al., 1996b) (Figure 14.91).

14.3.3.10 Dihydrocarveol

(+)-Neodihydrocarveol (**102a′**) was converted to *p*-menthane-2,8-diol (**50a′**), 8-*p*-menthene-2, 8-diol (**107a′**), and *p*-menthane-2,8,9-triols (**104a′** and **b′**) by *Aspergillus niger* TBUYN-2 (Noma et al., 1985a, 1985b; Noma and Asakawa, 1995) (Figures 14.92 and 14.93). In case of *Euglena gracilis* Z. mosquito repellent (+)-*p*-menthane-2,8-diol (**50a′**) was formed stereospecifically from (−)-carvone (**93′**) via (+)-dihydrocarvone (**101a′**) and (+)-neodihydrocarveol (**102a′**) (Noma et al., 1993; Noma, 2007). (−)-Neodihydrocarveol (**102a**) was also easily and stereospecifically converted by *Euglena gracilis* Z. to give (−)-*p*-menthane-2,8-diol (**50a**) (Noma et al., 1993).

On the other hand, *Absidia glauca* converted (−)-carvone (**93′**) stereospecifically to give (+)-8-*p*-menthene-2,8-diol (**107a′**) via (+)-dihydrocarvone (**101a′**) and (+)-neodihydrocarveol (**102a′**) (Demirci et al., 2004) (Figure 14.93).

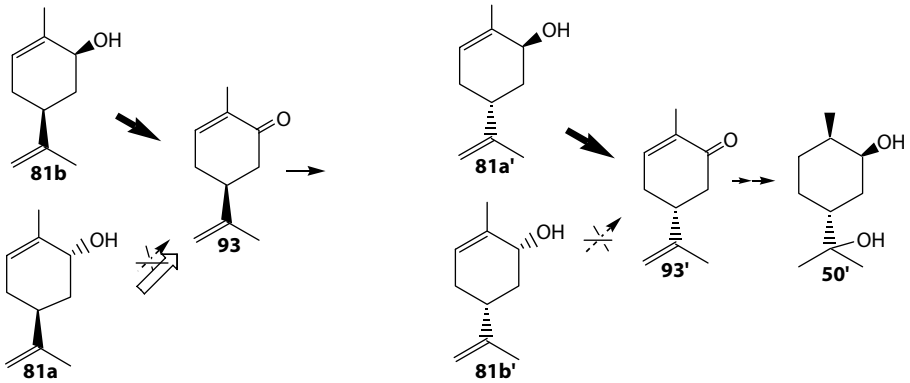


FIGURE 14.90 Enantio- and diastereoselective biotransformation of *trans*- (**81a** and **a′**) and *cis*-carveols (**81b** and **b′**) by *Euglena gracilis* Z and *Chlorella pyrenoidosa* IAM C-28. (Modified from Noma, Y., and Y. Asakawa, 1992. *Phytochem.*, 31: 2009–2011; Noma, Y. et al., 1997. *Proc. 41st TEAC*, pp. 227–229.)

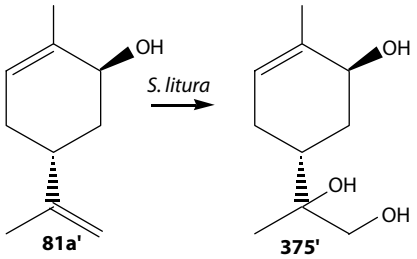


FIGURE 14.91 Biotransformation of (4*R*)-*trans*-carveol (**81a′**) by *Spodptera litura*. (Modified from Miyazawa, M. et al., 1996b. *Proc. 40th TEAC*, pp. 80–81.)

(+)- (**102b**) and (−)-Dihydrocarveol (**102b′**) were converted by 10 kinds of *Aspergillus* spp. to give mainly (+)- (**107b′**) and (−)-10-hydroxydihydrocarveol (**107b**, 8-*p*-menthene-2,10-diol) and (+)- (**50b′**) and (−)-8-hydroxydihydrocarveol (**50b**, *p*-menthane-2,8-diol), respectively (Figure 14.94). The metabolic pattern of dihydrocarveols is shown in Table 14.4.

In case of the biotransformation of *Streptomyces bottropensis*, SY-2-1 (+)-dihydrocarveol (**102b**) was converted to (+)-dihydrobottrospicatol (**105aa**) and (+)-dihydroisobottrospicatol (**105ab**), whereas (−)-dihydrocarveol (**102b′**) was metabolized to (−)-dihydrobottrospicatol (**105aa′**) and (−)-dihydroisobottrospicatol (**105ab′**). (+)-Dihydroisobottrospicatol (**105ab**) and (−)-dihydrobottrospicatol (**105aa′**) are the major products (Noma, 1984) (Figure 14.95).

Euglena gracilis Z. converted (−)-iso- (**102c**) and (+)-isodihydrocarveol (**102c′**) to give the corresponding 8-hydroxyisodihydrocarveols (**50c** and **50c′**), respectively (Noma et al., 1993) (Figure 14.96).

In case of the biotransformation of *Streptomyces bottropensis*, SY-2-1 (−)-neoisodihydrocarveol (**102d**) was converted to (+)-isodihydrobottrospicatol (**105ba**) and (+)-isodihydroisobottrospicatol (**105bb**), whereas (+)-neoisodihydro-carveol (**102d′**) was metabolized to (−)-isodihydrobottrospicatol (**105ba′**) and (−)-isodihydroisobottrospicatol (**105bb′**). (+)-Isodihydroisobottrospicatol (**105bb**) and (−)-isodihydrobottrospicatol (**105ba′**) are the major products (Noma, 1984) (Figure 14.97).

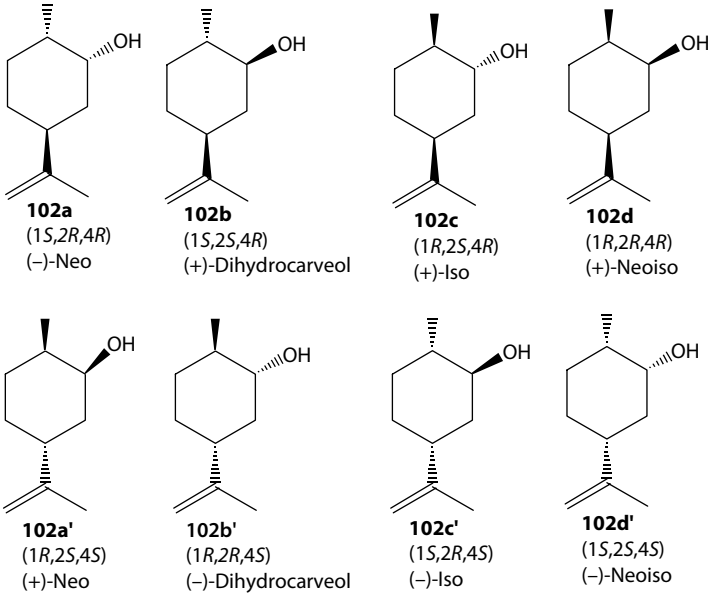


FIGURE 14.92 Chemical structure of eight kinds of dihydrocarveols.

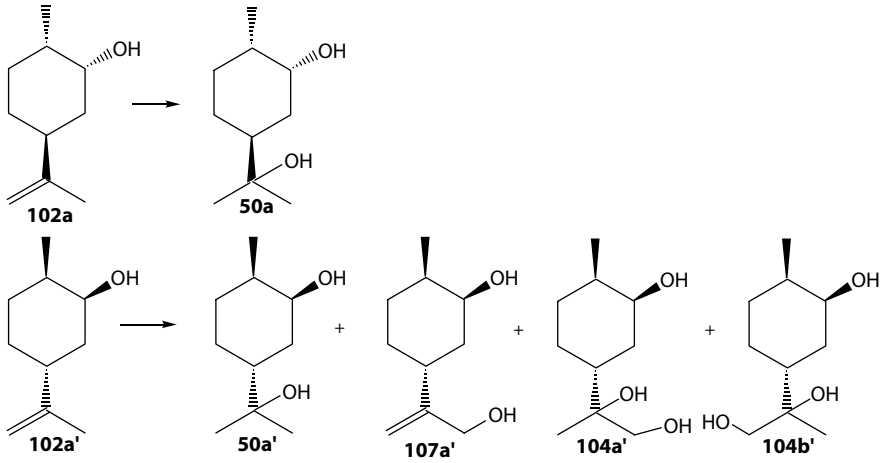


FIGURE 14.93 Biotransformation of (–)- and (+)-neodihydrocarveol (**102a** and **a′**) by *Euglena gracilis* Z, *Aspergillus niger* TBUYN-2, and *Absidia glauca*. (Modified from Noma, Y. et al., 1985a. *Annual Meeting of Agricultural and Biological Chemistry*, Sapporo, p. 68; Noma, Y. et al., 1985b. *Proc. 29th TEAC*, pp. 235–237; Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25; Noma, Y., 2007. *Aromatic Plants from Asia their Chemistry and Application in Food and Therapy*, L. Jiarovetz, N.X. Dung, and V.K. Varshney, pp. 169–186. Dehradun: Har Krishan Bhalla & Sons; Noma, Y. and Y. Asakawa, 1995. *Biotechnology in Agriculture and Forestry*, *Vol. 33. Medicinal and Aromatic Plants VIII*, Y.P.S. Bajaj, ed., pp. 62–96. Berlin: Springer; Demirci, F. et al., 2004. *Naturforsch.*, 59c: 389–392.)

Euglena gracilis Z. converted (–)- (**102d**) and (+)-neoisodihydrocarveol (**102d′**) to give the corresponding 8-hydroxyneoisodihydrocarveols (**50d** and **50d′**), respectively (Noma et al., 1993) (Figure 14.98).

Eight kinds of 8-hydroxydihydrocarveols (**50a**–**d** and **50a′**–**d′**; 8-*p*-menthane-2,8-diols) were obtained from carvone (**93** and **93′**), dihydrocarvones (**101a**–**b** and **101a′**–**b′**), and dihydrocarveols (**102a**–**d**, **102a′**–**d′**) by *Euglena gracilis* Z as shown in Figure 14.99 (Noma et al., 1993).

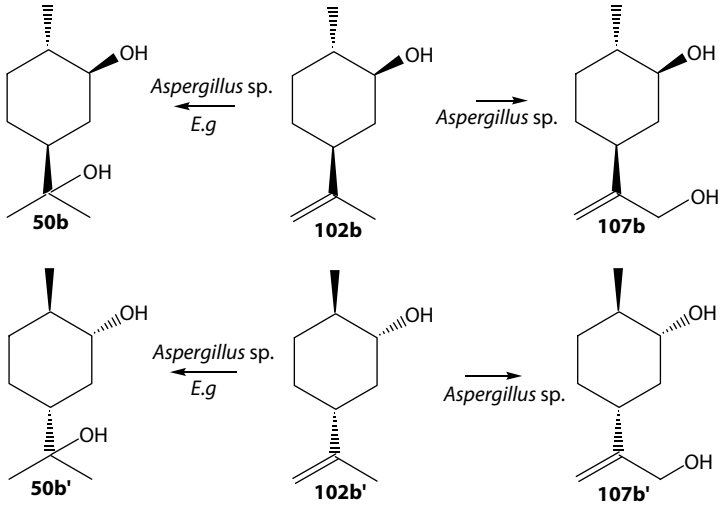


FIGURE 14.94 Biotransformation of (+)- (**102b**) and (–)-dihydrocarveol (**102b′**) by 10 kinds of *Aspergillus* spp. (Modified from Noma, Y., 1988. *The Meeting of Kansai Division of The Agricultural* and *Chemical Society of Japan*, Kagawa, p. 28) and *Euglena gracilis* Z (Modified from Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25).

TABLE 14.4
Metabolic Pattern of Dihydrocarveols (102b and 102b′) by 10 Kinds of *Aspergillus* spp.

Microorganisms	Compounds					
	107b′	50b′	C.r. (%)	107b	50b	C.r. (%)
A. awamori, IFO 4033	0	98	99	3	81	94
A. fumigatus, IFO 4400	0	14	34	+	6	14
A. sojae, IFO 4389	0	47	59	1	50	85
A. usami, IFO 4338	0	32	52	+	5	7
A. cellulosae, M-77	0	27	52	+	7	14
A. cellulosae, IFO 4040	0	30	55	1	5	8
A. terreus, IFO 6123	0	79	92	+	18	46
A. niger, IFO 4034	0	29	49	+	8	12
A. niger, IFO 4049	4	50	67	9	34	59
A. niger, TBUYN-2	29	68	100	30	53	100

C.r.—conversion ratio.

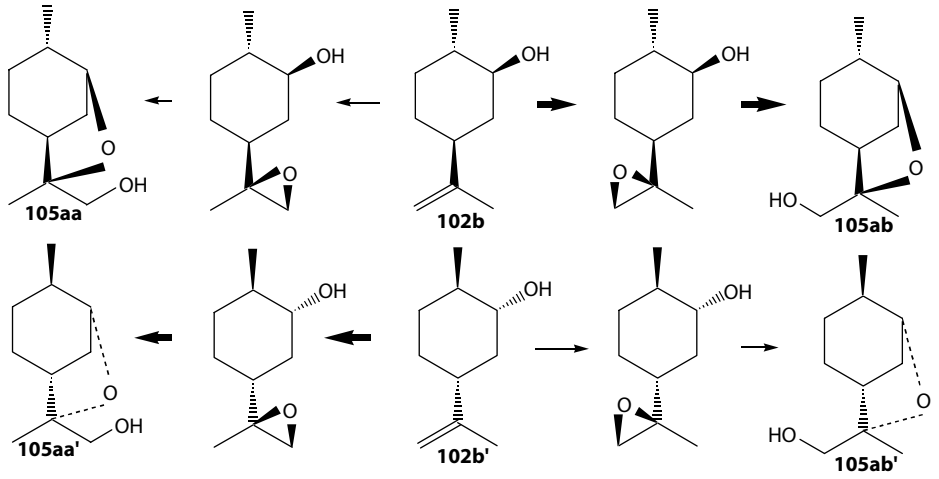


FIGURE 14.95 Biotransformation of (+)- (**102b**) and (–)-dihydrocarveol (**102b′**) by *Streptomyces bottropensis*, SY-2-1. (Modified from Noma, Y., 1984. *Kagaku to Seibutsu*, 22: 742–746.)

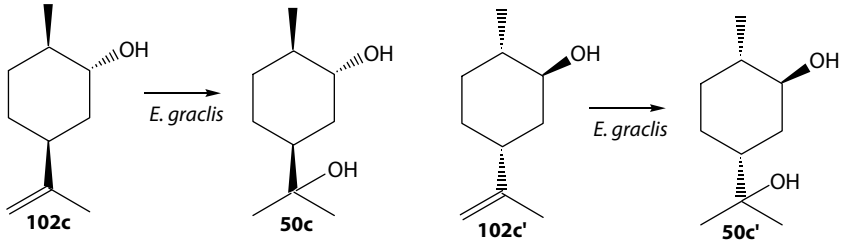


FIGURE 14.96 Biotransformation of (+)-iso- (**102c**) and (–)-dihydrocarveol (**102c′**) by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25.)

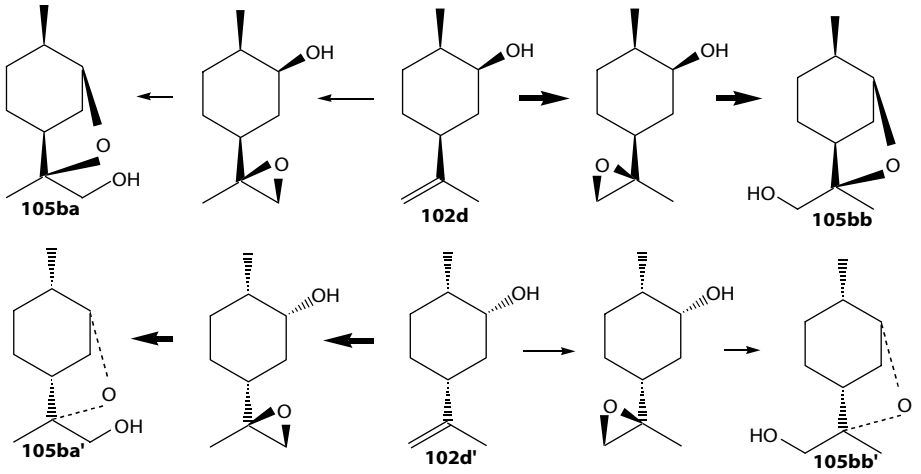


FIGURE 14.97 Formation of dihydroisobottrospicatols (**105**) from neoisodihydrocarveol (**102d** and **d′**) by *Streptomyces bottropensis*, SY-2-1. (Modified from Noma, Y., 1984. *Kagaku to Seibutsu*, 22: 742–746.)

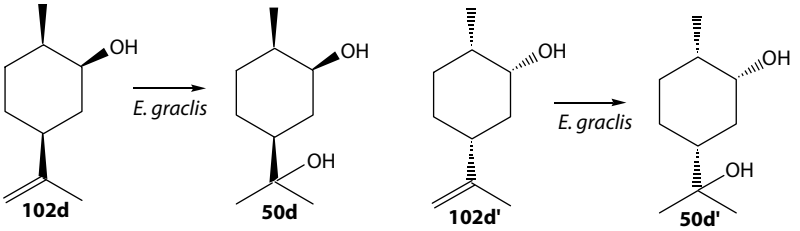
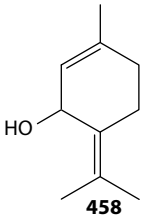


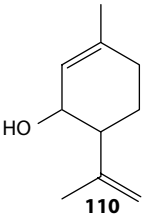
FIGURE 14.98 Biotransformation of (+)- (**102c**) and (−)-neoisodihydrocarveol (**102c′**) by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25.)

14.3.3.11 Piperitenol



Incubation of piperitenol (**458**) with *Aspergillus niger* gave a complex metabolites whose structures have not yet been determined (Noma, 2000).

14.3.3.12 Isopiperitenol



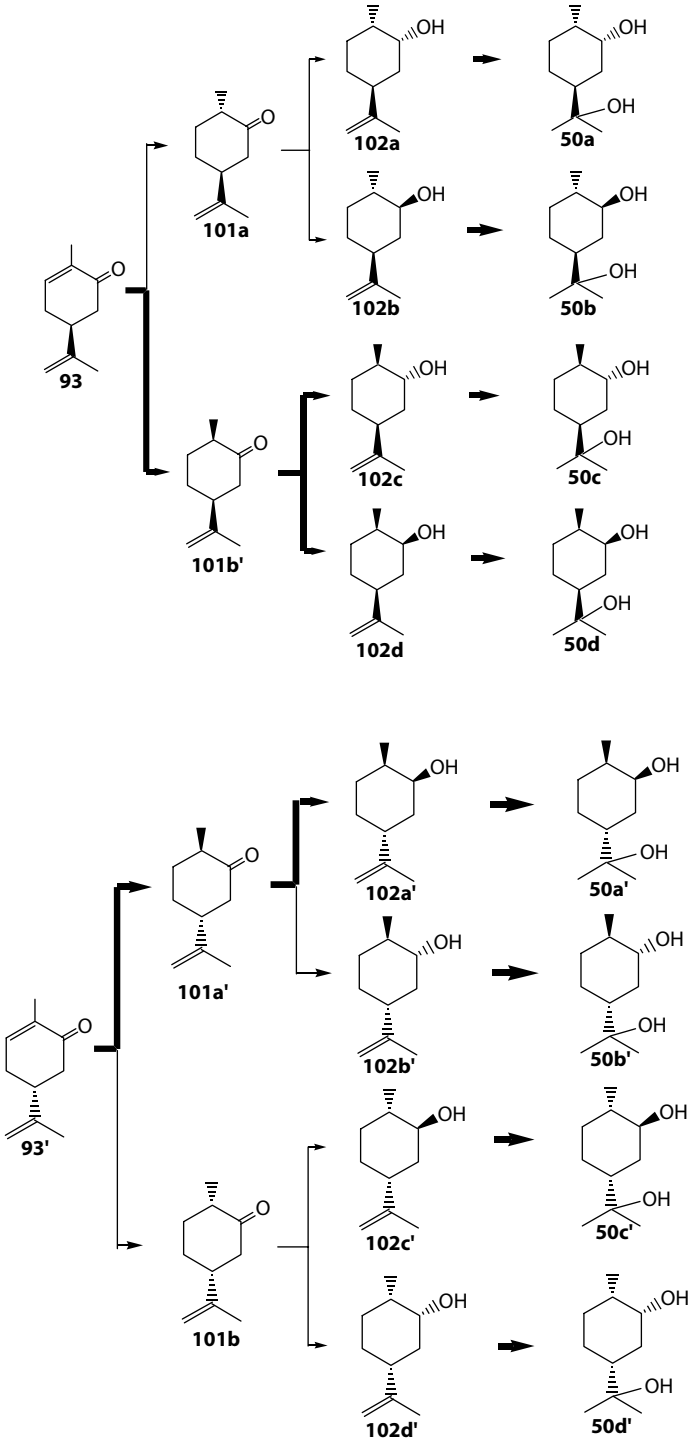
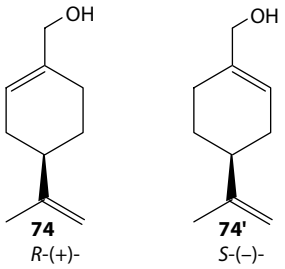


FIGURE 14.99 Formation of eight kinds of 8-hydroxydihydrocarveols (**50a**–**50d**, **50a′**–**50d′**), dihydrocarvones (**101a**–**101b** and **101a′**–**101b′**), and dihydrocarveols (**102a**–**102d** and **102a′**–**102d′**) from (+)- (**93**) and (–)-carvone (**93′**) by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25.)

Piperitenol (**458**) was metabolized by *Aspergillus niger* to give a complex alcohol mixtures whose structures have not yet been determined (Noma, 2000).

14.3.3.13 Perillyl Alcohol



(–)-Perillyl alcohol (**74′**) was epoxidized by *Streptomyces ikutamanensis* Ya-2-1 to give 8,9-epoxy-(–)-perillyl alcohol (**77′**) (Noma et al., 1986) (Figure 14.100).

(–)-Perillyl alcohol (**74′**) was glycosylated by *Eucalyptus perriniana* suspension cells to (–)-perillyl alcohol monoglucoside (**376′**) and diglucoside (**377′**) (Hamada et al., 2002; Yonemoto et al., 2005) (Figure 14.101).

Furthermore, 1-perillly-β-glucopyranoside (**376**) was converted into the corresponding oligosaccharides (**377–381**) using a cyclodextrin glucanotransferase (Yonemoto et al., 2005) (Figure 14.102).

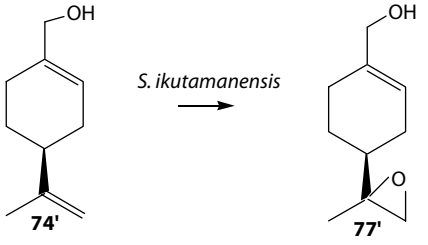


FIGURE 14.100 Biotransformation of (–)-perillyl alcohol (**74′**) by *Streptomyces ikutamanensis*, Ya-2-1. (Modified from Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206.)

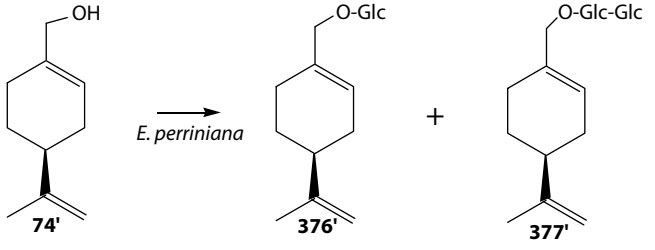


FIGURE 14.101 Biotransformation of (–)-perillyl alcohol (**74′**) by *Eucalyptus perriniana* suspension cell. (Modified from Hamada, H. et al., 2002. *Proc. 46th TEAC*, pp. 321–322; Yonemoto, N. et al., 2005. *Proc. 49th TEAC*, pp. 108–110.)

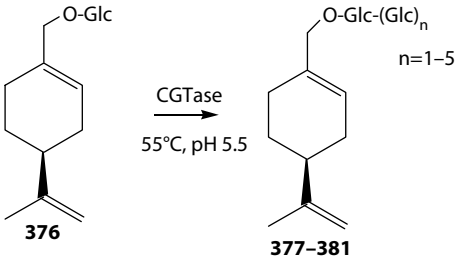
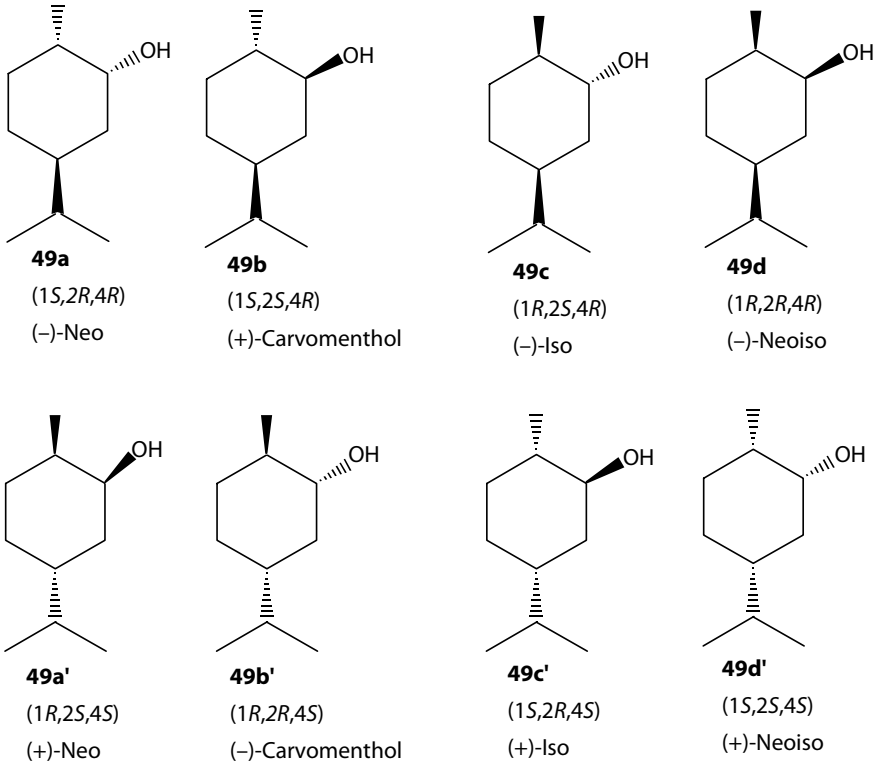


FIGURE 14.102 Biotransformation of (–)-perillyl alcohol monoglucoside (**376**) by CGTase. (Modified from Yonemoto, N. et al., 2005. *Proc. 49th TEAC*, pp. 108–110.)

14.3.3.14 Carvomenthol



(+)-Iso- (**49c**) and (+)-neoisocarvomenthol (**49d**) were formed from (+)-carvotanacetone (**47**) via (–)-isocarvomenthone (**48b**) by *Pseudomonas ovalis*, strain 6-1, whereas (+)-neocarvomenthol (**49a′**) and (–)-carvomenthol (**49b′**) were formed from (–)-carvotanacetone (**47′**) via (+)-carvomenthone (**48a′**) by the same bacteria; of which **48b**, **48a′**, and **49d** were the major products (Noma et al., 1974a) (Figure 14.103).

Microbial resolution of carvomenthols was carried out by selected microorganisms such as *Trichoderma S* and *Bacillus subtilis* var. *niger* (Oritani and Yamashita, 1973d). Racemic carvomenthyl acetate, racemic isocarvomenthyl acetate, and racemic neoisocarvomenthyl acetate were asymmetrically hydrolyzed to (–)-carvomenthol (**49b′**) with (+)-carvomenthyl acetate, (–)-isocarvomenthol (**49c**) with (+)-isocarvomenthyl acetate, and (+)-neoisocarvomenthol (**49d′**) with (–)-neoisocarvomenthyl acetate, respectively; racemic neocarvomenthyl acetate was not hydrolyzed (Oritani and Yamashita, 1973d) (Figure 14.104).

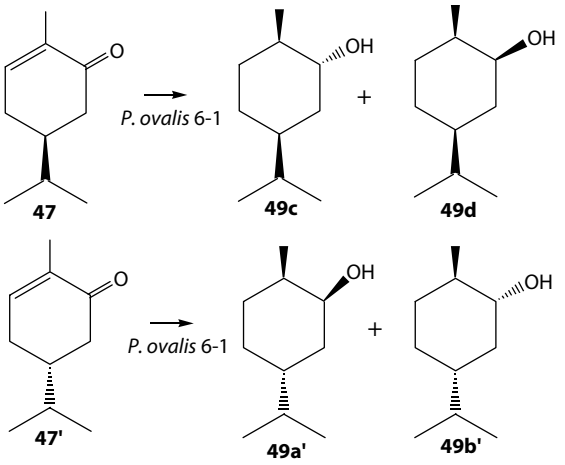


FIGURE 14.103 Formation of (–)-iso- (**49c**), (–)-neoiso- (**49d**), (+)-neo- (**49a′**), and (–)-carvomenthol (**49b′**) from (+)- (**47**) and (–)-carvotanacetone (**47′**) by *Pseudomonas ovalis*, strain 6-1. (Modified from Noma, Y. et al., 1974a. *Agric. Biol. Chem*., 38: 1637–1642.)

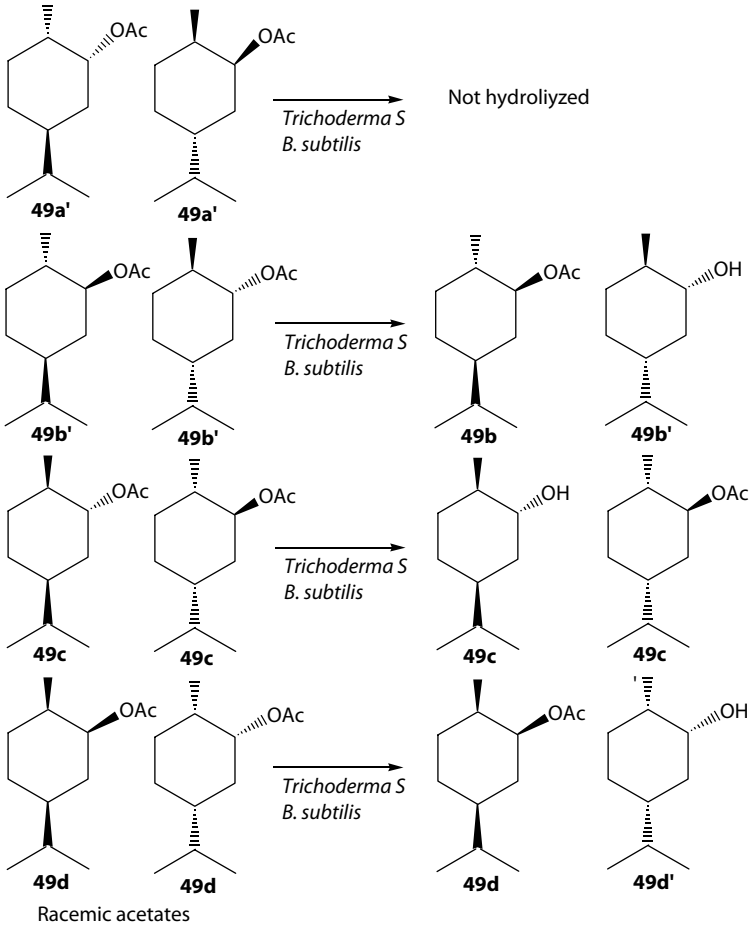
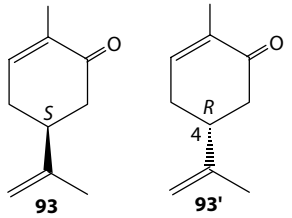


FIGURE 14.104 Microbial resolution of carvomenthols by *Trichoderma S* and *Bacillus. subtilis* var. *niger.* (Modified from Oritani, T. and K. Yamashita, 1973d. *Agric. Biol. Chem*., 37: 1691–1694.)

14.3.4 Monocyclic Monoterpene Ketone

14.3.4.1 α, β-Unsaturated Ketone

14.3.4.1.1 Carvone



93 (4*S*)-(+)-form

93' (4*R*)-(–)-form

Carvone occurs as (+)-carvone (**93**), (–)-carvone (**93′**), or racemic carvone. (*S*)-(+)-Carvone (**93**) is the main component of caraway oil (*ca*. 60%) and dill oil and has a herbaceous odour reminiscent of caraway and dill seeds. (*R*)-(–)-Carvone (**93′**) occurs in spearmint oil at a concentration of 70–80% and has a herbaceous odour similar to spearmint (Bauer et al., 1990).

The distribution of carvone convertible microorganisms is summarized in Table 14.5. When ethanol was used as a carbon source, 40% of bacteria converted (+)- (**93**) and (–)-carvone (**93'**). On the other hand, when glucose was used, 65% of bacteria converted carvone. In case of yeasts, 75% converted (+)- (**93**) and (–)-carvone (**93′**). Of fungi, 90% and 85% of fungi converted **93** and **93′**, respectively. In actinomycetes, 56% and 90% converted **93** and **93′**, respectively.

Many microorganisms except for some strains of actinomycetes were capable of hydrogenating the C=C double bond at C-1, 2 position of (+)- (**93**) and (–)-carvone (**93′**) to give mainly (–)-isodihydrocarvone (**101b**) and (+)-dihydrocarvone (**101a′**), respectively (Noma and Tatsumi, 1973; Noma et al., 1974b; Noma and Nonomura, 1974; Noma, 1976, 1977) (Figure 14.105) (Tables 14.6 and 14.7).

Furthermore, it was found that (–)-carvone (**93′**) was converted via (+)-isodihydrocarvone (**101b′**) to (+)-isodihydrocarveol (**102c′**) and (+)-neoisodihydrocarveol (**102d′**) by some strains of actinomycetes (Noma, 1979a, 1979b). (–)-Isodihydro- carvone (**101b**) was epimerized to (–)-dihydrocarvone (**101a**) after the formation of (–)-isodihydrocarvone (**101b**) from (+)-carvone (**93**) by the growing cells, the resting cells, and the cell-free extracts of *Pseudomonas fragi*, IFO 3458 (Noma et al., 1975).

TABLE 14.5
The Distribution of (+)- (93) and (–)-Carvone (93′) Convertible Microorganisms

Microorganisms	Number of Microorganisms Used	Numbers of Carvone Convertible Microorganisms	Ratio (%)
Bacteria	40	16 (Ethanol, **93**)	40
		16 (Ethanol, **93′**)	40
		26 (Glucose, **93**)	65
		26 (Glucose, **93′**)	65
Yeasts	68	51 (**93**)	75
		51 (**93′**)	75
Fungi	40	34 (**93**)	85
		36 (**93′**)	90
Actinomycetes	48	27 (**93**)	56
		43 (**93′**)	90

Source: Noma, Y. et al., 1993. Part VIII. *Proc. 37th TEAC*, pp. 23–25.

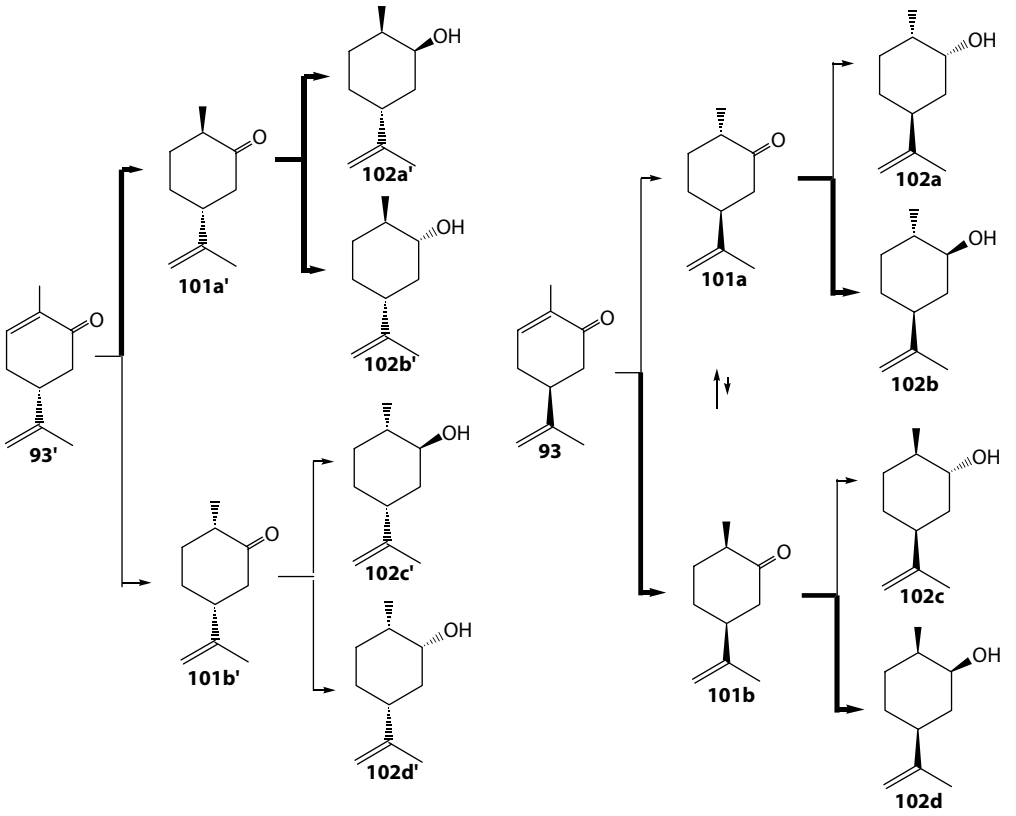


FIGURE 14.105 Biotransformation of (+)- (**93**) and (–)-carvone (**93′**) by various kinds of microorganisms. (Modified from Noma, Y. and C. Tatsumi, 1973. *Nippon Nogeikagaku Kaishi*, 47: 705–711; Noma, Y. et al., 1974b. *Agric. Biol. Chem*., 38: 735–740; Noma, Y. et al., 1974c. *Proc. 18th TEAC*, pp. 20–23; Noma, Y. and S. Nonomura, 1974. *Agric. Biol. Chem*., 38: 741–744; Noma, Y., 1976. *Ann. Res. Stud. Osaka Joshigakuen Junior College*, 20: 33–47; Noma, Y., 1977. *Nippon Nogeikagaku Kaishi*, 51: 463–470.)

Consequently, the metabolic pathways of carvone by microorganisms were summarized as the following eight groups (Figure 14.105).

Group 1. (–)-Carvone (**93′**)- (+)-dihydrocarvone (**101a′**)-(+)-neodihydrocarveol (**102a′**)
Group 2. **93′**–**101a′**-(–)-Dihydrocarveol (**102b′**)

TABLE 14.6
Ratio of Microorganisms that Carried Out the Hydrogenation of C=C Double Bond of Carvone by *Si* Plane Attack toward Microorganisms that Converted Carvone

Microorganisms	Ratio (%)
Bacteria	100[a]
	96[b]
Yeasts	74
Fungi	80
Actinomycetes	39

[a] When ethanol was used.
[b] When glucose was used.

Group 3. **93′**–**101a′**-**102a′** and **102b′**
Group 4. **93′**-(+)-Isodihydrocarvone (**101b′**)–**102c′** and **102d**'
Group 5. (+)-Carvone (**93**)-(−)-isodihydrocarvone (**101b**)-(−)-neoisodihydrocarveol (**102d**)
Group 6. **93**–**101b**–**102c**
Group 7. **93**–**101b**–**102c** and **102d**
Group 8. **93**–**101b**–**101a**

The result of the mode action of both the hydrogenation of carvone and the reduction for dihydrocarvone by microorganism is as follows. In bacteria, only two strains were able to convert (−)-carvone (**93′**) via (+)-dihydrocarvone (**101a′**) to (−)-dihydrocarveol (**102b′**) as the major product (Group 3, when ethanol was used as a carbon source, 12.5% of (−)-carvone (**93′**) convertible microorganisms belonged to this group and when glucose was used, 8% belonged to this group) (Noma and Tatsumi 1973; Noma et al., 1975), whereas when (+)-carvone (**93**) was converted, one strain converted it to a mixture of (−)-isodihydrocarveol (**102c**) and (−)-neoisodihydrocarveol (**102d**) (Group 7, 6% and 4% of **93** convertible bacteria belonged to this group, when ethanol and glucose were used, respectively.) and four strains converted it via (−)-isodihydrocarvone (**101b**) to (−)-dihydrocarvone (**101a**) (Group 8, 6% and 15% of (+)-carvone (**93′**) convertible bacteria belonged to this group, when ethanol and glucose were used, respectively.) (Noma et al., 1975). In yeasts, 43% of carvone convertible yeasts belong to group 1, 14% to group 2, and 33% to group 3 (of this group, three strains are close to group 1) and 12% to group 5, 4% to group 6, and 27% to group 7 (of this group, three strains are close to group 5 and one strain is close to group 6). In fungi, 51% of fungi metabolizing (−)-carvone (**93′**) by way of group 1 and 3% via group 3, but there was no strain capable of metabolizing (−)-carvone (**93′**) via group 2, whereas 20% of fungi metabolized (+)-carvone (**93**) via group 5 and 29% via group 7, but there was no strain capable of metabolizeing (+)-carvone (**93**) via group 6. In actinomycetes, (−)-carvone (**93′**) was converted to dihydrocarveols via group 1 (49%), group 2 (0%), group 3 (9%), and group 4 (28%), whereas (+)-carvone (**93**) was converted to dihydrocarveols via group 5 (7%), group 6 (0%), group 7 (19%), and group 8 (0%).

Furthermore, (+)-neodihydrocarveol (**102a′**) stereospecifically formed from (−)-carvone (**93′**) by *Aspergillus niger* TBUYN-2 was further biotransformed to mosquito repellent (1*R*,2*S*,4*R*)-(+)-*p*-menthane-2,8-diol (**50a′**), (1*R*,2*S*,4*R*)-(+)-8-*p*-menthene-2,10-diol (**107a′**), and the mixture of (1*R*,2*S*,4*R*,8*S/R*)-(+)-*p*-menthane-2,8,9-triols (**104aa′** and **104ab′**), while *Absidia glauca* ATCC 22752 gave **107a′** stereoselectively from **102a′** (Demirci et al., 2001) (Figure 14.106).

On the other hand, (−)-carvone (**93′**) was biotransformed stereoselectively to (+)-neodihydrocarveol (**102a′**) via (+)-dihydrocarvone (**101a′**) by a strain of *Aspergillus niger* (Noma and Nonomura 1974), *Euglena gracilis* Z. (Noma et al., 1993), and *Chlorella miniata* (Gondai et al., 1999). Furthermore, in *Euglena gracilis* Z., mosquito repellent (1*R*,2*S*,4*R*)-(+)-*p*-menthane-2,8-diol (**50a′**) was obtained stereospecifically from (−)-carvone (**93′**) via **101a′** and **102a′** (Figure 14.107).

As the microbial method for the formation of mosquito repellentl **50a′** was established, the production of (+)-dihydrocarvone (**101a′**) and (+)-neodihydrocarveol (**102a′**) as the precursor of mosquito repellent **50a′** was investigated by using 40 strains of bacteria belonging to *Escherichia*, *Aerobacter*, *Serratia*, *Proteus*, *Alcaligenes*, *Bacillus*, *Agrobacterium*, *Micrococcus*, *Staphylococcus*, *Corynebacterium*, *Sarcina*, *Arthrobacter*, *Brevibacterium*, *Pseudomonas*, and *Xanthomonas* spp., 68 strains of yeasts belonged to *Schzosaccharomyces*, *Endomycopsis*, *Saccharomyces*, *Schwanniomyces*, *Debaryomyces*, *Pichia*, *Hansenula*, *Lipomyces*, *Torulopsis*, *Saccharomycodes*, *Cryptococcus*, *Kloeckera*, *Trigonopsis*, *Rhodotorula*, *Candida*, and *Trichosporon* spp., 40 strains of fungi belonging to *Mucor*, *Absidia*, *Penicillium*, *Rhizopus*, *Aspergillus*, *Monascus*, *Fusarium*, *Pullularia*, *Keratinomyces*, *Oospora*, *Neurospora*, *Ustilago*, *Sporotrium*, *Trichoderma*, *Gliocladium,* and *Phytophythora* spp., and 48 strains of actinomycetes belonging to *Streptomyces*, *Actinoplanes*, *Nocardia*, *Micromonospora*, *Microbispora*, *Micropolyspora*, *Amorphosporangium*, *Thermopolyspora*, *Planomonospora*, and *Streptosporangium* spp.

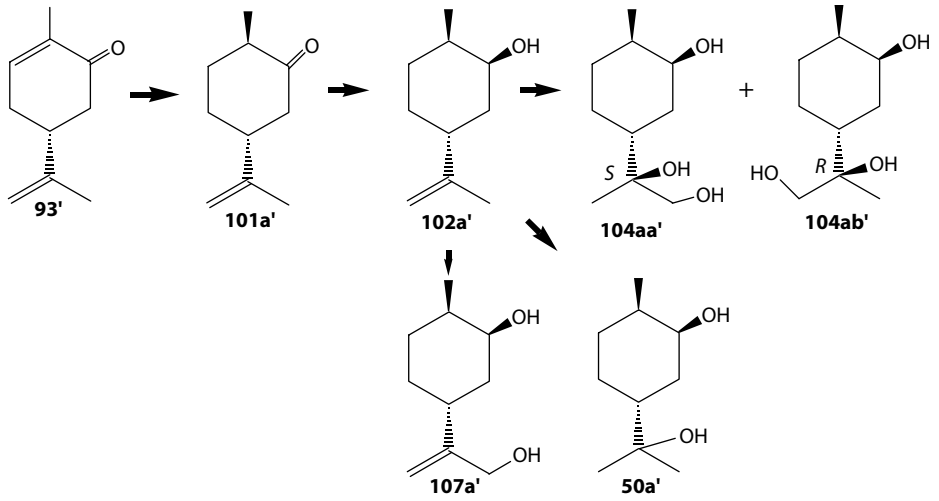


FIGURE 14.106 Metabolic pathways of (–)-carvone (**93′**) by *Aspergillus niger* TBUYN-2 and *Absidia glauca* ATCC 22752. (Modified from Demirci, F. et al., 2001. *XII Biotechnology Congr.*, Book of abstracts, p. 47.)

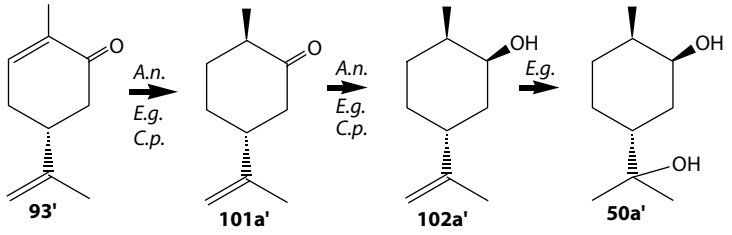


FIGURE 14.107 Metabolic pathway of (–)-carvone (**93′**) by *Aspergillus niger*, *Euglena gracilis* Z, and *Chlorella miniata*. (Modified from Noma, Y. and S. Nonomura, 1974. *Agric. Biol. Chem*., 38: 741–744; Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25; Gondai, T. et al., 1999. *Proc. 43rd TEAC*, pp. 217–219.)

As a result, 65% of bacteria, 75% of yeasts, 90% of fungi, and 90% of actinomycetes converted (–)-carvone (**93′**) to (+)-dihydrocarvone (**101a′**) or (+)-neodihydrocarveol (**102a′**) (Figure 14.105). Many microorganisms are capable of converting (–)-carvone (**93′**) to (+)-neodihydrocarveol (**102a′**) stereospecifically. Some of the useful microorganisms are listed in Tables 14.7 and 14.8. There is no good chemical method to obtain (+)-neodihydrocarveol (**102a′**) in large quantity. It was considered that the method utilizing microorganisms is a very useful means and better than the chemical synthesis for the production of mosquito repellent precursor (+)-neodihydrocarveol (**102a′**).

(–)-Carvone (**93**) was biotransformed by *Aspergillus niger* TBUYN-2 to give mainly (+)-8-hydroxyneodihydrocarveol (**50a′**), (+)-8,9-epoxyneodihydrocarveol (**103a′**), and (+)-10-hydroxyneodihydrocarveol (**107a′**) via (+)-dihydrocarvone (**101a′**) and (+)-neodihydrocarveol (**102a′**). *Aspergillus niger* TBUYN-2 dehydrogenated (+)-*cis*-carveol (**81b**) to give (+)-carvone (**93**), which was further converted to (–)-isodihydrocarvone (**101b**). Compound **101b** was further metabolized by four pathways to give 10-hydroxy- (–)-isodihydrocarvone (**106b**), (1*S*,2*S*,4*S*)-*p*-menthane-1,2-diol (**71d**) via 1α-hydroxy- (–)-isodihydrocarvone (**72b**) as intermediate, (–)-isodihydrocarveol (**102c**), and (–)-neoisodihydrocarveol (**102d**). Compound **102d** was further converted to isodihydroisobottrospicatol (**105bb**) via 8,9-epoxy-(–)-neoisodihydrocarveol (**103d**); Compound **105′** was a major product (Noma et al., 1985a) (Figure 14.109).

In case of the plant pathogenic fungus *Absidia glauca* (–)-carvone (**93′**) was metabolized to give the diol, 10-hydroxy-(+)-neodihydrocarveol (**107a′**) (Nishimura et al., 1983b).

(+)-Carvone (**93**) was converted by five bacteria and one fungus (Verstegen-Haaksma et al., 1995) to give (–)-dihydrocarvone (**101a**), (–)-isodihydrocarvone (**101b**), and (–)-neoisodihydrocarveol (**102d**).

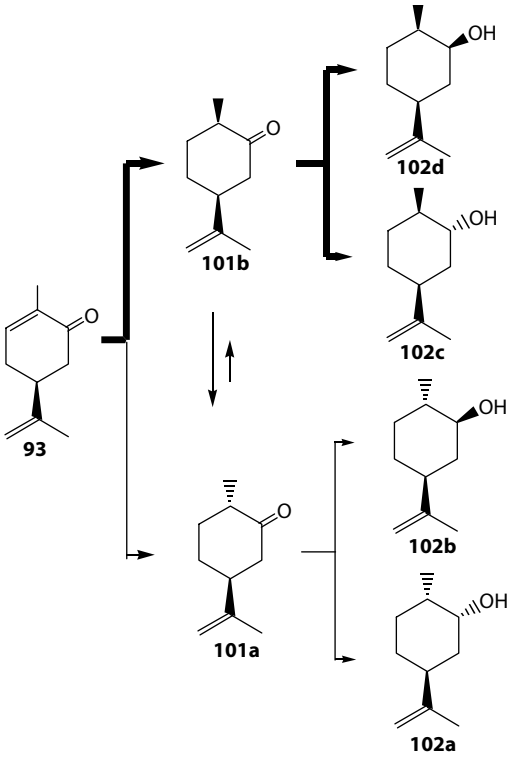


FIGURE 14.108 Metabolic pathways of (+)-carvone (**93**) by *Pseudomonas ovalis*, strain 6-1 and other many microorganisms. (Modified from Noma, Y. et al., 1974b. *Agric. Biol. Chem*., 38: 735–740.)

TABLE 14.7
Summary of Microbial and Chemical Hydrogenation of (–)-Carvone (93′) for the Formation of (+)-Dihdyrocarvone (101a′) and (+)-Isodihydrocarvone (101b′)

	Compounds	
Microorganisms	**101a′**	**101b′**
Amorphosporangium auranticolor	100	0
Microbiospora rosea IFO 3559	86	0
Bacillus subtilis var. *niger*	85	13
Bacillus subtilis IFO 3007	67	11
Pseudomonas polycolor IFO 3918	75	15
Pseudomonas graveolens IFO 3460	74	17
Arthrobacter pascens IFO 121139	73	12
Picha membranaefaciens IFO 0128	70	16
Saccharomyces ludwigii IFO 1043	69	18
Alcalygenes faecalis IAM B-141-1	70	13
Zn-25% KOH–EtOH	73	27
Raney-10% NaOH	71	19

Source: Noma, Y., 1976. *Ann. Res. Stud. Osaka Joshigakuen Junior College*, 20: 33–47.

TABLE 14.8
Summary of Microbial and Chemical Reduction of (–)-Carvone (93′) for the Formation of (+)-Neodihydrocarveol (102a′)

	Compounds					
Microorganisms	**101a′**	**101b′**	**102a′**	**102b′**	**102c′**	**102d′**
Torulopsis xylinus IFO 454	0	0	100	0	0	0
Monascus anka var. *rubellus* IFO 5965	0	0	100	0	0	0
Fusarium anguioides Sherbakoff IFO 4467	0	0	100	0	0	0
Phytophthora infestans IFO4872	0	0	100	0	0	0
Kloeckera magna IFO 0868	0	0	98	2	0	0
Kloeckera antillarum IFO 0669	19	4	72	0	0	0
Streptomyces rimosus	+	0	98	0	0	0
Penicillium notatum Westling IFO 464	6	2	92	0	0	0
Candida pseudotropicalis IFO 0882	17	4	79	0	0	0
Candida parapsilosis IFO 0585	16	4	80	0	0	0
$LiAlH_4$	0	0	17	67	2	13
Meerwein–Ponndorf–Verley reduction	0	0	29	55	9	5

Source: Noma, Y., 1976. *Ann. Res. Stud. Osaka Joshigakuen Junior College*, 20: 33–47.

Sensitivity of the microorganism to (+)-carvone (**93**) and some of the products prevented yields exceeding 0.35 g/L in batch cultures. The fungus *Trychoderma pseudokoningii* gave the highest yield of (–)-neoisodihydrocarveol (**102d**) (Figure 14.110). (+)-Carvone (**93**) is known to inhibit fungal growth of *Fusarium sulphureum* when it was administered via the gas phase (Oosterhaven et al., 1995a, 1995b). Under the same conditions, the related fungus, *Fusarium solani* var. *coeruleum* was not inhibited. In liquid medium, both fungi were found to convert (+)-carvone (**93**), with the same rate, mainly to (–)-isodihydrocarvone (**101b**), (–)-isodihydrocarveol (**102c**), and (–)-neoisodihydrocarveol (**102d**).

14.3.4.1.1.1 Biotransformation of Carvone to Carveols by Actinomycetes The distribution of actinomycetes capable of reducing carbonyl group of carvone containing α, β-unsaturated ketone to (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) was investigated. Of 93 strains of actinomycetes, 63 strains were capable of converting (–)-carvone (**93′**) to carveols. The percentage of microorganisms that produced carveols from (–)-carvone (**93′**) to total microorganisms was about 71%. Microorganisms that produced carveols were classified into three groups according to the formation of (–)-*trans*-carveol (**81a′**) and (–)-*cis*-carveol (**81b′**): group 1, (–)-carvone-**81b′** only; group 2, (–)-carvone-**81a′** only; and group 3, (–)-carvone-mixture of **81a′** and **81b′**. Three strains belonged to group 1 (4.5%), 34 strains belonged to group 2 (51.1%), and 29 strains belonged to group 3 (44%; of this group two strains were close to group 1 and 14 strains were close to group 2).

Streptomyces, A-5-1 isolated from soil converted (–)-carvone (**93′**) to **101a′**–**102d′** and (–)-*trans*-carveol (**81a′**), whereas *Nocardia*, 1-3-11 converted (–)-carvone (**93′**) to (–)-*cis*-carveol (**81b′**) together with **101a′**–**81a′** (Noma, 1980). In case of *Nocardia*, the reaction between **93′** and **81a′** was reversible and the direction from **81a′** to **93′** is predominantly (Noma, 1979a, 1979b; 1980) (Figure 14.111).

(–)-Carvone (**93′**) was metabolized by actinomycetes to give (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) and (+)-dihydrocarvone (**101a′**) as reduced metabolites. Compound **81b′** was further metabolized to (+)-bottrospicatol (**92a′**). Furthermore, **93′** was hydroxylated at C-5 position and C-8, 9 position to give 5β-hydroxy-(–)-carvone (**98a′**) and (–)-carvone-8,9-epoxide (**96′**), respectively. Compound **98a′** was further metabolized to 5β-hydroxyneodihydrocarveol (**100aa′**) via 5β-hydroxy-dihydrocarvone (**99a′**) (Noma, 1979a, 1979b; 1980) (Figure 14.111).

Metabolic pattern of (+)-carvone (**93**) is similar to that of (–)-carvone (**93′**) in *Streptomyces bottropensis*. (+)-Carvone (**93**) was converted by *Streptomyces bottropensis* to give (+)-carvone-8,9-epoxide

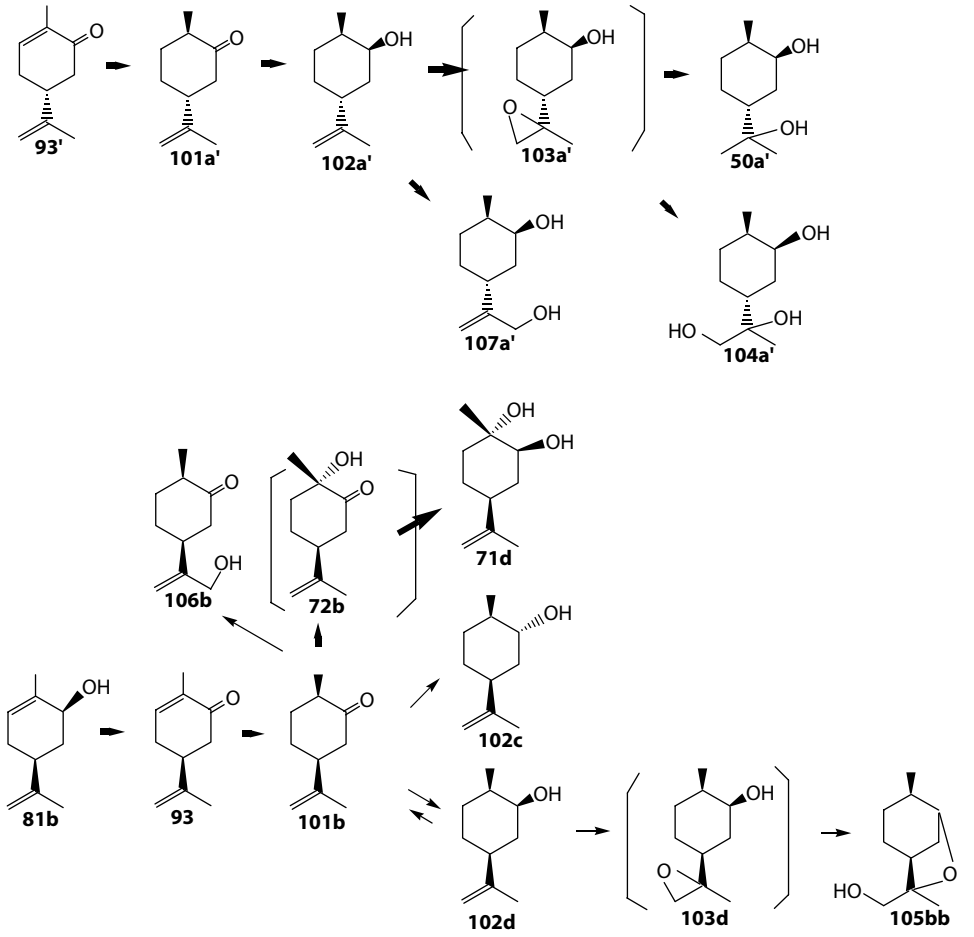


FIGURE 14.109 Possible main metabolic pathways of (–)-carvone (**93′**) and (+)-carvone (**93**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 1985a. *Annual Meeting of Agricultural and Biological Chemistry*, Sapporo, p. 68.)

(**96**) and (+)-5α-hydroxycarvone (**98a**) (Figure 14.112). (+)-Carvone-8,9-epoxide (**96**) has light sweet aroma and has strong inhibitory activity for the germination of lettuce seeds (Noma and Nishimura, 1982).

The investigation of (–)-carvone (**93′**) and (+)-carvone (**93**) conversion pattern was carried out by using rare actinomycetes. The conversion pattern was classified as follows (Figure 14.113):

Group 1. Carvone (**93**)–dihydrocarvones (**101**)–dihydrocarveol (**102**)–dihydrocarveol-8,9-epoxide (**103**)–dihydrobottrospicatols (**105**)–5-hydroxydihydrocarveols (**100**)
Group 2. Carvone (**93**)–carveols (**89**)–bottrospicatols (**92**)–5-hydroxy-*cis*-carveols (**12**)

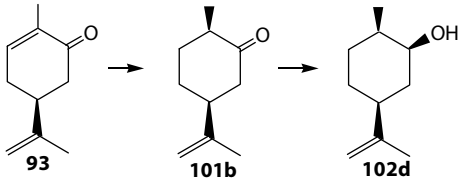


FIGURE 14.110 Biotransformation of (+)-carvone (**93**) by *Trychoderma pseudokoningii*. (Modified from Verstegen-Haaksma, A.A. et al., 1995. *Ind. Crops Prod.*, 4: 15–21.)

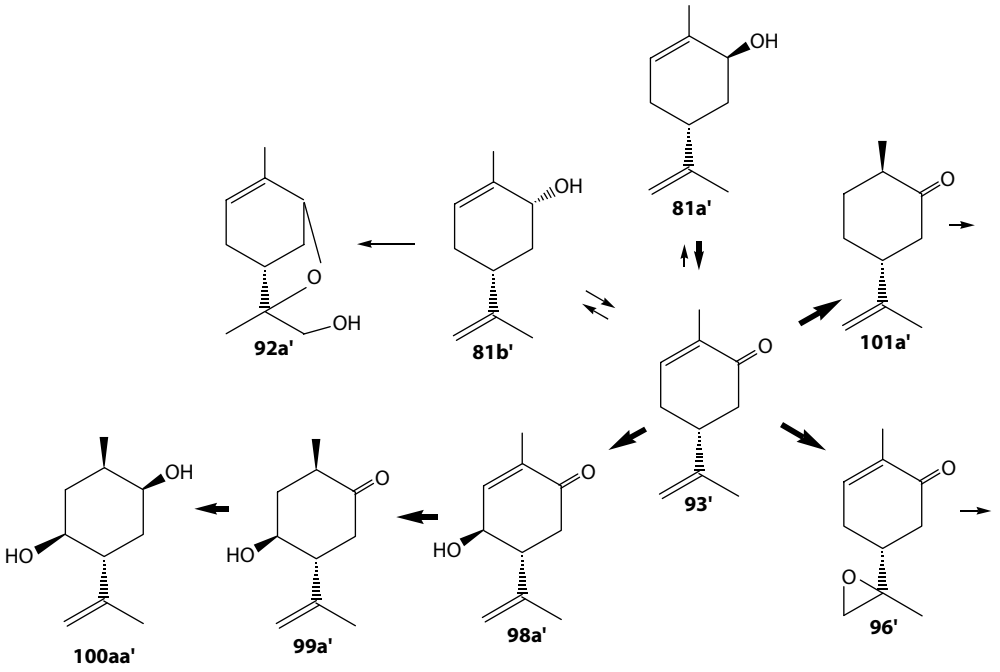


FIGURE 14.111 Metabolic pathways of (–)-carvone (**93′**) by *Streptomyces bottropensis* SY-2-1**,** *Streptomyces ikutamanensis* Ya-2-1, *Streptomyces*, A-5-1, and *Nocardia*, 1-3-11. (Modified from Noma, Y., 1979a. *Nippon Nogeikagaku Kaishi*, 53: 35–39; Noma, Y., 1979b. *Ann. Res. Stud. Osaka Joshigakuen Junior College*, 23: 27–31; Noma, Y., 1980. *Agric. Biol. Chem*., 44: 807–812; Noma, Y. and H. Nishimura, 1983a. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 390; Noma, Y. and H. Nishimura, 1983b. *Proc. 27th TEAC*, pp. 302–305.)

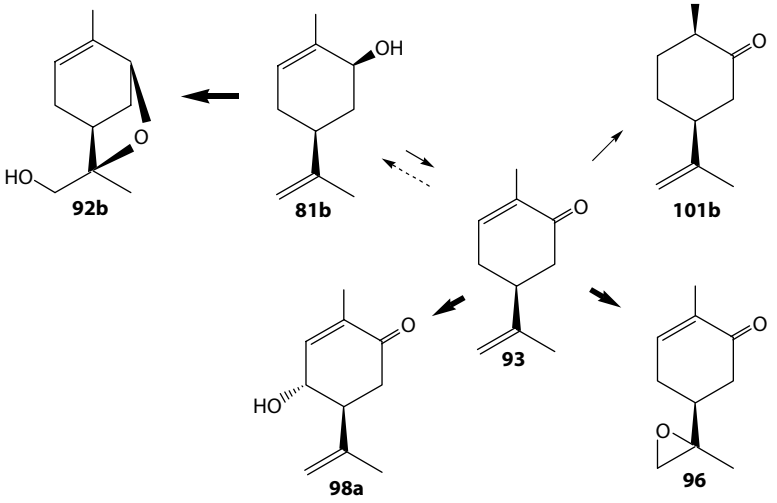


FIGURE 14.112 Metabolic pathways of (+)-carvone (**93′**) by *Streptomyces bottropensis* SY-2-1 and *Streptomyces ikutamanensis* Ya-2-1. (Modified from Noma, Y. and H. Nishimura, 1982. *Proc. 26th TEAC*, pp. 156–159l; Noma, Y. and H. Nishimura, 1983a. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 390; Noma, Y. and H. Nishimura, 1983b. *Proc. 27th TEAC*, pp. 302–305; Noma, Y., 1984. *Kagaku to Seibutsu*, 22: 742–746.)

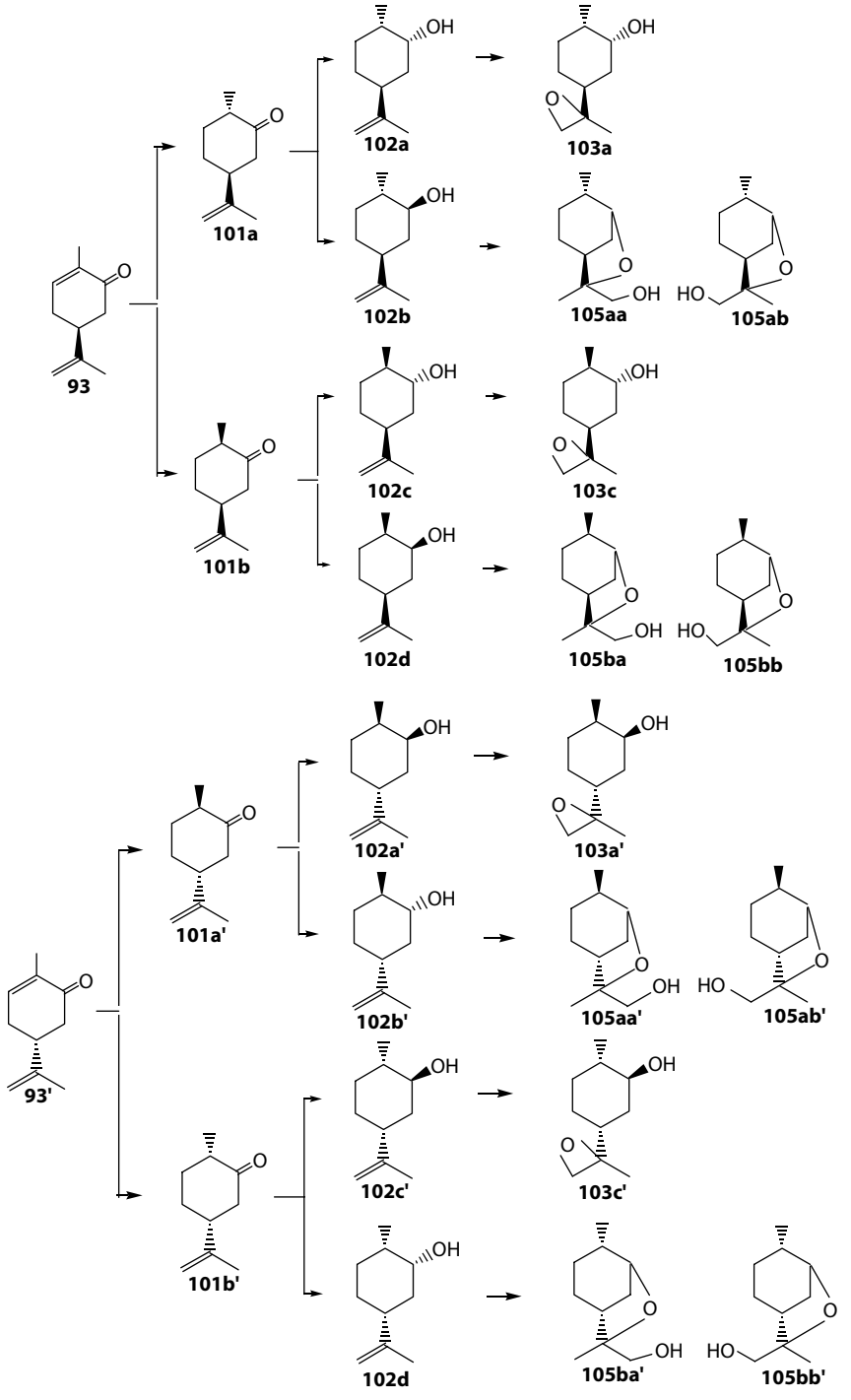


FIGURE 14.113 Metabolic pathways of (+)- (**93**) and (–)-carvone (**93′**) and dihydrocarveols (**102a-d** and **102a′-d′**) by *Streptomyces bottropensis*, SY-2-1 and *Streptomyces ikutamanesi*s, Ya-2-1. (Modified from Noma, Y., 1984. *Kagaku to Seibutsu*, 22: 742–746.)

Group 3. Carvone (**93**)–5-hydroxycarvone (**98**)–5-hydroxyneodihydrocarveols (**15**)
Group 4. Carvone (**93**)–carvone-8,9-epoxides (**96**).

Of 50 rare actinomycets, 22 strains (44%) were capable of converting (–)-carvone (**93′**) to give (–)-carvone-8,9-epoxide (**96′**) via pathway 4 and (+)-5β-hydroxycarvone (**98a′**), (+)-5α-hydroxycarvone (**98b′**), and (+)-5β-hydroxyneodihydrocarveol (**100aa′**) via pathway 3 (Noma and Sakai, 1984).

On the other hand, in case of (+)-carvone (**93**) conversion, 44% of rare actinomycetes were capable of converting (+)-carvone (**93**) to give (+)-carvone-8,9-epoxide (**96**) via pathway 4 and (–)-5α-hydroxycarvone (**98a**), (–)-5β-hydroxycarvone (**98b**), and (–)-5α-hydroxyneodihydrocarveol (**100aa**) via pathway 3 (Noma and Sakai, 1984).

14.3.4.1.1.2 Biotransformation of Carvone by Citrus Pathogenic Fungi, Aspergillus niger Tiegh TBUYN *Citrus* pathogenic *Aspergillus niger* Tiegh (CBAYN) and *Aspergillus niger* TBUYN-2 hydrogenated C=C double bond at C-1, 2 position of (+)-carvone (**93**) to give (–)-isodihydrocarvone (**101b**) as the major product together with a small amount of (–)-dihydrocarvone (**101a**), of which **101b** was further metabolized through two kinds of pathways as follows; namely one is the pathway to give (+)-1α-hydroxyneoisodihydrocarveol (**71**) via (+)-1α-hydroxyisodihydrocarvone (**72**) and the other one is the pathway to give (+)-4α-hydroxy-isodihydrocarvone (**378**) (Noma and Asakawa, 2008) (Figure 14.114).

The biotransformation of enones such as (–)-carvone (**93′**) by the cultured cells of *Chlorella miniata* was examined. It was found that the cells reduced stereoselectively the enones from *si*-face at α-position of the carbonyl group and then the carbonyl group from *re*-face (Figure 14.115).

Stereospecific hydrogenation occurs independent of the configuration and the kinds of the substituent at C-4 position, so that the methyl group at C-1 position is fixed mainly at *R* configuration. [2-^{2}H]- (–)-Carvone ([2-^{2}H]-**93′**) was synthesized in order to clear up the hydrogenation mechanism at C-2 by microorganisms. (Compound [2-^{2}H]-**93** was also easily biotransformed to [2-^{2}H]-8-hydroxy-(+)-neodihydro-carveol (**50a′**) via [2-^{2}H]-(+)-neodihydrocarveol (**102a′**). On the basis of ^{1}H-NMR spectral data of compounds **102a′** and **50a′**, the hydrogen addition of the carbon–carbon double bond at the C_1 and C_2 position by *Aspergillus niger* TBUYN-2, *Euglena gracilis* Z., and *Dunaliella tertiolecta* occurs from the *si* face and *re* face, respectively, namely, *anti* addition (Noma et al., 1995) (Figure 14.115) (Table 14.9).

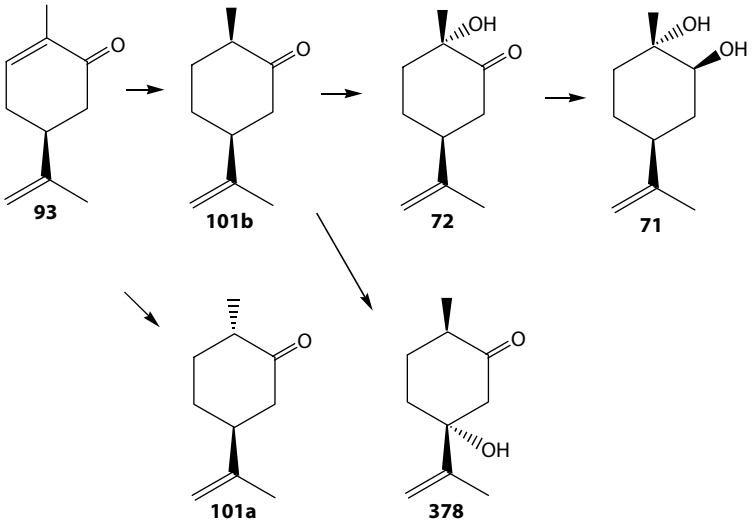


FIGURE 14.114 Metabolic pathways of (+)-carvone (**93**) by *Citrus* pathogenic fungi, *Aspergillus niger* Tiegh CBAYN and *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2008. *Proc. 52nd TEAC*, pp. 206–208.)

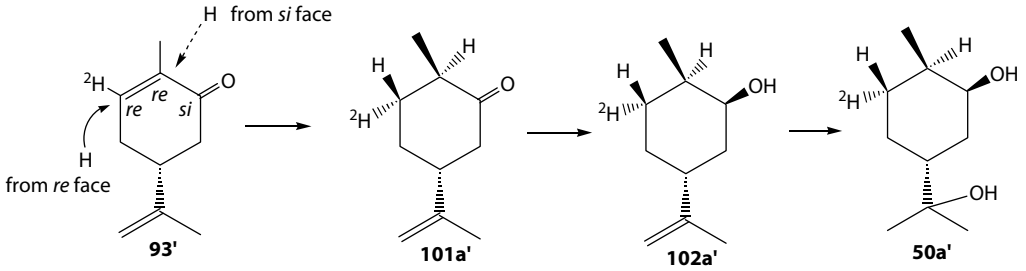


FIGURE 14.115 The stereospecific hydrogenation of the C=C double bond of α, β-unsaturated ketones, the reduction of saturated ketone, and the hydroxylation by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1995. *Proc. 39th TEAC*, pp. 367–368; Noma, Y. and Y. Asakawa, 1998. *Biotechnology in Agriculture and Forestry, Vol. 41. Medicinal and Aromatic Plants X*, Y.P.S. Bajaj, ed., pp. 194–237. Berlin Heidelberg: Springer.)

14.3.4.1.1.3 Hydrogenation Mechanisms of C=C Double Bond and Carbonyl Group In order to understand the mechanism of the hydrogenation of α-, β-unsaturated ketone of (–)-carvone (**93′**) and the reduction of carbonyl group of dihydrocarvone (**101a′**) (–)-carvone (**93′**), (+)-dihydrocarvone (**101a′**) and the analogues of (–)-carvone (**93′**) were chosen and the conversion of the analogues was carried out by using *Pseudomonas ovalis*, strain 6-1. As the analogues of carvone (**93** and **93′**), (–)- (**47′**) and (+)-carvotanacetone (**47**), 2-methyl-2-cyclohexenone (**379**), the mixture of (–)-*cis*- (**81b′**) and (–)-*trans*-carveol (**81a′**), 2-cyclohexenone, racemic menthenone (**148**), (–)-piperitone (**156**), (+)-pulegone (**119**), and 3-methyl-2-cyclohexenone (**381**) were chosen. Of these analogues, (–)- (**47′**) and (+)-carvotanacetone (**47**) were reduced to give (+)-carvomenthone (**48a′**) and (–)-isocarvomenthone (**48b′**), respectively. 2-Methyl-2-cyclohexenone (**379**) was mainly reduced to (–)-2-methylcyclohexanone. But other compounds were not reduced.

The efficient formation of (+)-dihydrocarvone (**101a**), (–)-isodihydrocarvone (**101b′**), (+)-carvomenthone (**48a**), (–)-isocarvomenthone (**48b′**), and (–)-2-methylcyclohexanone from (–)-carvone (**93**), (+)-carvone (**93′**), (–)-carvotanacetone (**47**), (+)-carvotanacetone (**47′**), and 2-methyl-2-cyclohexenone (**379**) suggested at least that C=C double bond conjugated with carbonyl group may be hydrogenated from behind (*si* plane) (Noma, 1977; Noma et al., 1974b) (Figure 14.116).

14.3.4.1.1.4 What is Hydrogen Honor in the Hydrogenation of Carvone to Dihydrocarvone? What is Hydrogen Donor in Carvone Reductase? Carvone reductase prepared from *Euglena gracilis* Z, which catalyzes the NADH-dependent reduction of the C=C bond adjacent to the carbonyl group, was characterized with regard to the stereochemistry of the hydrogen transfer into the substrate. The reductase was isolated from *Euglena gracilis* Z and was found to reduce stereospecifically the C=C double bond of carvone by *anti*-addition of hydrogen from the *si* face at α-position to the carbonyl group and the *re* face at β-position (Table 14.9). The hydrogen atoms participating in the enzymatic reduction at α- and β-position to the carbonyl group originate from the medium and the *pro-4R* hydrogen of NADH, respectively (Shimoda et al., 1998) (Figure 14.117).

TABLE 14.9
The Summary for the Stereospecificity of the Reduction of the C=C Double Bond of [2-^{2}H]-(–)-Carvone ([2-^{2}H]-93) by Various Kinds of Microorganisms

	Stereochemistry at C-2H of Compounds	
Microorganisms	**102a**	**50a**
Aspergillus niger TBUYN-2	β	
Euglena gracilis Z	β	β
Dunaliella tertiolecta	β	
The cultured cells of *Nicotiana tabacum* (Suga et al., 1986)	β	

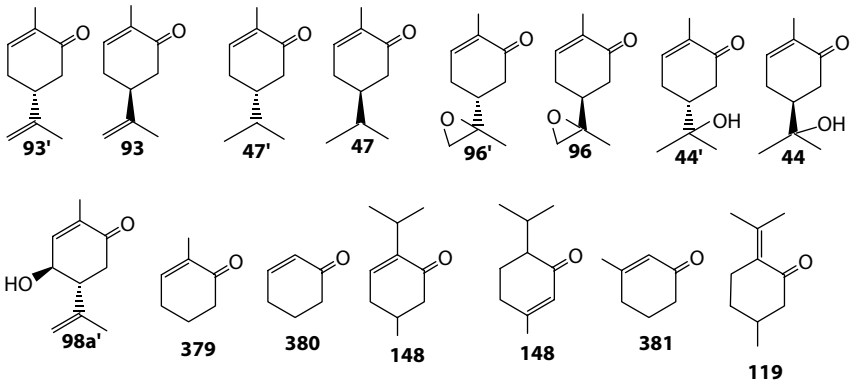


FIGURE 14.116 Substrates used for the hydrogenation of C=C double bond with *Pseudomonas ovalis*, strain 6-1, *Streptomyces bottropensis* SY-2-1, *Streptomyces ikutamanensis* Ya-2-1, and *Euglena gracilis* Z.

TABLE 14.10
Purification of the Reductase from *Euglena gracilis* Z.

	Total Protein (mg)	Total Activity Unit × 10^4	Sp. Act Units per Gram Protein	Fold
Crude extract	125	2.2	1.7	1
DEAE Toyopearl	7	1.5	21	12
AF-Blue Toyopearl	0.1	0.03	30	18

In the case of biotransformation by using *Cyanobacterium* (+)- (**93**) and (–)-carvone (**93′**) were converted with a different type of pattern to give (+)-isodihydrocarvone (**101b′**, 76.6%) and (–)-dihydrocarvone (**101a**, 62.2%), respectively (Kaji et al., 2002) (Figure 14.118). On the other hand, *Catarantus rosea* cultured cell biotransformed (–)-carvone (**93′**) to give 5β-hydroxy- (+)-neodihydrocarveol (**100aa′**, 57.5%), 5α-hydroxy-(+)-neodihydrocarveol (**100ab′**, 18.4%), 5α-hydroxy-(–)-carvone (**98b′**), 4β-hydroxy-(–)-carvone (**384′**, 6.3%), 10-hydroxycarvone (**390′**), 5β-hydroxycarvone (**98′**), 5α-hydroxyneodihydrocarveol (**100ab′**), 5β-hydroxyneodihydrocarveol (**100aa′**), and 5α-hydroxydihydrocarvone (**99b′**) as the metabolites as shown in Figure 14.119, whereas (+)-carvone (**93**) gave 5α-hydroxy-(+)-carvone (**98a**, 65.4%) and 4α-hydroxy-(+)-carvone (**384**, 34.6%) (Hamada and Yasumune, 1995; Hamada et al., 1996; Kaji et al., 2002) (Figure 14.119) (Table 14.11).

(–)-Carvone (**93′**) was incubated with *Cyanobacterium*, enone reductase (43 kDa) isolated from the bacterium and microsomal enzyme to afford (+)-isodihydrocarvone (**101b′**) and (+)-dihydrocarvone

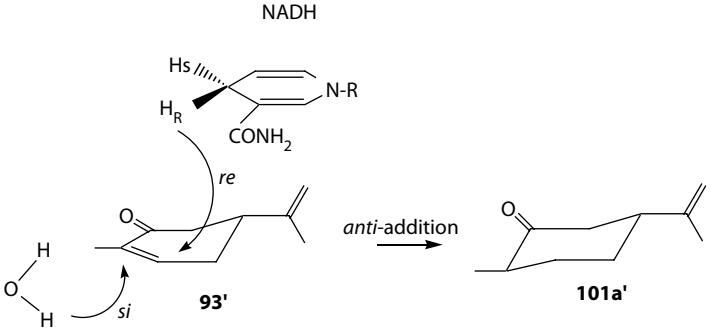


FIGURE 14.117 Stereochemistry in the reduction of (–)-carvone (**93′**) by the reductase from *Euglena gracilis* Z. (Modified from Shimoda, K. et al., 1998. *Phytochem.*, 49: 49–53.)

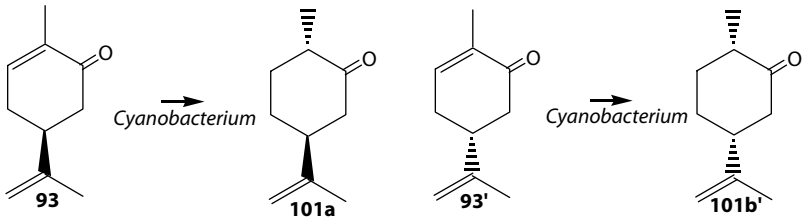


FIGURE 14.118 Biotransformation of (–)- and (+)-carvone (**93** and **93′**) by *Cyanobacterium*. (Modified from Kaji, M. et al., 2002. *Proc. 46th TEAC*, pp. 323–325.)

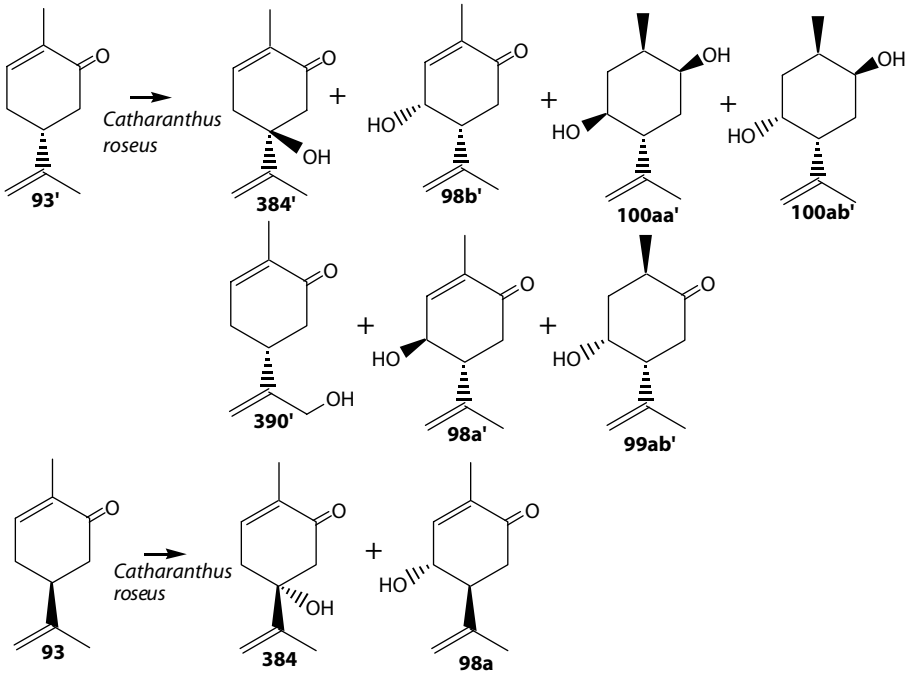


FIGURE 14.119 Biotransformation of (+)- and (–)-carvone (**93** and **93′**) by *Cataranthus roseus*. (Modified from Hamada, H. and H. Yasumune, 1995. *Proc. 39th TEAC*, pp. 375–377; Hamada, H. et al., 1996. *Proc. 40th TEAC*, pp. 111–112; Kaji, M. et al., 2002. *Proc. 46th TEAC*, pp. 323–325.)

(**101a′**). Cyclohexenone derivatives (**379** are **385**) were treated in the same enone reductase with microsomal enzyme to give the dihydro derivative (**382a**, **386a**) with *R*-configuration in excellent *ee* (over 99%) and the metabolites (**382b**, **386b**) with *S*-configuration in relatively high *ee* (85% and 80%) (Shimoda et al., 2003) (Figure 14.120).

TABLE 14.11
Enantioselectivity in the Reduction of Enones (379 and 385) by Enone Reductase

Microsomal Enzyme	Substrate	Product	ee	Configuration[a]
–	**379**	**382a**	>99	R
–	**385**	**386a**	>99	R
+	**379**	**382b**	85	S
+	**385**	**386b**	80	S

[a] Preferred configuration at α-position to the carbonyl group of the products.

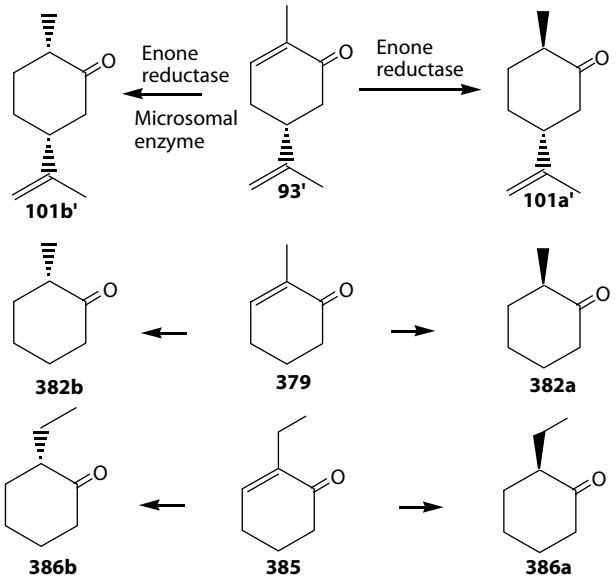
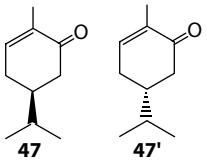


FIGURE 14.120 Biotransformation of 2-methyl-2-cyclohexenone (**379**) and 2-ethyl-2-cyclohexenone (**385**) by enone reductase.

In contrast, almost all the yeasts tested showed reduction of carvone, although the enzyme activity varied. The reduction of (–)-carvone (**93′**) was often much faster than the reduction of (+)-carvone (**93**). Some yeasts only reduced the carbon–carbon double bond to yield the dihydrocarvone isomers (**101a′** and **b′** and **101a** and **b**) with the stereochemistry at C-1 with *R* configuration, while others also reduced the ketone to give the dihydrocarveols with the stereochemistry at C-2 always with *S* for (–)-carvone (**93′**), but sometimes *S* and sometimes *R* for (+)-carvone (**93**). In the case of (–)-carvone (**93′**) yields increased up to 90% within 2 h (van Dyk et al., 1998).

14.3.4.1.2 Carvotanacetone



In the conversion of (+)- (**47**) and (–)-carvotanacetone (**47′**) by *Pseudomonas ovalis*, strain 6-1, (–)-carvotanacetone (**47′**) is converted stereospecifically to (+)-carvomenthone (**48a′**) an the latter compound is further converted to (+)-neocarvomenthol (**49a′**) and (–)-carvomenthol (**49b′**) in small amounts, whereas (+)-carvotanacetone (**47**) is converted mainly to (–)-isocarvomenthone (**48b**) and (–)-neoisocarvomenthol (**49d**), forming (–)-carvomenthone (**48a**) and (–)-isocarvomenthol (**49c**) in small amounts as shown in Figure 14.121 (Noma et al., 1974a).

Biotransformation of (–)-carvotanacetone (**47**) and (+)-carvotanacetone (**47′**) by *Streptomyces bottropensis*, SY-2-1 was carried out (Noma et al., 1985c).

As shown in Figure 14.122, (+)-carvotanacetone (**47**) was converted by *Streptomyces bottropensis,* SY-2-1 to give 5β-hydroxy-(+)-neoisocarvomenthol (**139db**), 5α-hydroxy-(+)-carvotanacetone (**51a**), 5β-hydroxy- (–)-carvomenthone (**52ab**), 8-hydroxy-(+)-carvotanacetone (**44**), and 8-hydroxy-(–)-carvomenthone (**45a**), whereas (–)-carvotanacetone (**47′**) was converted to give 5β-hydroxy-(–)-carvotanacetone (**51a′**) and 8-hydroxy-(–)-carvotanacetone (**44′**).

Aspergillus niger TBUYN-2 converted (–)-carvotanacetone (**47′**) to (+)-carvomenthone (**48a′**), (+)-carvomenthone (**49a′**), diastereoisomeric *p*-menthane-2,9-diols [**55aa′** (8*R*) and **55ab′** (8*S*) in the

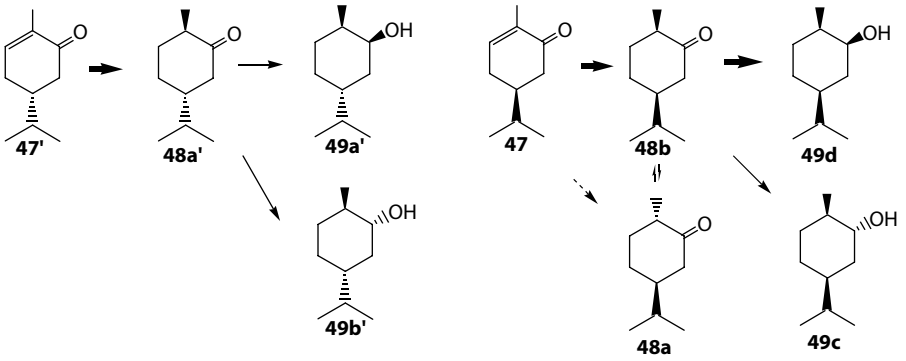


FIGURE 14.121 Metabolic pathways of (–)-carvotanacetone (**47′**) and (+)-carvotanacetone (**47**) by *Pseudomonas ovalis*, strain 6-1. (Modified from Noma, Y. et al., 1974a. *Agric. Biol. Chem*., 38: 1637–1642.)

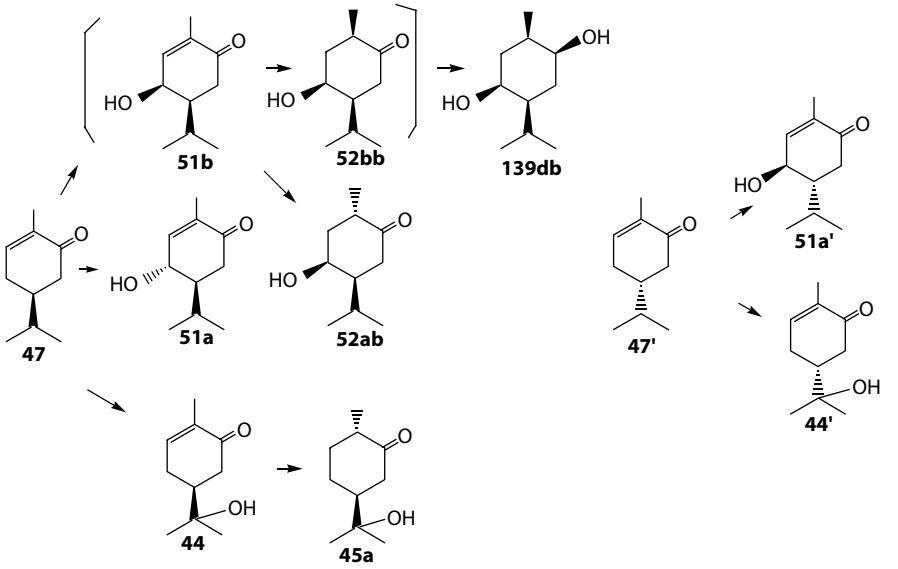
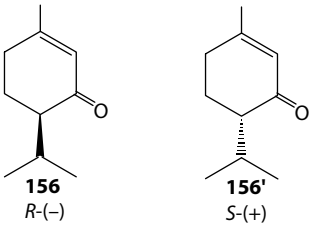


FIGURE 14.122 Proposed the metabolic pathways of (+)-carvotanacetone (**47**) and (–)-carvotanacetone (**47′**) by *Streptomyces bottropensis*, SY-2-1. (Modified from Noma, Y. et al., 1985c. *Proc. 29th TEAC*, pp. 238–240.)

ratio of 3:1], and 8-hydroxy-(+)-neocarvomenthol (**102a′**). On the other hand, the same fungus converted (+)-carvotanacetone (**47**) to (–)-isocarvomenthone (**48b**), 1α-hydroxy-(+)- neoisocarvomenthol (**54**) via 1α-hydroxy-(+)-isocarvomenthone (**53**) and 8-hydroxy-(–)-isocarvomenthone (**45b**) as shown in Figure 14.123 (Noma et al., 1988b).

14.3.4.1.3 Piperitone



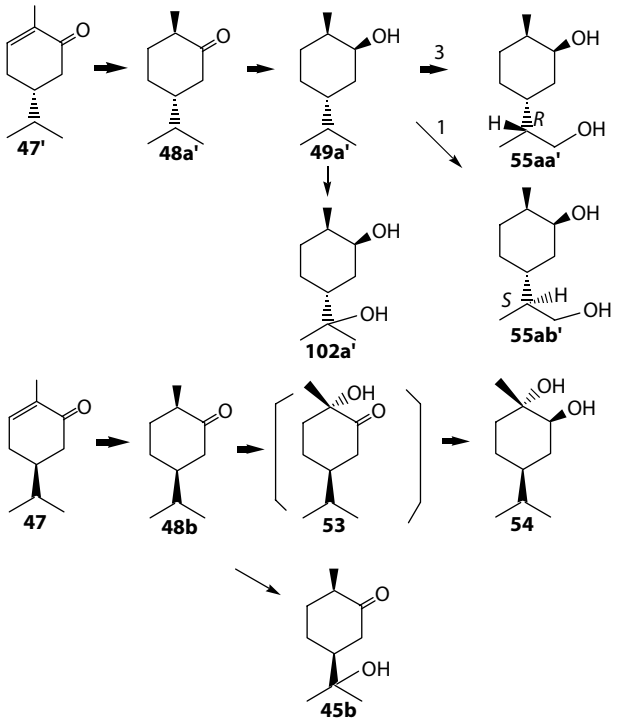


FIGURE 14.123 Proposed metabolic pathways of (–)-carvotanacetone (**47**) and (+)-carvotanacetone (**47′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 1988b. *Proc. 32nd TEAC*, pp. 146–148.)

A large number of yeasts were screened for the biotransformation of (–)-piperitone (**156**). A relatively small number of yeasts gave hydroxylation products of (–)-piperitone (**156**). Products obtained from (–)-piperitone (**156**) were 7-hydroxypiperitone (**161**), *cis*-6-hydroxypiperitone (**158b**), *trans*-6-hydroxypiperitone (**158a**), and 2-isopropyl-5-methylhydroquinone (**180**). Yields for the hydroxylation reactions varied between 8% and 60%, corresponding to the product concentrations of 0.04–0.3 g/L. Not one of the yeasts tested reduced (–)-piperitone (**156**) (van Dyk et al., 1998). During the initial screen with (–)-piperitone (**156**) only hydroxylation products were obtained. The hydroxylation products (**161**, **158a**, and **158b**) obtained with nonconventional yeasts from the genera

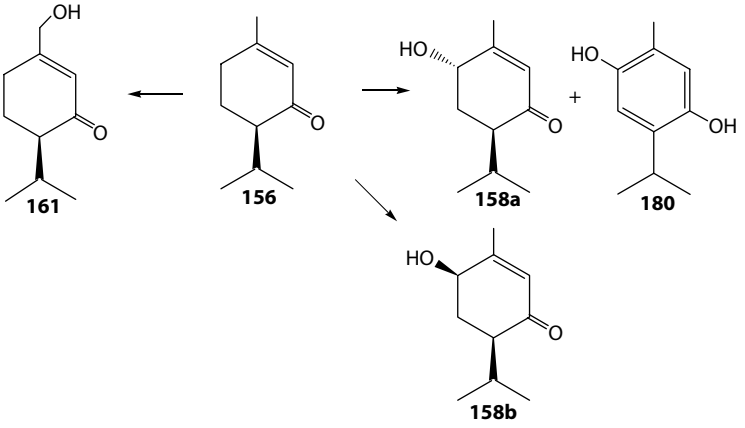


FIGURE 14.124 Hydroxylation products of (*R*)-(–)-piperitone (**156**) by yeast. (Modified from van Dyk, M.S. et al., 1998. *J. Mol. Catal. B: Enzym.*, 5: 149–154.)

Arxula, *Candida*, *Yarrowia*, and *Trichosporon* have recently been described (van Dyk et al., 1998) (Figure 14.124).

14.3.4.1.4 Pulegone

(*R*)-(+)-Pulegone (**119**), with a mint-like odour monoterpene ketone, is the main component (up to 80–90%) of *Mentha pulegium* essential oil (Pennyroyal oil), which is sometimes used in beverages and food additive for human consumption and occasionally in herbal medicine as an abortifacient drug. The biotransformation of (+)-pulegone (**119**) by fungi was investigated (Ismaili-Alaoui et al., 1992). Most fungal strains grown in a usual liquid culture medium were able to metabolize (+)-pulegone (**119**) to some extent in a concentration range of 0.1–0.5 g/L; higher concentrations were generally toxic, except for a strain of *Aspergillus* sp. isolated from mint leaves infusion, which was able to survive to concentrations of up to 1.5 g/L. The predominant product was generally l-hydroxy-(+)-pulegone (**384**) (20–30% yield). Other metabolites were present in lower amounts (5% or less) (see Figure 14.125). The formation of 1-hydroxy-(+)-pulegone (**387**) was explained by hydroxylation at a tertiary position. Its dehydration to piperitenone (**112**), even under the incubation conditions, during isolation or derivative reactions precluded any tentative determination of its optical purity and absolute configuration.

Botrytis allii converted (+)-pulegone (**119**) to (−)-(1*R*)-8-hydroxy-4-*p*-menthen-3-one (**121**) and piperitenone (**112**) (Miyazawa et al., 1991a, 1991b). *Hormonema isolate* (UOFS Y-0067) quantitatively reduced (+)-pulegone (**119**) and (−)-menthone (**149a**) to (+)-neomenthol (**137a**) (van Dyk et al., 1998) (Figure 14.125).

Biotrasformation by the recombinant reductase and the transformed *Escherichia coli* cells were examined with pulegone, carvone, and verbenone as substrates (Figure 14.126). The recombinant reductase catalyzed the hydrogenation of the exocylic C=C double bond of pulegone (**119**) to give menthone derivatives (Watanabe et al., 2007) (Tables 14.12 and 14.13).

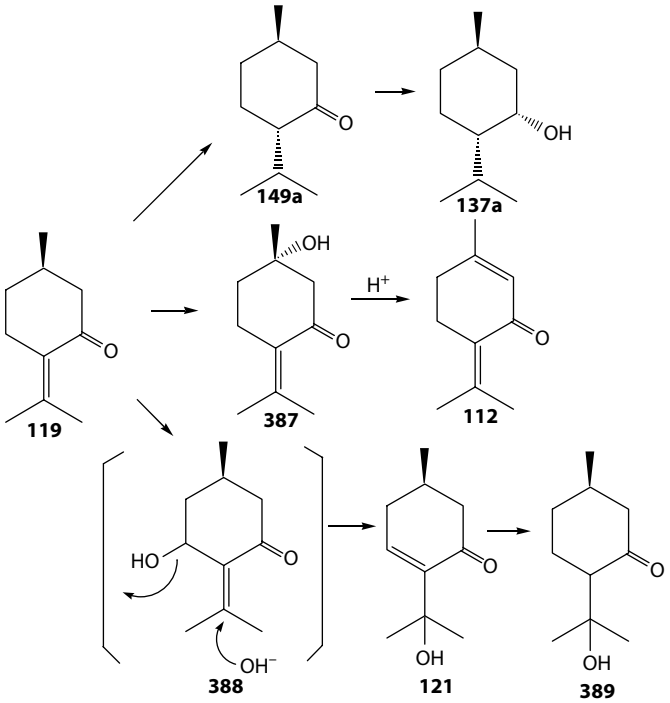


FIGURE 14.125 Biotransformation of (+)-pulegone (**119**) by *Aspergillus* sp., *Botrytis allii*, and *H. isolate* (UOFS Y-0067). (Modified from Miyazawa, M. et al., 1991a. *Chem. Express*, 6: 479–482; Miyazawa, M. et al., 1991b. *Chem. Express*, 6: 873; Ismaili-Alaoui, M. et al., 1992. *Tetrahedron Lett.*, 33: 2349–2352; van Dyk, M.S. et al., 1998. *J. Mol. Catal. B: Enzym.*, 5: 149–154.)

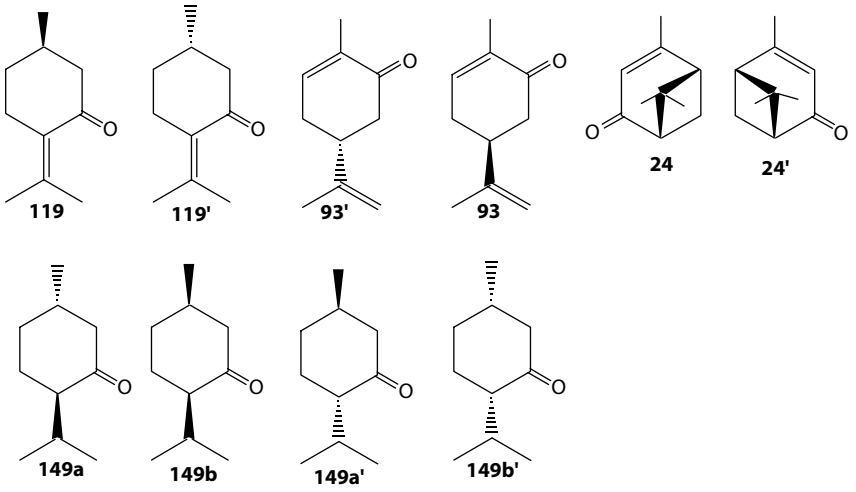


FIGURE 14.126 Chemical structures of substrate reduced by the recombinant pulegone reductase and the transformed *Escherichia coli* cells.

14.3.4.1.5 Piperitenone and Isopiperitenone

Piperitenone (**112**) is metabolized to 5-hydroxypiperitenone (**117**), 7-hydroxypiperitenone (**118**), and 7,8-dihydroxypiperitone (**157**). Isopiperitenol (**110**) is reduced to give isopiperitenone (**111**), which is further metabolized to piperitenone (**112**), 7-hydroxy- (**113**), 10-hydroxy- (**115**), 4-hydroxy- (**114**), and 5-hydroxy-isopiperitenone (**116**). Compounds **111** and **112** are isomerized to each other. Pulegone (**119**) was metabolized to **112**, 8,9-dehydromenthenone (**120**) and 8-hydroxymenthenone (**121**) as shown in the biotransformation of the same substrate using *Botrytis allii* (Miyazawa et al., 1991b) (Figure 14.127).

H. isolate (UOFS Y-0067) reduced (4*S*)-isopiperitenone (**111**) to (3*R*,4*S*)-isopiperitenol (**110**), a precursor of (−)-menthol (**137b**) (van Dyk et al., 1998) (Figure 14.128).

TABLE 14.12
Substrate Specificity in the Reduction of Eenones with the Recombinant Pulegone Reductase

Entry No. (Reaction Time)	Substrates	Products	Conversions (%)
1 (3 h)	(*R*)-(+)-Pulegone (**119**)	(1*R*, 4*R*)-Isomenthone (**149b**)	4.4
2 (12 h)	(*R*)-Pulegone (**119**)	(1*S*, 4*R*)-Menthone (**149a**)	6.8
3 (3 h)	(*S*)-(−)-Pulegone (**119′**)	(1*R*, 4*R*)-Isomenthone (**149b**)	14.3
4 (12 h)	(*S*)-Pulegone (**119′**)	(1*S*, 4*R*)-Menthone (**149a**)	15.7
5 (12 h)	(*R*)-(−)-Carvone (**93′**)	(1*S*, 4*S*)-Isomenthone (**149b′**)	0.3
6 (12 h)	(*S*)-(+)-Carvone (**93**)	(1*R*, 4*S*)-Menthone (**149a′**)	0.5
7 (12 h)	(1*S*, 5*S*)-Verbenone (**24**)	(1*S*, 4*S*)-Isomenthone (**149b′**)	1.6
8 (12 h)	(1*R*, 5*R*)-Verbenone (**24′**)	(1*R*, 4*S*)-Menthone (**149a′**)	2.1
			N.d.
		—	N.d.
		—	N.d.
		—	N.d.
		—	

N.d.—denotes not detected.

TABLE 14.13
Biotransformation of Pulegone (119 and 119′) with the Transformed *Escherichia coli* cells[a]

Substrates	Products	Conversion (%)
(*R*)-(+)-Pulegone (**119**)	(1*R*, 4*R*)-Isomenthone (**149b**)	26.8
(*S*)-(−)-Pulegone (**119′**)	(1*S*, 4*R*)-Menthone (**149a**)	30.0
	(1*S*, 4*S*)-Isomenthone (**149b′**)	32.3
	(1*R*, 4*S*)-Menthone (**149a′**)	7.1

[a] Reaction times of the transformation reaction are 12 h.

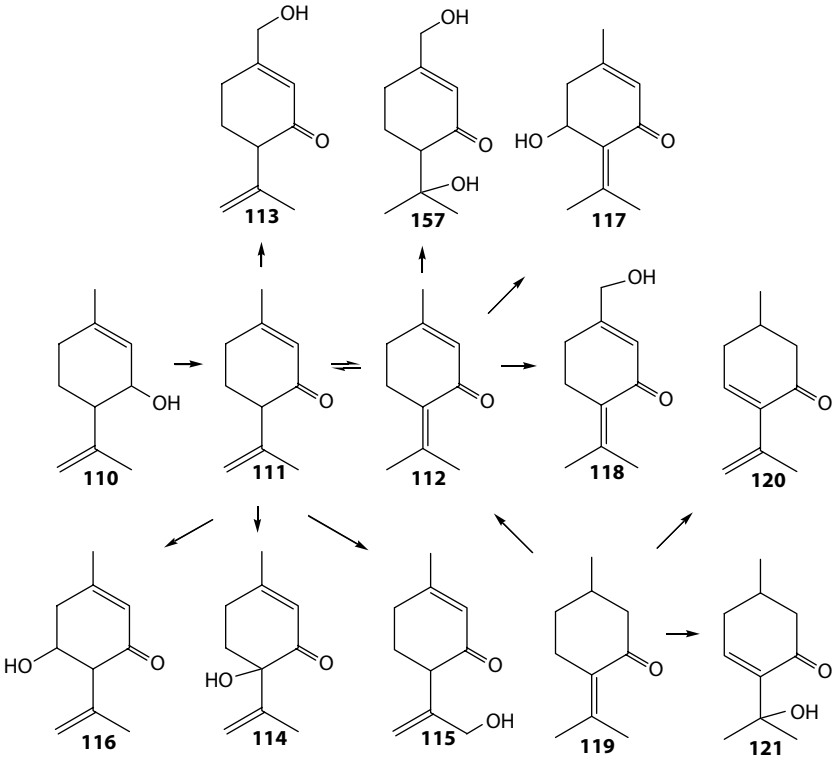


FIGURE 14.127 Biotransformation of isopiperitenone (**111**) and piperitenone (**112**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 1992c. *Proc. 37th TEAC*, pp. 26–28.)

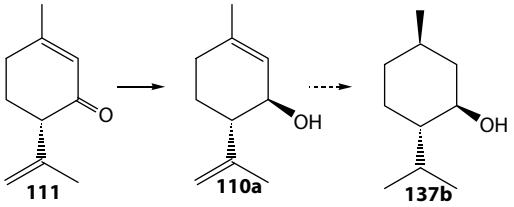
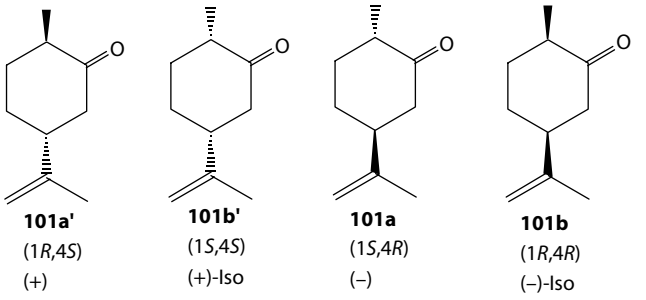


FIGURE 14.128 Biotransformation of isopiperitenone (**111**) by *H. isolate* (UOFS Y-0067). (Modified from van Dyk, M.S. et al., 1998. *J. Mol. Catal. B: Enzym.*, 5: 149–154.)

14.3.4.2 Saturated Ketone

14.3.4.2.1 Dihydrocarvone



In the reduction of saturated carbonyl group of dihydrocarvone by microorganism, (+)-dihydrocarvone (**101a′**) is converted stereospecifically to either (+)-neodihydrocarveol (**102a′**) or (–)-dihydrocarveol (**102b′**) or nonstereospecifically to the mixture of **102a′** and **102b′**, whereas (–)-isodihydrocarvone (**101b**) is converted stereospecifically to either (–)-neoisodihydrocarveol (**102d**) or (–)-isodihydrocarveol (**102c**) or nonstereospecifically to the mixture of **102c** and **102d** by various microorganisms (Noma and Tatsumi, 1973; Noma et al., 1974c; Noma and Nonomura 1974; Noma, 1976, 1977).

(+)-Dihydrocarvone (**101a′**) and (+)-isodihydrocarvone (**101b′**) are easily isomerized chemically to each other. In the microbial transformation of (–)-carvone (**93′**), the formation of (+)-dihydrocarvone (**101a′**) is predominant. (+)-Dihydrocarvone (**101a′**) was reduced to both/either (+)-neodihydrocarveol (**102a′**) and/or (–)-dihydrocarveol (**102b**), whereas in the biotransformation of (+)-carvone (**93**), (+)-isodihydrocarvone (**101b**) was formed predominantly. (+)-Isodihydrocarvone (**101b**) was reduced to both (+)-isodihydrocarveol (**102c**) and (+)-neoisodihydrocarveol (**102d**) (Figure 14.129).

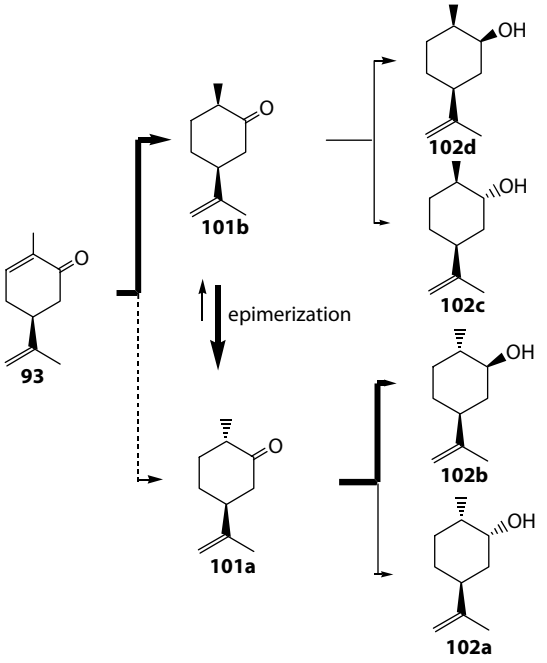


FIGURE 14.129 Proposed metabolic pathways of (+)-carvone (**93**) and (–)-isodihydrocarvone (**101b**) by *Pseudomonas fragi*, IFO 3458. (Modified from Noma, Y. et al., 1975. *Agric. Biol. Chem.*, 39: 437–441.)

However, *Pseudomonas fragi*, IFO 3458, *Pseudomonas fluorescens*, IFO 3081, and *Aerobacter aerogemes*, IFO 3319 and IFO 12059, formed (–)-dihydrocarvone (**101a**) predominantly from (+)-carvone (**93**). In the time course study of the biotransformation of (+)-carvone (**93**), it appeared that predominant formation of (–)-dihydrocarvone is due to the epimerization of (–)-isodihydrocarvone (**101b′**) by epimerase of *Pseudomonas fragi* IFO 3458 (Noma et al., 1975).

14.3.4.2.2 Isodihydrocarvone Epimerase

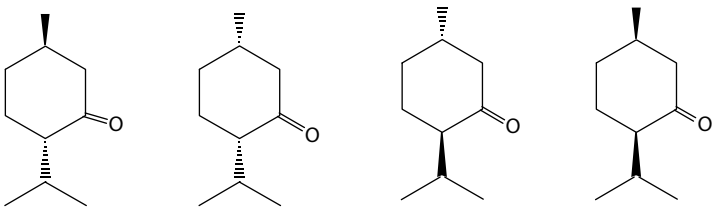
14.3.4.2.2.1 Preparation of Isodihydrocarvone Epimerase The cells of *Pseudomonas fragi* IFO 3458 were harvested by centrifugation and washed five times with 1/100 M KH_2PO_4–Na_2HPO_4 buffer (pH 7.2). Bacterial extracts were prepared from the washed cells (20 g from 3-L medium) by sonic lysis (Kaijo Denki Co., Ltd., 20Kc., 15 min, at 5–7°C) in 100 mL of the same buffer. Sonic extracts were centrifuged at 25, 500 *g* for 30 min at –2°C. The opalescent yellow supernatant fluid had the ability to convert (–)-isodihydrocarvone (**101b**) to (–)-dihydrocarvone (**101a**). On the other hand, the broken cell preparation was incapable of converting (–)-isodihydrocarvone (**101b**) to (–)-dihydrocarvone (**101a**). The enzyme was partially purified from this supernatant fluid about 56-fold with heat treatment (95–97°C for 10 min), ammonium sulfate precipitation (0.4–0.7 saturation), and DEAE-Sephadex A-50 column chromatography.

The reaction mixture consisted of a mixture of (–)-isodihydrocarvone (**101b**) and (–)-dihydrocarvone (**101a**) (60:40 or 90:10), 1/30 M KH_2PO_4–Na_2HPO_4 buffer (pH 7.2), and the crude or partially purified enzyme solution. The reaction was started by the addition of the enzyme solution and stopped by the addition of ether. The ether extract was applied to analytical GLC (Shimadzu Gas Chromatograph GC-4A 10% PEG-20M, 3 m × 3 mm, temperature 140–170°C at the rate of 1°C a min, N_2 35 mL/min), and epimerization was assayed by measuring the peak areas of (–)-isodihydrocarvone (**101b**) and (–)-dihydrocarvone (**101a**) in gas liquid chromatography (GLC) before and after the reaction.

The crude extract and the partially purified preparation were found to be very stable to heat treatment; 66% and 36% of the epimerase activity remained after treatment at 97°C for 60 and 120 min, respectively (Noma et al., 1975).

A strain of *Aspergillus niger* TBUYN-2 hydroxylated at C-1 position of (–)-isodihydrocarvone (**101b**) to give 1α-hydroxyisodihydrocarvone (**72b**), which was easily and smoothly reduced to (1*S*, 2*S*, 4*S*)-(–)-8-*p*-menthene-1,2-*trans*-diol (**71d**), which was also obtained from the biotransformation of (–)-*cis*-limonene-1,2-epoxide (**69**) by microorganisms and decomposition by 20% HCl (Figure 14.127) (Noma et al., 1985a, 1985b). Furthermore, *Aspergillus niger* TBUYN-2 and *Aspergillus niger* Tiegh (CBAYN) biotransformed (–)-isodihydrocarvone (**101b**) to give (–)-4α-hydroxyisodihydrocarvone (**378b**) and (–)-8-*p*-menthene-1,2-*trans*-diol (**71d**) as the major products together with a small amount of 1α-hydroxyisodihydrocarvone (**72b**) (Noma and Asakawa, 2008) (Figure 14.130).

14.3.4.2.3 Menthone and Isomenthone



149a (1*R*,4*S*) (–)-Menthone

149b (1*S*,4*S*) (–)-Isomenthone

149a' (1*S*,4*R*) (+)-Menthone

149b' (1*R*,4*R*) (+)-Isomenthone

The growing cells of *Pseudomonas fragi* IFO 3458 epimerized 17% of racemic isomenthone (**149b** and **b′**) to menthone (**149a** and **a′**) (Noma et al., 1975). (–)-Menthone (**149a**) was converted

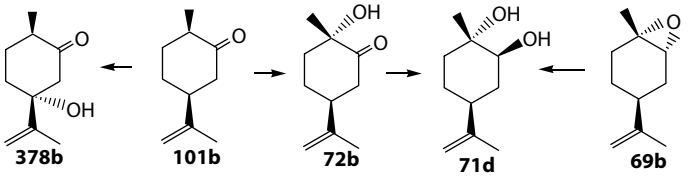
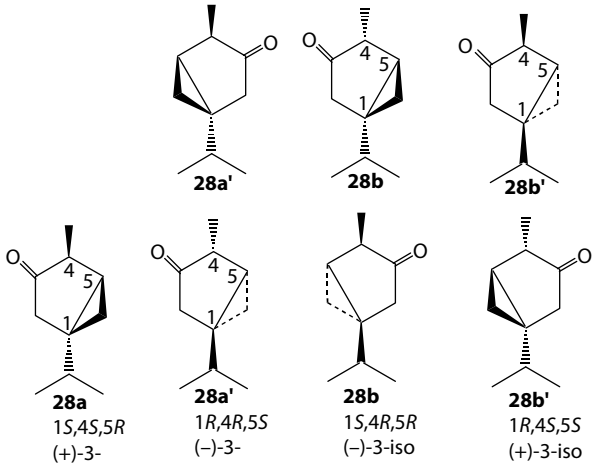


FIGURE 14.130 Biotransformation of (+)-carvone (**93**), (–)-isodihydrocarvone (**101b**), and (–)-*cis*-limonene-1,2-epoxide (**69b**) by *Aspergillus niger* TBUYN-2 and *Aspergillus niger* Tiegh (CBAYN). (Modified from Noma, Y. et al., 1985a. *Annual Meeting of Agricultural and Biological Chemistry*, Sapporo, p. 68; Noma, Y. and Y. Asakawa, 2008. *Proc. 52nd TEAC*, pp. 206–208.)

by *Pseudomonas fluorescens* M-2 to (–)-3-oxo-4-isopropyl-1-cyclohexanecarboxylic acid (**164a**), (+)-3-oxo-4-isopropyl-1-cyclohexanecarboxylic acid (**164b**), and (+)-3-hydroxy-4-isopropyl-1-cyclohexanecarboxylic acid (**165ab**). On the other hand, (+)-menthone (**149a′**) was converted to give (+)-3-oxo-4-isopropyl-1-cyclohexane carboxylic acid (**164a′**) and (–)-3-oxo-4-isopropyl-1-cyclohexane carboxylic acid (**164b′**). Racemic isomenthone (**149b** and **b′**) was converted to give racemic 1-hydroxy-1-methyl-4-isopropylcyclohexane-3-one (**150**), racemic piperitone (**156**), racemic 3-oxo-4-isopropyl-1-cyclohexene-1-carboxylic acid (**162**), 3-oxo-4-isopropyl-1-cyclohexane carboxylic acid (**164b**), 3-oxo-4-isopropyl-1-cyclohexane carbxylic acid (**164a**), and (+)-3-hydroxy-4-isopropyl-1-cyclohexane carboxylic acid (**165ab**) (Figure 14.131).

Soil plant pathogenic fungi, *Rhizoctonia solani* 189 converted (–)-menthone (**149a**) to 4β-hydroxy-(–)-menthone (**392**, 29%) and 1 α, 4 β-dihydroxy-(–)-menthone (**393**, 71%) (Nonoyama et al., 1999) (Figure 14.131). (–)-Menthone (**149a**) was transformed by *Spodoptera litura* to give 7-hydroxymenthone (**151a**), 7-hydroxyneomenthol (**165c**), and 7-hydroxy-9-carboxymenthone (**394a**) (Hagiwara et al., 2006) (Figure 14.132). (–)-Menthone (**149a**) gave 7-hydroxymenthone (**151a**) and (+)-neomenthol (**137c**) by human liver microsome (CYP2B6). Of 11 recombinant human P450 enzymes (express in *Trichoplusia ni* cells) tested, CYP2B6 catalyzed oxidation of (–)-menthone (**149a**) to 7-hydroxymenthone (**151a**) (Nakanishi and Miyazawa, 2004) (Figure 14.132).

14.3.4.2.4 Thujone



β-Pinene (**1**) is metabolized to 3-thujone (**28**) via α-pinene (**4**) (Gibbon and Pirt, 1971). α-Pinene (**4**) is metabolized to give thujone (**28**). Thujone (**28**) was biotransformed to thujoyl alcohol (**29**) by *Aspergillus niger* TBUYN-2 (Noma, 2000). Furthermore, (–)-3-isothujone (**28b**) prepared from *Armois* oil was biotransformed by plant pathogenic fungus, *Botrytis allii* IFO 9430 to give 4-hydroxythujone (**30**) and 4,6-dihydroxythujone (**31**) (Miyazawa et al., 1992a) (Figure 14.133).

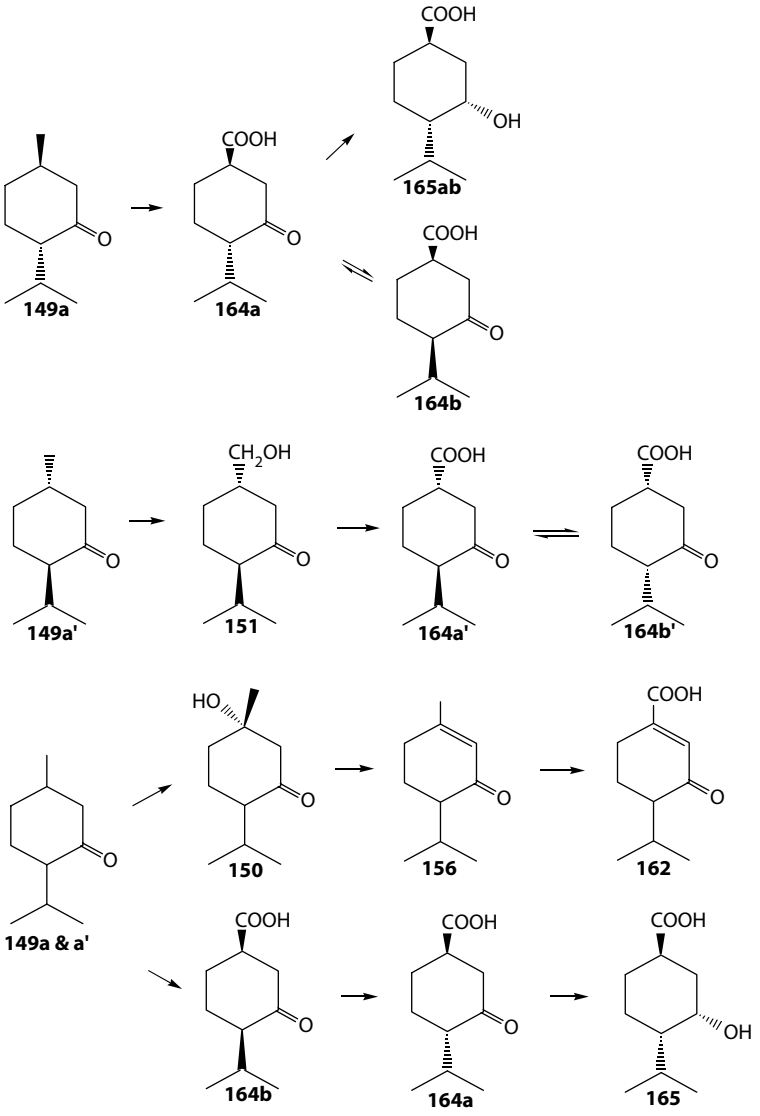


FIGURE 14.131 Biotransformation of (–)- (**149a**) and (+)-menthone (**149a′**) and racemic isomenthone (**149b** and **149b′**) by *Pseudomonas fluorescens* M-2. (Modified from Sawamura, Y. et al., 1974. *Proc. 18th TEAC*, pp. 27–29.)

14.3.4.3 Cyclic Monoterpene Epoxide

14.3.4.3.1 1,8-Cineole

1,8-Cineole (**122**) is a main component of the essential oil of *Eucalyptus adiata* var. *australiana* leaves, comprising *ca.* 75% in the oil, which corresponds to 31 mg/g fr.wt. leaves (Nishimura et al., 1980).

The most effective utilization of **122** is very important in terms of renewable biomass production. It would be of interest, for example, to produce more valuable substances, such as plant growth regulators, by the microbial transformation of **122**. The first reported utilization of **122** was presented by MacRae et al. (1979), who showed that it was a carbon source for *Pseudomonas flava* growing on *Eucalyptus* leaves. Growth of the bacterium in a mineral salt medium containing **122** resulted in the oxidation at the C-2 position of **122** to give the metabolites (1*S*,4*R*,6*S*)-(+)-2α-hydroxy-1,8-cineole (**225a**), (1*S*,4*R*,6*R*)-(–)-2β-hydroxy-1,8-cineole (**125a**), (1*S*,4*R*)-(+)-2-oxo-1,8-cineole (**126**), and (–)-(*R*)-5,5-dimethyl-4-(3′-oxobutyl)-4,5-dihydrofuran-2(3H)-one (**128**) (Figure 14.134).

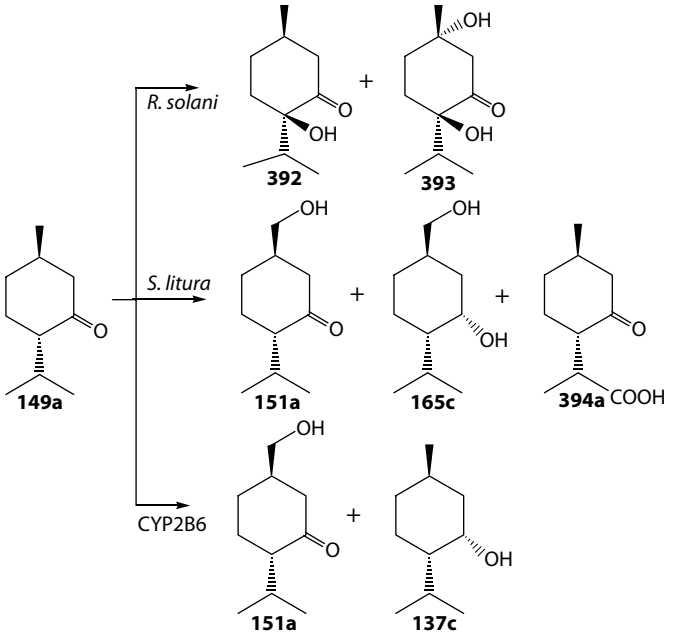


FIGURE 14.132 Metabolic pathway of (–)-menthone (**149a**) by *Rhizoctonia solani* 189, *Spodptera litura* and human liver microsome (CYP2B6). (Modified from Nonoyama, H. et al., 1999. *Proc. 43rd TEAC*, pp. 387–388; Nakanishi, K. and M. Miyazawa, 2004. *Proc. 48th TEAC*, pp. 401–402; Hagiwara, Y. et al., 2006. *Proc. 50th TEAC*, pp. 279–280.)

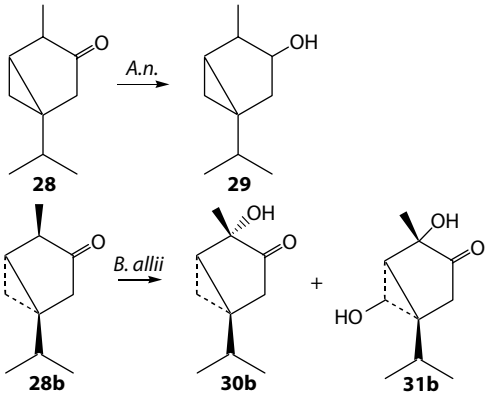


FIGURE 14.133 Biotransformation of (–)-3-isothujone (**28b**) by *Aspergillus niger* TBUYN-2 and plant pathogenic fungus, *Botrytis allii* IFO 9430. (Modified from Gibbon, G.H. and S.J. Pirt, 1971. *FEBS Lett.*, 18: 103–105; Miyazawa, M. et al., 1992a. *Proc. 36th TEAC*, pp. 197–198.)

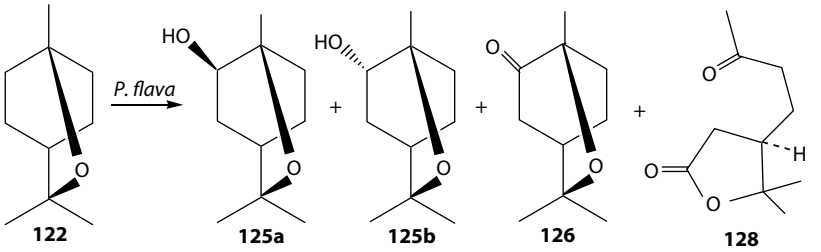


FIGURE 14.134 Biotransformation of 1,8-cineole (**84**) by *Pseudomonas flava*. (Modified from MacRae, I.C. et al., 1979. *Aust. J. Chem*., 32: 917–922.)

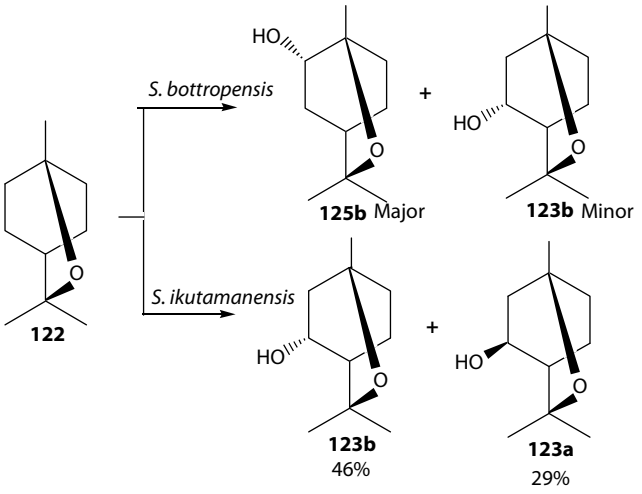


FIGURE 14.135 Biotransformation of 1,8-cineole (**122**) by *Streptomyces bottropensis* SY-2-1 and *Streptomyces ikutamanensis* Ya-2-1. (Modified from Noma, Y. and H. Nishimura, 1980. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 28; Noma, Y. and H. Nishimura, 1981. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 196.)

Streptomyces bottropensis, SY-2-1 biotransformed 1,8-cineole (**122**) stereochemically to (+)-2α-hydroxy-1,8-cineole (**125b**) as the major product and (+)-3α-hydroxy-1,8-cineole (**123b**) as the minor product. Recovery ratio of 1,8-cineole metabolites as ether extract was *ca.* 30% in *Streptomyces bottropensis*, SY-2-1 (Noma and Nishimura, 1980, 1981) (Figure 14.135).

In case of *Streptomyces ikutamanensis*, Ya-2-1 1,8-cineole (**122**) was biotransformed regioselectively to give (+)-3α-hydroxy-1,8-cineole (**123b**, 46%) and (+)-3β-hydroxy-1,8-cineole (**123b**, 29%) as the major product. Recovery ratio as ether extract was *ca.* 8.5% in *Streptomyces ikutamanensis*, Ya-2-1 (Noma and Nishimura, 1980, 1981) (Figure 14.135).

When (+)-3α-hydroxy-1,8-cineole (**123b**) was used as substrate in the cultured medium of *Streptomyces ikutamanensis*, Ya-2-1, (+)-3β-hydroxy-1,8-cineole (**123a**, 32%) was formed as the major product together with a small amount of (+)-3-oxo-1,8-cineole (**126a**, 1.6%). When (+)-3β-hydroxy-1,8-cineole (**123a**) was used, (+)-3-oxo-1,8-cineole (**126a**, 9.6%) and (+)-3α-hydroxy-1,8-cineole (**123b**, 2%) were formed. When (+)-3-oxo-1,8-cineole (**126a**) was used, (+)-3α-hydroxy- (**123b**, 19%) and (+)-3β-hydroxy-1,8-cineole (**123a**, 16%) were formed.

Based on the above results, it is obvious that (+)-3β-hydroxy-1,8-cineole (**123b**) is formed mainly in the biotransformation of 1,8-cineole (**122**), (+)-3α-hydroxy-1,8-cineole (**123b**), and (+)-3-oxo-1,8-cineole (**126a**) by *Streptomyces ikutamanensis*, Ya-2-1. The production of (+)-3β-hydroxy-1,8-cineole (**123b**) is interesting, because it is a precursor of mosquito repellent, *p*-menthane-3,8-diol (**142aa′**) (Noma and Nishimura, 1981) (Figure 14.136).

When *Aspergillus niger* TBUYN-2 was cultured in the presence of 1,8-cineole (**122**) for 7 days, it was transformed to three alcohols [racemic 2α-hydroxy-1,8-cineoles (**125b** and **b′**), racemic 3α-hydroxy- (**123b** and **b′**), and racemic 3β-hydroxy-1,8-cineoles (**123a** and **123a′**)] and two ketones [racemic 2-oxo- (**126** and **126′**) and racemic 3-oxo-1,8-cineoles (**124** and **124′**)] (Figure 14.135). The formation of 3α-hydroxy- (**123b** and **b′**) and 3β-hydroxy-1,8-cineoles (**123a** and **123a′**) is of great interest not only due to the possibility of the formation of *p*-menthane-3,8-diol (**142** and **142′**), the mosquito repellents and plant growth regulators that are synthesized chemically from 3α-hydroxy- (**123b** and **b′**) and 3β-hydroxy-1,8-cineoles (**123a** and **123a′**), respectively, but also from the viewpoint of the utilization of *Eucalyptus adiata* var. *australiana* leaves oil as biomass. An Et_2O extract of the culture broth (products and **122** as substrate) was recovered in 57% of substrate (w/w) (Nishimura et al., 1982; Noma et al., 1996) (Figure 14.137).

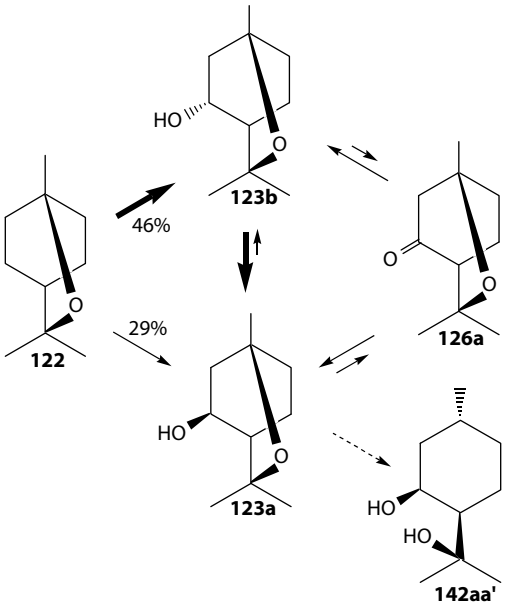


FIGURE 14.136 Biotransformation of 1,8-cineole (**122**), (+)-3α-hydroxy-1,8-cineole (**123b**), (+)-3β-hydroxy-1,8-cineole(**123a**), and (+)-3-oxo-1,8-cineole (**126a**) by *Streptomyces ikutamanensis*, Ya-2-1. (Modified from Noma, Y. and H. Nishimura, 1981. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 196.)

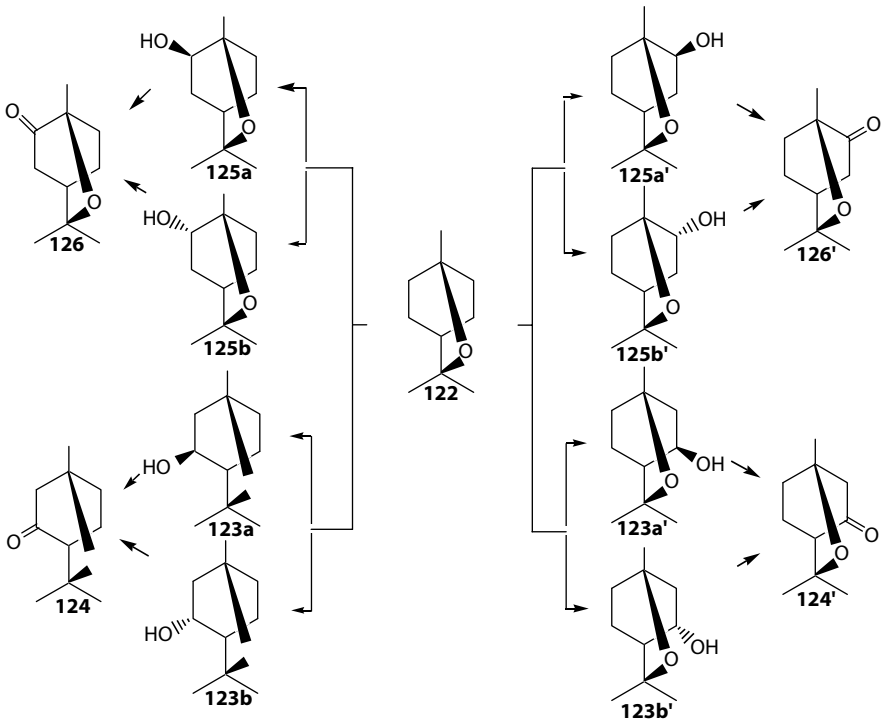


FIGURE 14.137 Biotransformation of 1,8-cineole (**122**) by *Aspergillus niger* TBUYN-2. (Modified from Nishimura, H. et al., 1982. *Agric. Biol. Chem.*, 46: 2601–2604; Noma, Y. et al., 1996. *Proc. 40th TEAC*, pp. 89–91.)

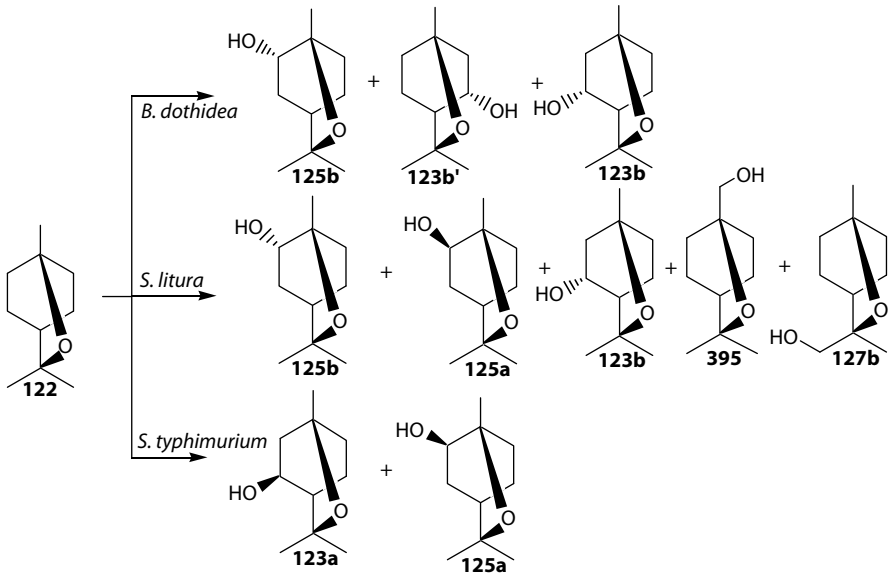


FIGURE 14.138 Biotransformation of 1,8-cineole (**122**) by *Botryosphaeria dothidea*, *Spodptera litura*, and *Salmonella typhimurium*. (Modified from Noma, Y. et al., 1996. *Proc. 40th TEAC*, pp. 89–91; Saito, H. and M. Miyazawa, 2006. *Proc. 50th TEAC*, pp. 275–276; Hagiwara, Y. and M. Miyazawa, 2007. *Proc. 51st TEAC*, pp. 304–305.)

Plant pathogenic fungus *Botryosphaeria dothidea* converted 1,8-cineole (**122**) to optical pure (+)-2α-hydroxy-1,8-cineole (**125b**) and racemic 3α-hydroxy-1,8-cineole (**123b** and **b′**), which were oxidized to optically active 2-oxo- (**126**) (100% ee) and racemic 3-oxo-1,8-cineole (**124** and **124′**), respectively (Table 14.14). Cytochrome P-450 inhibitor, 1-aminobenzotriazole, inhibited the hydroxylation of the substrate (Noma et al., 1996) (Figure 14.138). *Spodptera litura* also converted 1,8-cineole (**122**) to give three secondary alcohols (**123b, 125a,** and **b**) and two primary alcohols (**395** and **127**) (Hagihara and Miyazawa, 2007). *Salmonella typhimurium* OY1001/3A4 and NADPH-P450 reductase hydroxylated 1,8-cineole (**122**) to 2β-hydroxy-1,8-cineole (**125a**, $[\alpha]_D + 9.3$, 65.3% ee) and 3β-hydroxy-1, 8-cineole (**123a**, $[\alpha]_D$–27.8, 24.7% ee) (Saito and Miyazawa, 2006).

Extraction of the urinary metabolites from brushtail possums (*Trichosurus vulpecula*) maintained on a diet of fruit impregnated with 1,8-cineole (**122**) yielded *p*-cresol (**129**) and the novel C-9 oxidated products 9-hydroxy-1,8-cineole (**127a**) and 1,8-cineole-9-oic acid (**462a**) (Flynn and Southwell, 1979; Southwell and Flynn, 1980) (Figure 14.139).

1,8-Cineole (**122**) gave 2β-hydroxy-1,8-cineole (**125a**) by CYP-450 human and rat liver microsome. Cytochrome P450 molecular species responsible for metabolism of 1,8-cineole (**122**) was determined to be CYP3A4 and CYP3A1/2 in human and rat, respectively. Kinetic analysis showed that K_m and V_{max} values for the oxidation of 1,8-cineole (**122**) by human and rat treated with

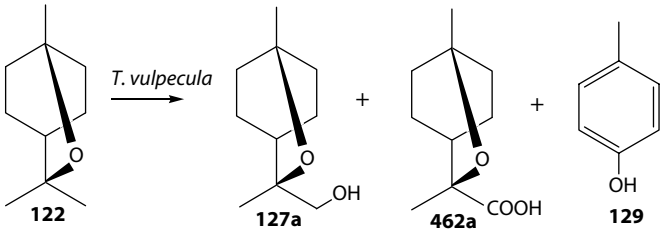


FIGURE 14.139 Metabolism of 1,8-cineole in *Trichosurus vulpecula*. (Modified from Southwell, I.A. and T.M. Flynn, 1980. *Xenobiotica*, 10: 17–23.)

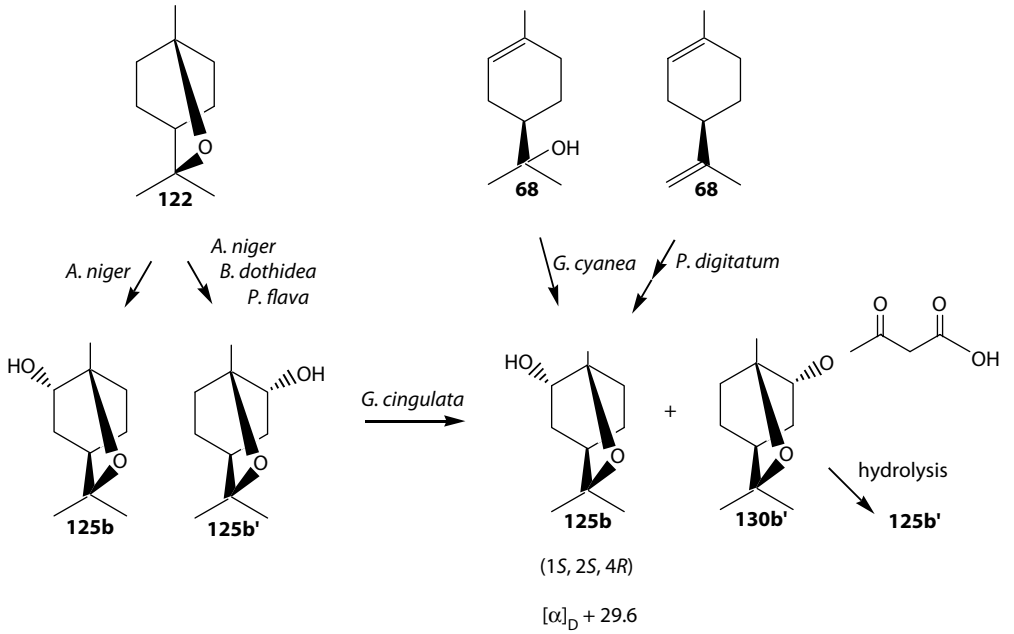


FIGURE 14.140 Formation of 2α-hydroxy-1,8-cineoles (**125b** and **b′**) from 1,8-cineole (**122**) and optical resolution by *Glomerella cingulata* and *Aspergillus niger* TBUYN-2 and **125b′** from (+)-limonene (**68**) by *Pencillium digitatum*. (Modified from Nishimura, H. et al., 1982. *Agric. Biol. Chem*., 46: 2601–2604; Abraham, W.-R. et al., 1986. *Appl. Microbiol. Biotechnol*., 24: 24–30; Miyazawa, M. et al., 1995b. *Proc. 39th TEAC*, pp. 352–353; Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206; Noma, Y. and Y. Asakawa, 2007a. *Book of Abstracts of the 38th ISEO*, p. 7.)

pregnenolone-16α-carbonitrile (PCN), recombinant CYP3A4 were determined to be 50 μM and 90.9 nmol/min/nmol P450, 20 μM and 11.5 nmol/min/nmol P450, and 90 μM and 47.6 nmol/min/nmol P450, respectively (Shindo et al., 2000).

Microbial resolution of racemic 2α-hydroxy-1,8-cineoles (**125b** and **b′**) was carried out by using *Glomerella cingulata*. The mixture of **125b** and **b′** was added to a culture of *Glomerella cingulata* and esterified to give after 24 h (1*R*,2*R*,4*S*)-2α-hydroxy-1,8-cineole-2-yl-malonate (**130b′**) in 45% yield (ee 100%). The recovered alcohol showed 100% ee of the (1*S*,2*S*,4*R*)-enantiomer (**125b**) (Miyazawa et al., 1995b). On the other hand, optically active (+)-2α-hydroxy-1,8-cineole **(125b)** was also formed from (+)-limonene (**68**) by a strain of *Citrus* pathogenic fungus *Penicillium digitatum* (Saito and Miyazawa 2006, Noma and Asakawa 2007a) (Figure 14.140).

Esters of racemic 2α-hydroxy-1,8-cineole (**125b** and **b′**) were prepared by a convenient method (Figure 14.141). Their odours were characteristic. Then products were tested against antimicrobial activity and their microbial resolution was studied (Hashimoto and Miyazawa, 2001) (Table 14.15).

1,8-Cineole (**122**) was glucosylated by *Eucalyptus perriniana* suspension cells to 2α-hydroxy-1,8-cineole monoglucoside (**404**, 16.0% and **404′**, 16.0%) and diglucosides (**405**, 1.4%) (Hamada et al., 2002) (Figure 14.142).

14.3.4.3.2 1,4-Cineole

Regarding the biotransformation of 1,4-cineole (**131**), *Streptomyces griseus* transformed it to 8-hydroxy-1,4-cineole (**134**), whereas *Bacillus cereus* transformed 1,4-cineole (**131**) to 2α-hydroxy-1,4-cineole (**132b**, 3.8%) and 2β-hydroxy-1,4-cineoles (**132a**, 21.3%) (Liu et al., 1988) (Figure 14.144). On the other hand, a strain of *Aspergillus niger* biotransformed 1,4-cineole (**131**) regiospecifically to 2α-hydroxy-1,4-cineole (**132b**) (Miyazawa et al., 1991c) and (+)-3α-hydroxy-1,4-cineole (**133b**) (Miyazawa et al., 1992b) along with the formation of 8-hydroxy-1,4-cineole (**134**) and 9-hydroxy-1,4-cineole (**135**) (Miyazawa et al., 1992c) (Figure 14.144).

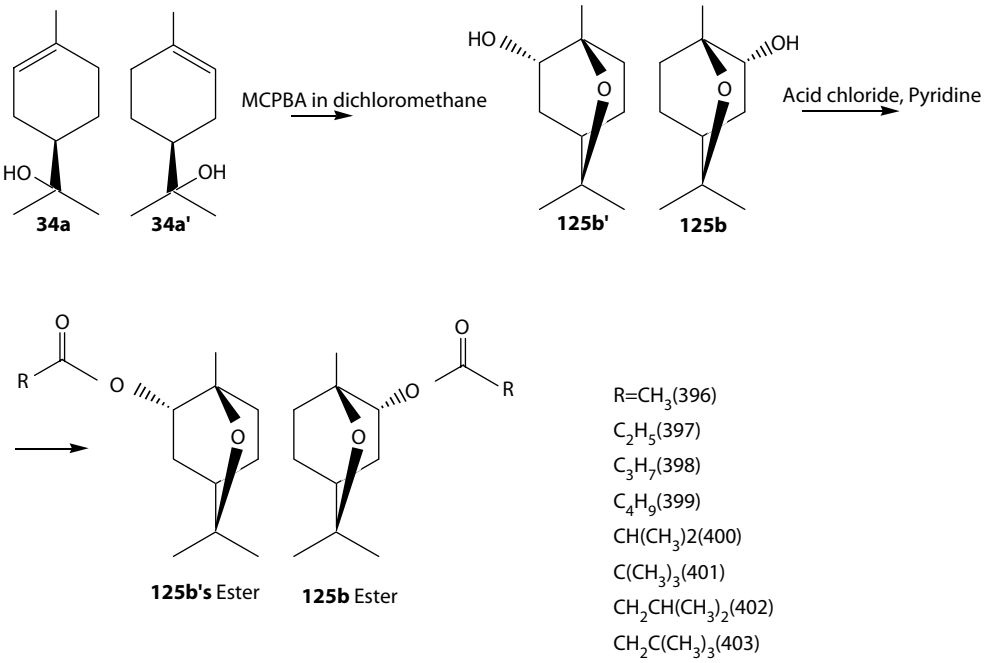


FIGURE 14.141 Chemical synthesis of esters of racemic 2α-hydroxy-1,8-cineole (**125b** and **b′**). (Modified from Hashimoto Y. and M. Miyazawa, 2001. *Proc. 45th TEAC*, pp. 363–365.)

Microbial optical resolution of racemic 2α-hydroxy-1,4-cineoles (**132b** and **b′**) was carried out by using *Glomerella cingulata* (Liu et al., 1988). The mixture of 2α-hydroxy-1,4-cineoles (**132b** and **b′**) was added to a culture of *Glomerella cingulata* and esterified to give after 24 h (1*R*,2*R*,4*S*)-2α-hydroxy-1,4-cineole-2-yl malonate (**136′**) in 45% yield (ee 100%). The recovered alcohol showed an ee of 100% of the (1*S*,2*S*,4*R*)-enantiomer (**132b**). On the other hand, optically active (+)-2α-hydroxy-1,4-cineole (**132b**) was also formed from (–)-terpinen-4-ol (**342**) by *Gibberella cyanea* DSM (Abraham et al., 1986) and *Aspergillus niger* TBUYN-2 (Noma and Asakawa, 2007b) (Figure 14.145).

TABLE 14.14
Stereoselectivity in the Biotransformation of 1,8-Cineole (122) by *Aspergillus niger, Botryosphaeria dothidea*, and *Pseudomonas flava*

	Products
Microorganisms	**125a and a′, 125b and b′, 123b and b′, 123a and a′**
Aspergillus niger TBUYN-2	2:43:49:6
	50:50 41:59
Botryosphaeria dothidea	4:59:34:3
	100:0 53:47
Pseudomonas flava	29:71:0:0
	100:0

Source: Noma, Y. et al., 1996. *Proc. 40th TEAC*, pp. 89–91.

TABLE 14.15
Yield and Enantiomer Excess of Esters of Racemic 2α-Hydroxy-1,8-Cineole (125b and b′) on the Microbial Resolution by *Glomerella cingulata*

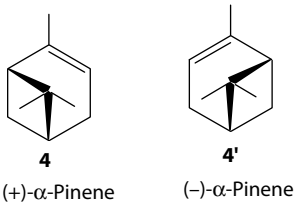
	0 h	24 h		48 h	
Compounds	**%ee**	**%ee**	**Yield (%)**	**%ee**	**Yield (%)**
396	(−)36.3	(+)85.0	24.0	(+)100	14.1
397	(−)36.9	(+)73.8	18.6	(+)100	8.6
398	(−)35.6	(+)33.2	13.7	(+)75.4	3.5
399	(−)36.8	(+)45.4	14.4	(+)100	2.3
400	(−)35.4	(−)21.4	25.2	(+)20.6	8.0
401	(−)36.7	(−)37.8	31.5	(−)40.6	15.2
402	(−)36.1	(−)29.8	46.8	(−)15.0	24.0
403	(−)36.3	(−)37.6	72.2	(−)39.0	36.9

Source: Hashimoto Y. and M. Miyazawa, 2001. *Proc. 45th TEAC*, pp. 363–365.

14.4 METABOLIC PATHWAYS OF BICYCLIC MONOTERPENOIDS

14.4.1 Bicyclic Monoterpene

14.4.1.1 α-Pinene



α-Pinene (**4** and **4′**) is the most abundant terpene in nature and obtained industrially by fractional distillation of turpentine (Krasnobajew, 1984). (+)-α-Pinene (**4**) occurs in oil of *Pinus palustris* Mill. at concentrations of up to 65%, and in oil of *Pinus caribaea* at concentrations of 70% (Bauer et al., 1990). On the other hand, *Pinus caribaea* contains (–)-α-pinene (**4′**) at the concentration of 70–80% (Bauer et al., 1990).

The biotransformation of (+)-α-pinene (**4**) was investigated by *Aspergillus niger* NCIM 612 (Bhattacharyya et al., 1960, Prema and Bhattacharyya, 1962). A 24 h shake culture of this strain metabolized 0.5% (+)-α-pinene (**4**) in 4–8 h. After the fermentation of the culture broth contained (+)-verbenone (**24**) (2–3%), (+)-*cis*-verbenol (**23b**) (20–25%), (+)-*trans*-sobrerol (**43a**) (2–3%), and

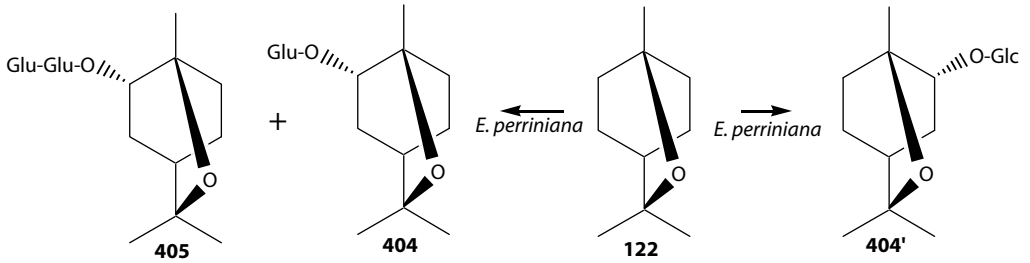


FIGURE 14.142 Biotransformation of 1,8-cineole (**122**) by *Eucalyptus perriniana* suspension cell. (Modified from Hamada, H. et al., 2002. *Proc. 46th TEAC*, pp. 321–322.)

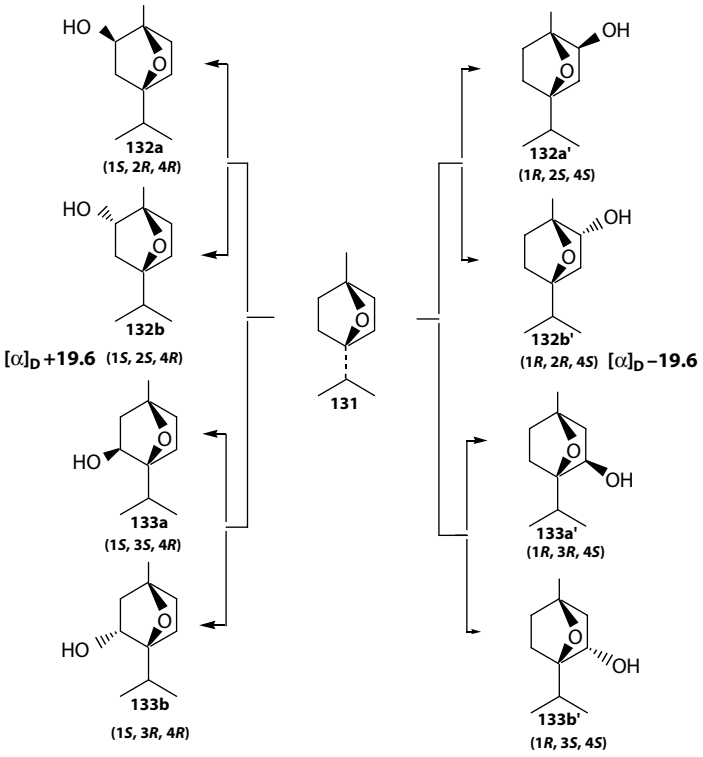


FIGURE 14.143 Metabolic pathways of 1,4-cineole (**131**) by microorganisms

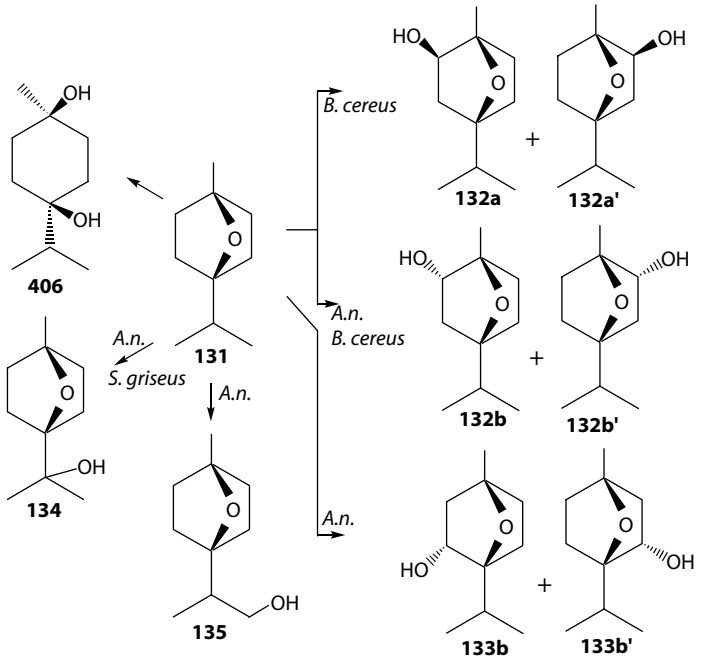


FIGURE 14.144 Metabolic pathways of 1,4-cineole (**131**) by *Aspergillus niger* TBUYN-2, *Bacillus cereus*, and *Streptomyces griseus*. (Modified from Liu, W. et al., 1988. *J. Org. Chem*., 53: 5700–5704; Miyazawa, M. et al., 1991c. *Chem. Express*, 6: 771–774; Miyazawa, M. et al., 1992b. *Chem. Express*, 7: 305–308; Miyazawa, M. et al., 1992c. *Chem. Express*, 7: 125–128; Miyazawa, M. et al., 1995b. *Proc. 39th TEAC*, pp. 352–353.)

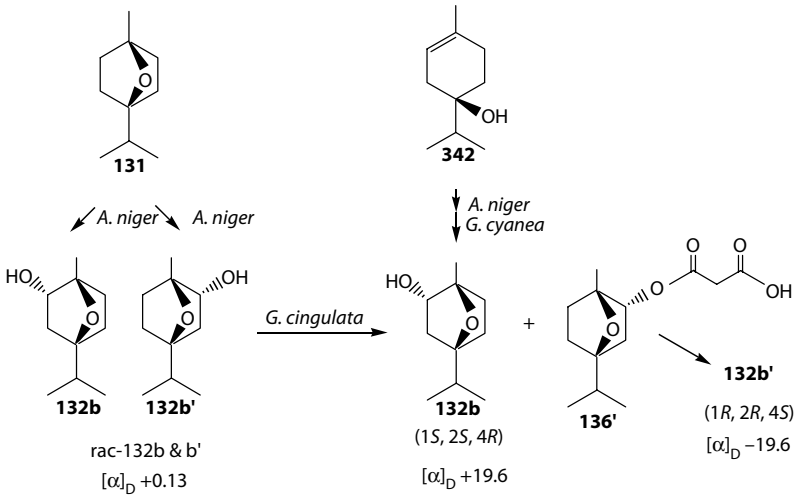


FIGURE 14.145 Formation of optically active 2α-hydroxycineole from 1,4-cineole (**131**) and terpinene-4-ol (**342**) by *Aspergillus niger* TBUYN-2, *Gibberella cyanea*, and *Glomerella cingulata*. (Modified from Abraham, W.-R. et al., 1986. *Appl. Microbiol. Biotechnol.*, 24: 24–30; Miyazawa, M. et al., 1991c. *Chem. Express*, 6: 771–774; Miyazawa, M. et al., 1995b. *Proc. 39th TEAC*, pp. 352–353; Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

(+)-8-hydroxycarvotanacetone (**44**) (Bhattacharyya et al., 1960; Prema and Bhattacharyya 1962 (Figure 14.146).

The degradation of (+)-α-pinene (**4**) by a soil *Pseudomonas* sp. (PL strain) was investigated by Hungund et al. (1970). A terminal oxidation pattern was proposed, leading to the formation of organic acids through ring cleavage. (+)-α-Pinene (**4**) was fermented in shake cultures by a soil *Pseudomonas* sp. (PL strain) that is able to grow on (+)-α-pinene (**4**) as the sole carbon source, and borneol (**36**), myrtenol (**5**), myrtenic acid (**84**), and α-phellandric acid (**65**) (Shukla and Bhattacharyya, 1968) (Figure 14.147) were obtained.

The degradation of (+)-α-pinene (**4**) by *Pseudomonas fluorescens* NCIMB11671 was studied and a pathway for the microbial breakdown of (+)-α-pinene (**4**) was proposed as shown in Figure 14.148 (Best et al., 1987; Best and Davis, 1988). The attack of oxygen is initiated by enzymatic oxygenation of the 1,2-double bond to form α-pinene epoxide (**38**), which then undergoes rapid rearrangement to produce a unsaturated aldehyde, occurring as two isomeric forms. The primary product of the reaction (*Z*)-2-methyl-5-isopropylhexa-2,5-dien-1-al (**39**, isonovalal) can undergo chemical isomerization to the *E*-form (novalal, **40**). Isonovalal (**39**), the native form of the aldehyde, possesses *citrus*, woody, spicy notes, whereas novallal (**40**) has woody, aldehydic, and cyclone notes. The same biotransformation was also carried out by *Nocardia* sp. strain P18.3 (Griffiths et al., 1987a, b).

Pseudomonas PL strain and PIN 18 degradated α-pinene (**4**) by the pathway proposed in Figure 14.149 to give two hydrocarbon, limonene (**68**) and terpinolene (**346**), and neutral metabolite,

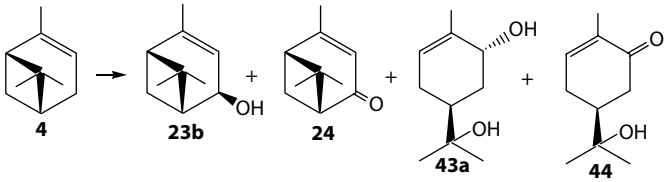


FIGURE 14.146 Biotransformation of (+)-α-pinene (**4**) by *Aspergillus niger* NCIM 612. (Modified from Bhattacharyya, P.K. et al., 1960. *Nature*, 187: 689–690; Prema, B.R. and P.K. Bhattachayya, 1962. *Appl. Microbiol.*, 10: 524–528.)

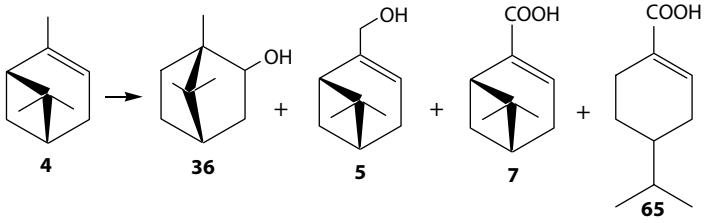


FIGURE 14.147 Biotransformation of (+)-α-pinene (**4**) by *Pseudomonas* sp. (PL strain). (Modified from Shukla, O.P., and P.K. Bhattacharyya, 1968. *Indian J. Biochem*., 5: 92–101.)

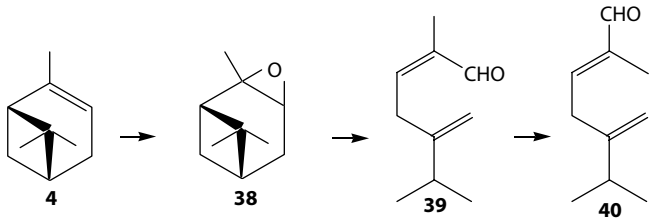


FIGURE 14.148 Biotransformation of (+)-α-pinene (**4**) by *Pseudomonas fluorescens* NCIMB 11671. (Modified from Best, D.J. et al., 1987. *Biotransform*., 1: 147–159.)

borneol (**36**). A probable pathway has been proposed for the terminal oxidation of β-isopropylpimelic acid (**248**) in the PL strain and PIN 18 (Shukala and Bhattacharyya, 1968).

Pseudomonas PX 1 biotransformed (+)-α-pinene (**4**) to give (+)-*cis*-thujone (**29**) and (+)-*trans*-carveol (**81a**) as major compounds. Compounds **81a**, **171**, **173**, and **178** have been identified as fermentation products (Gibbon and Pirt, 1971; Gibbon et al., 1972) (Figure 14.150).

Aspergillus niger TBUYN-2 biotransformed (–)-α-pinene (**4′**) to give (–)-α-terpineol (**34′**) and (–)-*trans*-sobrerol (**43a′**) (Noma et al., 2001). The mosquitocidal (+)-(1*R*,2*S*,4*R*)-1-*p*-menthane-2,8-diol (**50a′**) was also obtained as a crystal in the biotransformation of (–)-α-pinene (**4′**) by *Aspergillus niger* TBUYN-2 (Noma et al., 2001; Noma, 2007) (Figure 14.151).

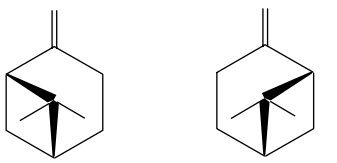
(1*R*)-(+)-α-Pinene (**4**) and its enantiomer (**4′**) were fed to *Spodptera litura* to give the corresponding (+)- and (–)-verbenones (**24** and **24′**) and (+)- and (–)-myrtenols (**5** and **5′**) (Miyazawa et al., 1996c) (Figure 14.152).

(–)-α-Pinene (**4′**) was treated in human liver microsomes CYP 2B6 to afford (–)-*trans*-verbenol (**23′**) and (–)-myrtenol (**5′**) (Sugie and Miyazawa, 2003) (Figure 14.153).

In rabbit, (+)-α-pinene (**4**) was metabolized to (–)-*trans*-verbenols (**23**) as the main metabolites together with myrtenol (**5**) and myrtenic acid (**7**). The purities of (–)-verbenol (**23**) from (–)- (**4′**), (+)- (**4**), and (+/–)-α-pinene (**4** and **4′**) was 99%, 67%, and 68%, respectively. This means that the biotransformation of (–)-**4′** in rabbit is remarkably efficient in the preparation of (–)-*trans*-verbenol (**23a**) (Ishida et al., 1981b) (Figure 14.154).

(–)-α-Pinene (**4′**) was biotransformed by the plant pathogenic fungus *Botrytis cinerea* to afford 3α-hydroxy-(–)-β-pinene (**2a′**, 10%), 8-hydroxy-(–)-α-pinene (**434′**, 12%), 4β-hydroxy-(–)-pinene-6-one (**468′**, 16%), and (–)-verbenone (**24′**) (Farooq et al., 2002) (Figure 14.155).

14.4.1.2 β-Pinene



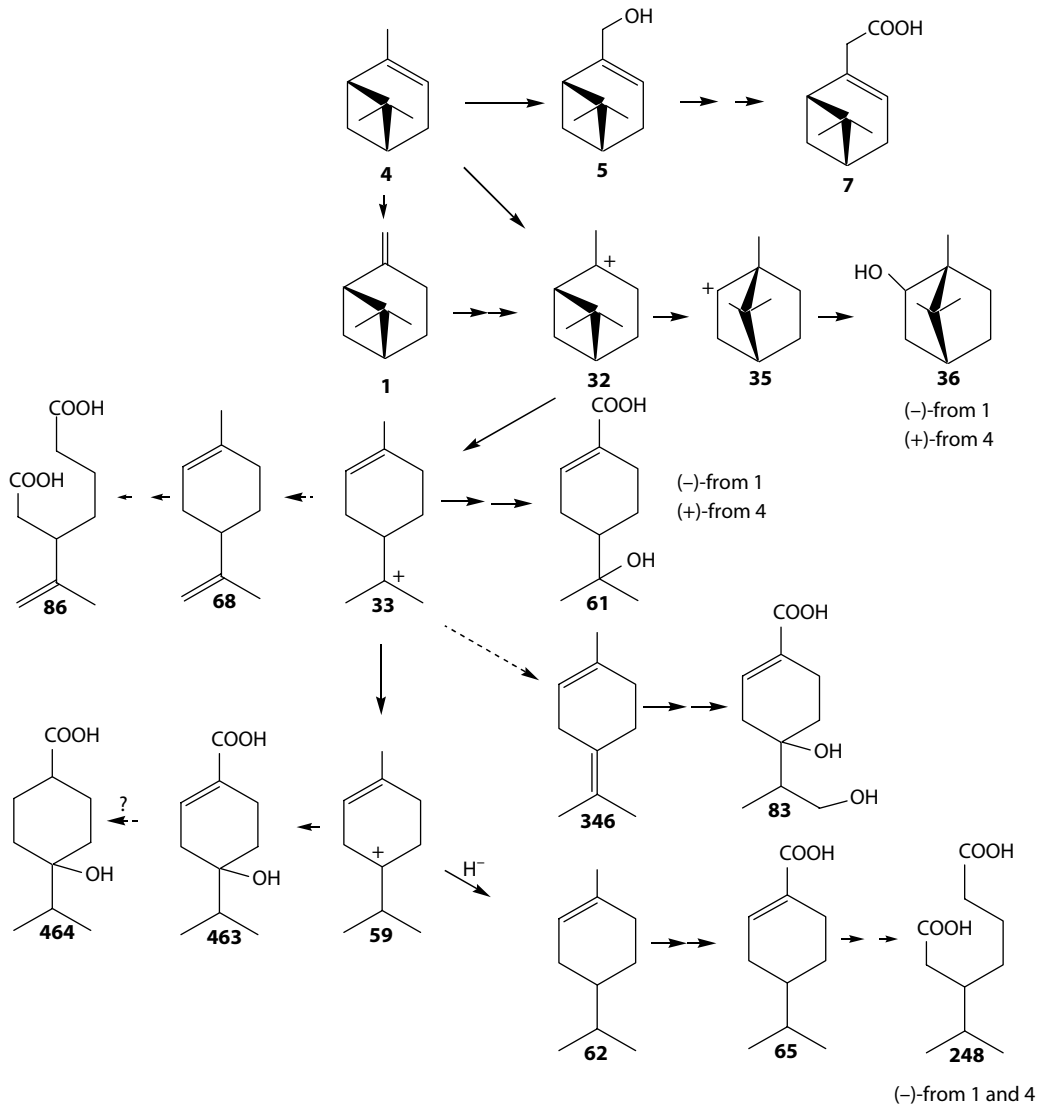


FIGURE 14.149 Metabolic pathways of degradation of α- and β-pinene by a soil Pseudomonad (PL strain) and *Pseudomonas* PIN 18. (Modified from Shukla, O.P., and P.K. Bhattacharyya, 1968. *Indian J. Biochem*., 5: 92–101.)

(+)-β-Pinene (**1**) is found in many essential oils. Optically active and racemic β-pinene are present in turpentine oils, although in smaller quantities than (+)-α-pinene (**4**) (Bauer et al., 1990).

Shukla et al. (1968) obtained a similarly complex mixture of transformation products from (–)-β-pinene (**1′**) through degradation by a *Pseudomonas* sp/(PL strain). On the other hand, Bhattacharyya and Ganapathy (1965) indicated that *Aspergillus niger* NCIM 612 acts differently and more specifically on the pinenes by preferably oxidizing (–)-β-pinene (**1′**) in the allylic position to form the interesting products pinocarveol (**2′**) and pinocarvone (**3′**), besides myrtenol (**5′**) (see Figure 14.156). Furthermore, the conversion of (–)-β-pinene (**1′**) by *Pseudomonas putida-arvilla* (PL strain) gave borneol (**36′**) (Rama Devi and Bhattacharyya, 1978) (Figure 14.156).

Pseudomonas pseudomallai isolated from local sewage sludge by the enrichment culture technique utilized caryophyllene as the sole carbon source (Dhavlikar et al., 1974). Fermentation of (–)-β-pinene (**1′**) by *Pseudomonas pseudomallai* in a mineral salt medium (Seubert's medium) at

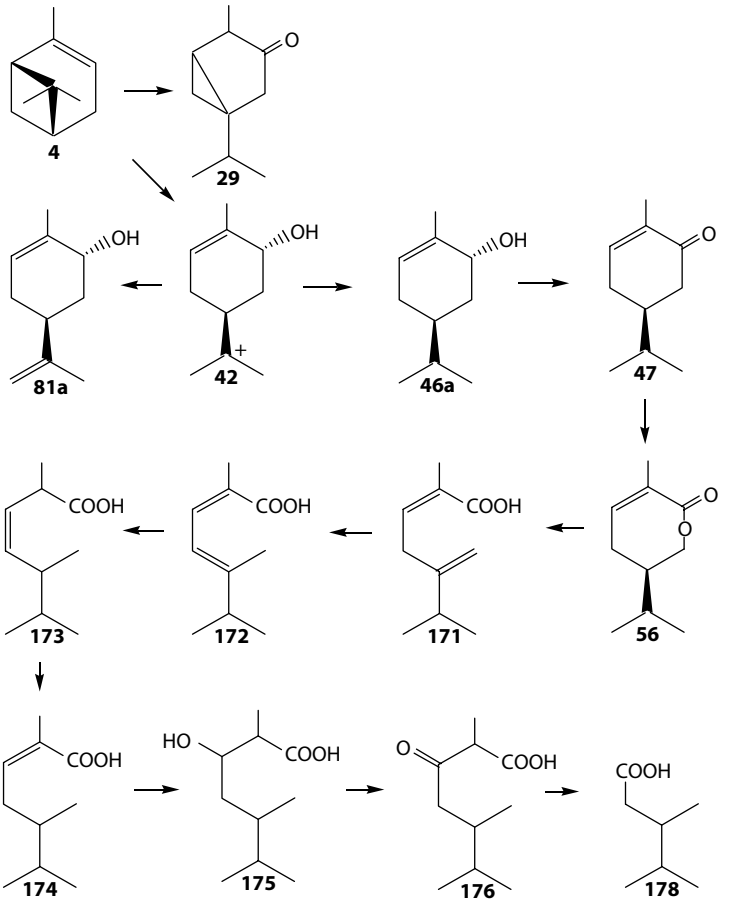


FIGURE 14.150 Proposed metabolic pathways for (+)-α-pinene (**4**) degradation by *Pseudomonas* PX 1. (Modified from Gibbon, G.H. and S.J. Pirt, 1971. *FEBS Lett.*, 18: 103–105; Gibbon, G.H. et al., 1972. *Proc. IV IFS, Ferment. Technol. Today*, pp. 609–612.)

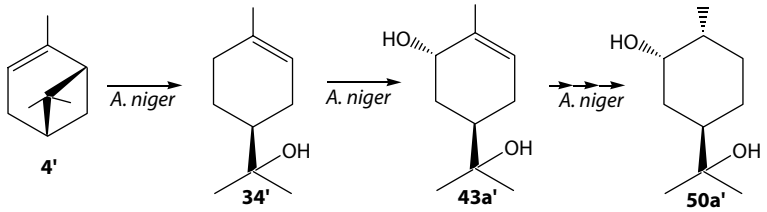


FIGURE 14.151 Biotransformation of (–)-α-pinene (**4**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 2001. *Proc. 45th TEAC*, pp. 88–90.)

30°C with agitation and aeration for 4 days yielded camphor (**37′**), borneol (**36a′**), isoborneol (**36b′**), α-terpineol (**34′**), and β-isopropyl pimelic acid (**248′**) (see Figure 14.154). Using modified Czapek-Dox medium and keeping the other conditions the same, the pattern of the metabolic products was dramatically changed. The metabolites were *trans*-pinocarveol (**2′**), myrtenol (**5′**), α-fenchol (**11′**), α-terpineol (**34′**), myrtenic acid (**7′**), and two unidentified products (see Figure 14.157).

(–)-β-Pinene (**1′**) was converted by plant pathogenic fungi, *Botrytis cinerea*, to give four new compounds such as (–)-pinane-2α,3α-diol (**408′**), (–)-6β-hydroxypinene (**409′**), (–)-4α,5-dihydroxypinene (**410′**), and (–)-4α-hydroxypinen-6-one (**411′**) (Figure 14.158).

This study progressed further biotransformation of (–)-pinane-2α,3α-diol (**408′**) and related compounds by microorganisms as shown in Figure 14.158.

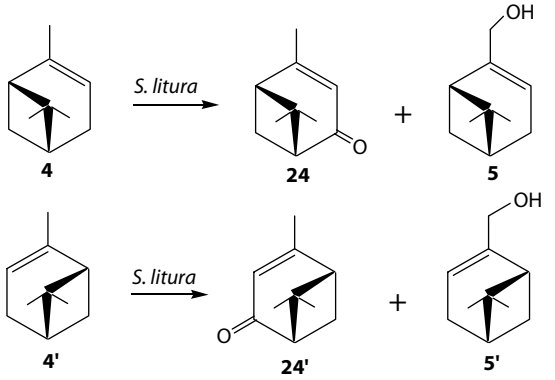


FIGURE 14.152 Biotransformation of (+)- (**4**) and (–)-α-pinene (**4′**) by *Spodptera litura*. (Modified from Miyazawa, M. et al., 1996c. *Proc. 40th TEAC*, pp. 84–85.)

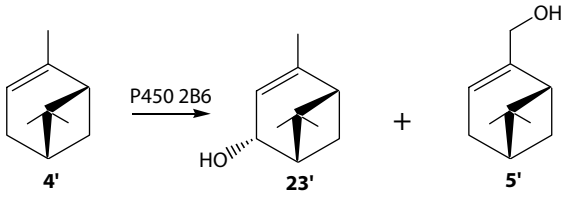


FIGURE 14.153 Biotransformation of (–)-α-pinene (**4′**) by human liver microsomes CYP 2B6. (Modified from Sugie, A. and M. Miyazawa, 2003. *Proc. 47th TEAC*, pp. 159–161.)

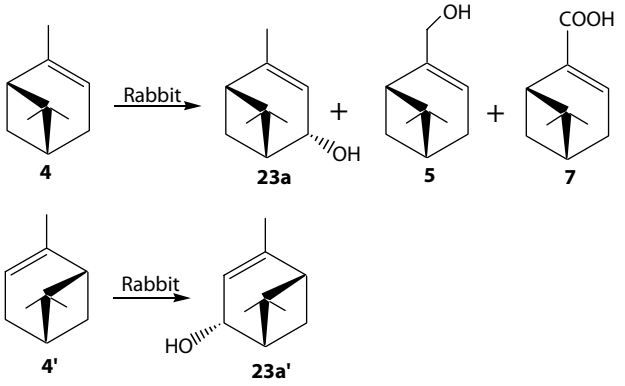


FIGURE 14.154 Biotransformation of α-pinene by rabbit. (Modified from Ishida, T. et al., 1981b. *J. Pharm. Sci.*, 70: 406–415.)

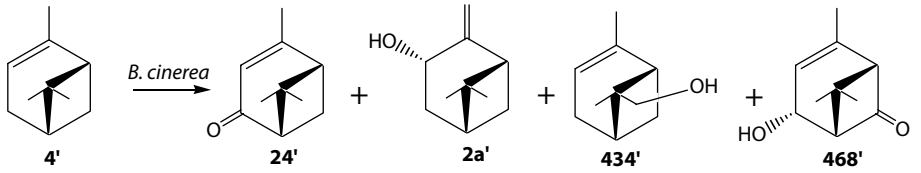


FIGURE 14.155 Microbial transformation of (–)-α-pinene (**4′**) by *Botrytis cinerea*. (Modified from Farooq, A. et al., 2002. *Z. Naturforsch.*, 57c: 686–690.)

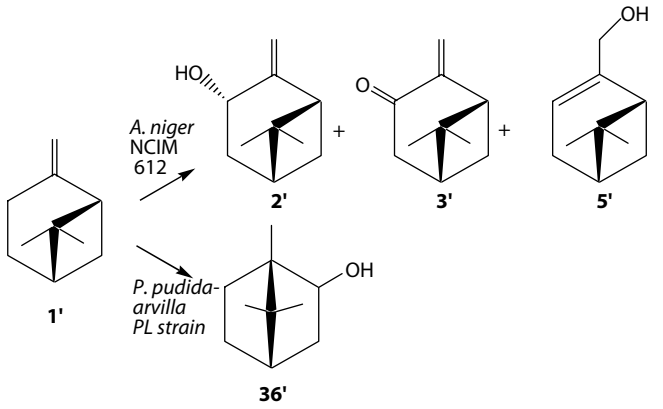


FIGURE 14.156 Biotransformation of (–)-β-pinene (**1′**) by *Aspergillus niger* NCIM 612 and *Pseudomonas putida-arvilla* (PL strain). (Modified from Bhattacharyya, P.K. and K. Ganapathy, 1965. *Indian J. Biochem.*, 2: 137–145; Rama Devi, J. and P.K. Bhattacharyya, 1978. *J. Indian Chem. Soc*., 55: 1131–1137.)

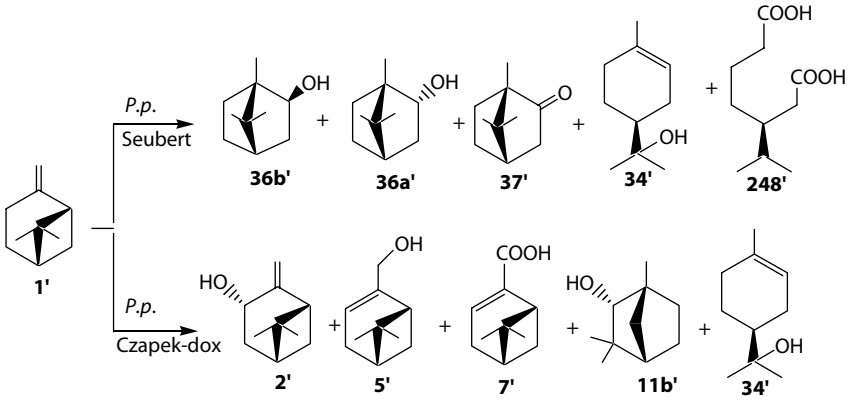


FIGURE 14.157 Biotransformation of (–)-β-pinene (**1′**) by *Pseudomonas pseudomallai*. (Modified from Dhavalikar, R.S. et al., 1974. *Dragoco Rep.*, 3: 47–49.)

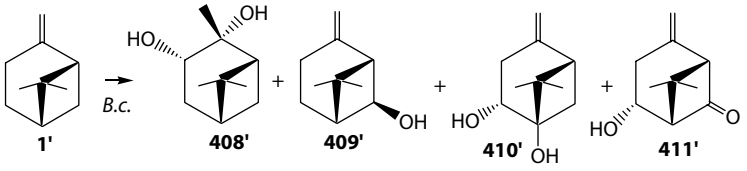


FIGURE 14.158 Biotransformation of (–)-β-pinene (**1′**) by *Botrytis cinerea*. (Modified from Farooq, A. et al., 2002. *Z. Naturforsch*., 57c: 686–690.)

As shown in Figure 14.159, (+)- (**1**) and (–)-β-pinenes (**1′**) were biotransformed by *Aspergillus niger* TBUYN-2 to give (+)-α-terpineol (**34**) and (+)-oleuropeyl alcohol (**204**) and their antipodes (**34′** and **204′**), respectively. The hydroxylation process of α-terpineol (**34**) to oleuropeyl alcohol (**204**) was completely inhibited by 1-aminotriazole as cyt.P-450 inhibitor.

(–)-β-Pinene (**1′**) was at first biotransformed by *Aspergillus niger* TBUYN-2 to give (+)-*trans*-pinocarveol (**2a′**) (**274**). (+)-*trans*-Pinocarveol (**2a′**) was further transformed by three pathways: firstly, (+)-*trans*-pinocarveol (**2a′**) was metabolized to (+)-pinocarvone (**3′**), (–)-3-isopinanone (**413′**), (+)-2α-hydroxy-3-pinanone (**414′**), and (+)-2α,5-dihydroxy-3-pinanone (**415′**). Secondly, (+)-*trans*-pinocarveol (**2a′**) was metabolized to (+)-6β-hydroxyfenchol (**349ba′**) and thirdly (+)-*trans*-pinocarveol (**2a′**) was metabolized to (–)-6β,7-dihydroxyfenchol (**412ba′**) via epoxide and diol as intermediates (Noma and Asakawa, 2005a) (Figure 14.160).

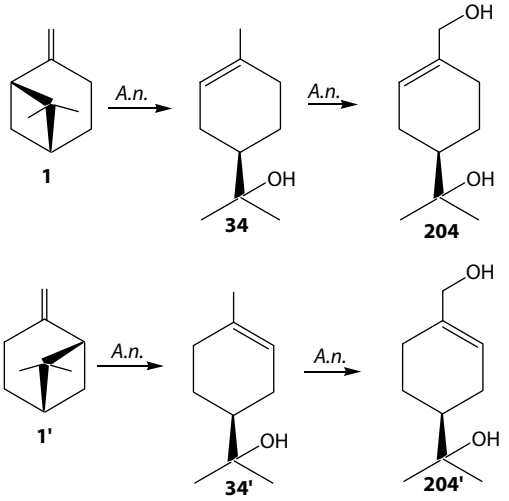


FIGURE 14.159 Biotransformation of (+)- (**1**) and (–)-β-pinene (**1′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 2001. *Proc. 45th TEAC*, pp. 88–90.)

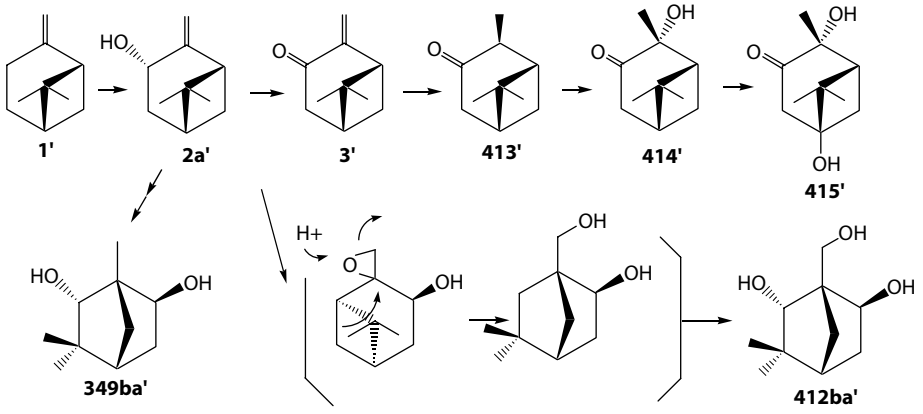


FIGURE 14.160 The metabolism of (–)-β-pinene (**1′**) and (+)-*trans*-pinocarveol (**2a′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

(–)-β-Pinene (**1′**) was metabolized by *Aspergillus niger* TBUYN-2 with three pathways as shown in Figure 14.154 to give (–)-α-pinene (**4′**), (–)-α-terpineol (**34**'), and (+)-*trans*-pinocarveol (**2a′**). (–)-α-Pinene (**4′**) is further metabolized by three pathways. At first (–)-α-pinene (**4′**) was metabolized via (–)-α-pinene epoxide (**38′**), *trans*-sobrerol (**43a′**), (–)-8-hydroxycarvotanacetone (**44′**), (+)-8-hydroxycarvomenthone (**45a**) to (+)-*p*-menthane-2,8-diol (**50a′**), which was also metabolized in (–)-carvone (**93′**) metabolism. Secondly, (–)-α-pinene (**4′**) is metabolized to myretenol (**83′**), which is metabolized by rearrangement reaction to give (–)-oleuropeyl alcohol (**204′**). (–)-α-Terpineol (**34′**), which is formed from β-pinene (**1′**), was also metabolized to (–)-oleuropeyl alcohol (**204′**) and (+)-*trans*-pinocarveol (**2a′**), formed from (–)-β-pinene (**1′**), was metabolized to pinocarvone (**3′**), 3-pinanone (**413′**), 2α-hydroxy-3-pinanone (**414′**), 2α,5-dihydroxy-3-pinanone (**415′**), and 2α,9-dihydroxy-3-pinanone (**416′**). Furthermore, (+)-*trans*-pinocarveol (**2a′**) was metabolized by rearrangemet reaction to give 6β-hydroxyfenchol (**349ba′**) and 6β,7-dihydroxyfenchol (**412ba′**) (Noma and Asakawa, 2005a) (Figure 14.161).

(–)-β-Pinene (**1′**) was metabolized by *Aspergillus niger* TBUYN-2 to give (+)-*trans*-pinocarveol (**2a′**), which was further metabolized to 6β-hydroxyfenchol (**349ba′**) and 6β, 7-dihydroxyfenchol

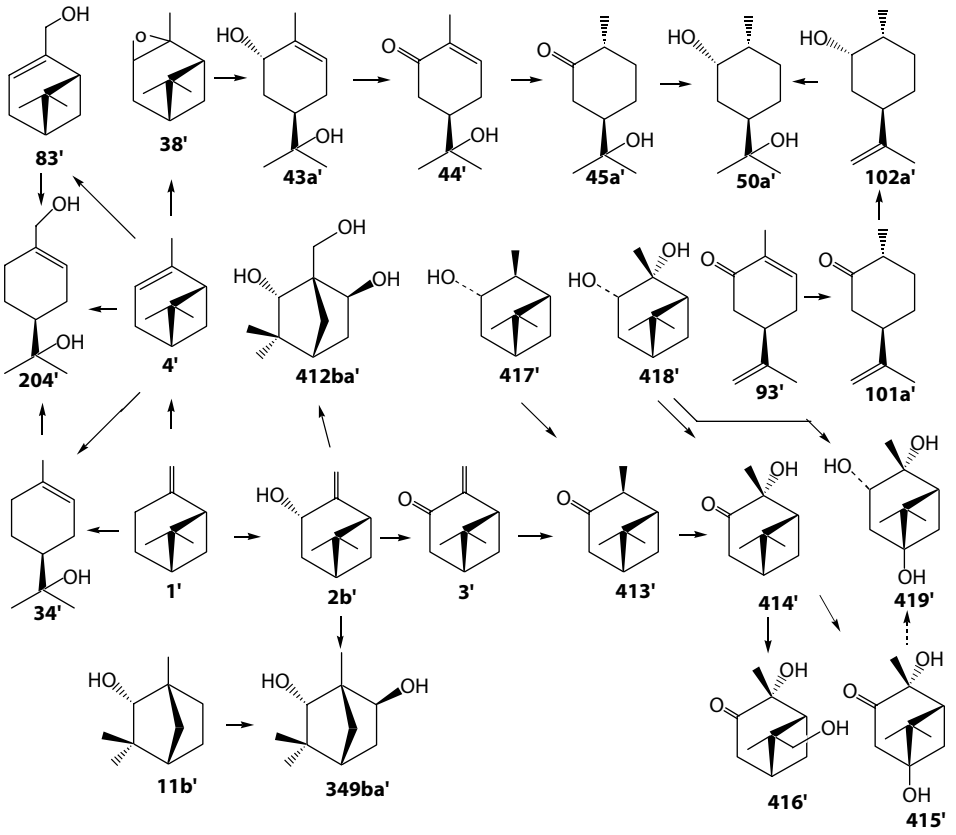


FIGURE 14.161 Biotransformation of (–)-β-pinene (**1′**), (–)-α-pinene (**4′**), and related compounds by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

(**412ba′**) by rearrangement reaction (Noma and Asakawa, 2005a) (Figure 14.162). 6β-Hydroxy-fenchol (**349ba′**) was also obtained from (–)-fenchol (**11b′**). (–)-Fenchone was hydroxylated by the same fungus to give 6β- (**13a′**) and 6α-hydroxy-(–)-fenchone (**13b′**). There is a close relationship between the metabolism of (–)-β-pinene (**1′**) and those of (–)-fenchol (**11′**) and (–)-fenchone (**12′**).

(–)-β-Pinene (**1′**) and (–)-α-pinene (**4′**) were isomerized to each other. Both are metabovlized via (–)-α-terpineol (**34**') to (–)-oleuropeyl alcohol (**204′**) and (–)-oleuropeic acid (**61′**). (–)-Myrtenol (**5′**) formed from (–)-α-pinene (**1′**) was further metabolized via cation to (–)-oleuropeyl alcohol (**204′**) and (–)-oleuropeic acid (**61′**). (–)-α-Pinene (**4′**) is further metabolized by *Aspergillus niger* TBUYN-2 via (–)-α-pinene epoxide (**38′**) to *trans*-sobrerol (**43a′**), (–)-8-hydroxycarvotanacetone (**44′**), (+)-8-hydroxycarvomenthone (**45a**), and mosquitocidal (+)-*p*-menthane-2,8-diol (**50a′**) (Battacharyya et al., 1960; Noma et al., 2001, 2002, 2003) (Figure 14.163).

The major metabolites of (–)-β-pinene (**1′**) were *trans*-10-pinanol (myrtanol) (**8ba′**) (39%) and (–)-1-*p*-menthene-7,8-diol (oleuropeyl alcohol) (**204′**) (30%). In addition, (+)-*trans*-pinocarveol (**2a′**) (11%) and (–)-α-terpineol (**34′**) (5%), verbenol (**23a** and **23b**) and pinocarveol (**2a′**) were oxidation products of α- (**4**) and β-pinene (**1**), respectively, in bark beetle, *Dendroctonus frontalis*. (–)-*Cis*- (**23b′**) and (+)-*trans*-verbenols (**23a′**) have pheromonal activity in *Ips paraconfussus* and *Dendroctonus brevicomis*, respectively (Ishida et al., 1981b) (Figure 14.164).

14.4.1.3 (±)-Camphene

Racemate camphene (**437** and **437′**) is a bicyclic monoterpene hydrocarbon found in *Liquidamar* species, *Chrysanthemum*, *Zingiber officinale*, *Rosmarinus officinalis*, and among other plants. It

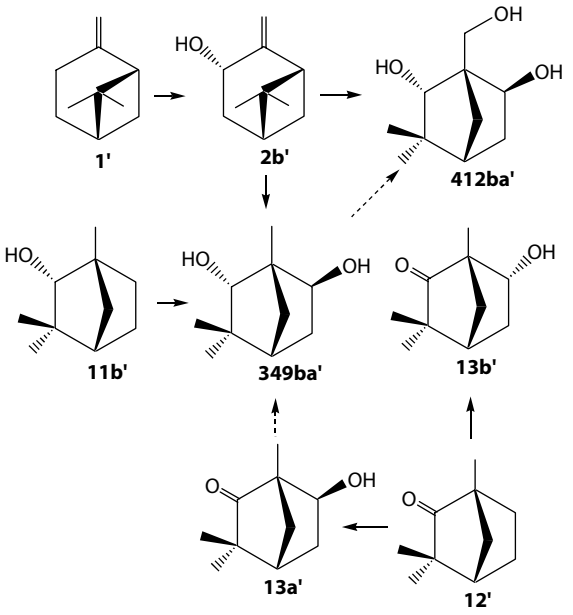


FIGURE 14.162 Relationship of the metabolism of (–)-β-pinene (**1′**), (+)-fenchol (**11′**) and (–)-fenchone (**12′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

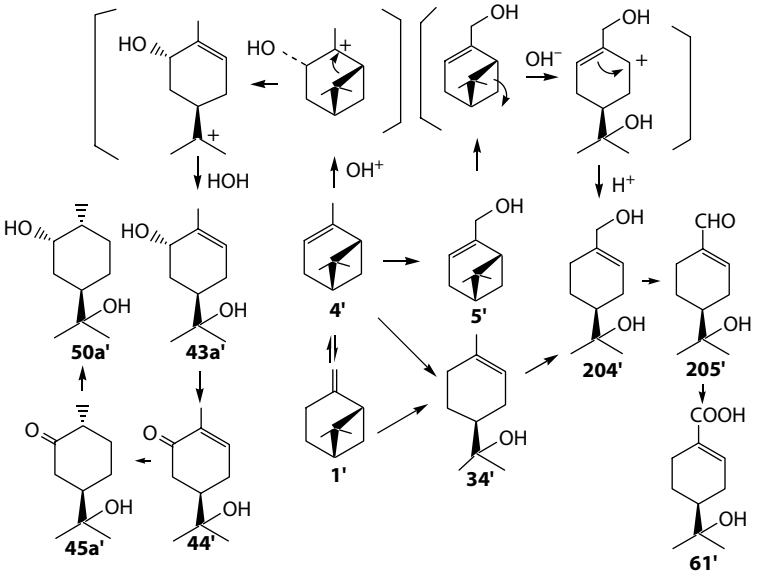


FIGURE 14.163 Metabolic pathways of (–)-β-pinene (**1′**) and related compounds by *Aspergillus niger* TBUYN-2 . (Modified from Bhattacharyya, P.K. et al., 1960. *Nature*, 187: 689–690; Noma, Y. et al., 2001. *Proc. 45th TEAC*, pp. 88–90; Noma, Y. et al., 2002. *Book of Abstracts of the 33rd ISEO*, p. 142; Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93.)

was administered into rabbits. Six metabolites, camphene-2,10-glycols (**438a, 438b**), which were the major metabolites, together with 10-hydroxytricyclene (**438c**), 7-hydroxycamphene (**438d**), 6-exo-hydroxycamphene **(438e**), and 3-hydroxytricyclene (**438f**) were obtained (Ishida et al., 1979). On the basis of the production of the glycols (**438a** and **438b**) in good yield, these alcohols might be formed through their epoxides as shown in Figure 14.165. The homoallyl camphene oxidation products (**438c**–**f**) apparently were formed through the non-classical cation as the intermediate.

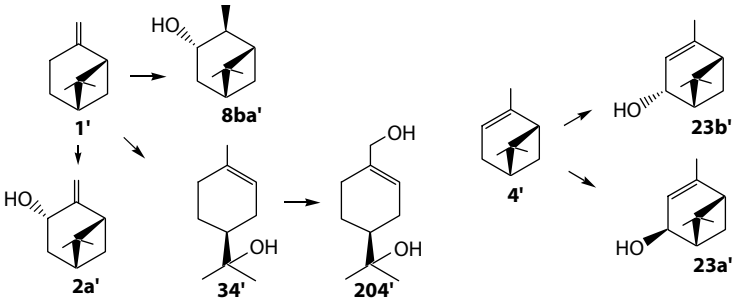


FIGURE 14.164 Metabolism of β-pinene (**1**) by bark beetle, *Dendroctonus frontalis*. (Modified from Ishida, T. et al., 1981b. *J. Pharm. Sci.*, 70: 406–415.)

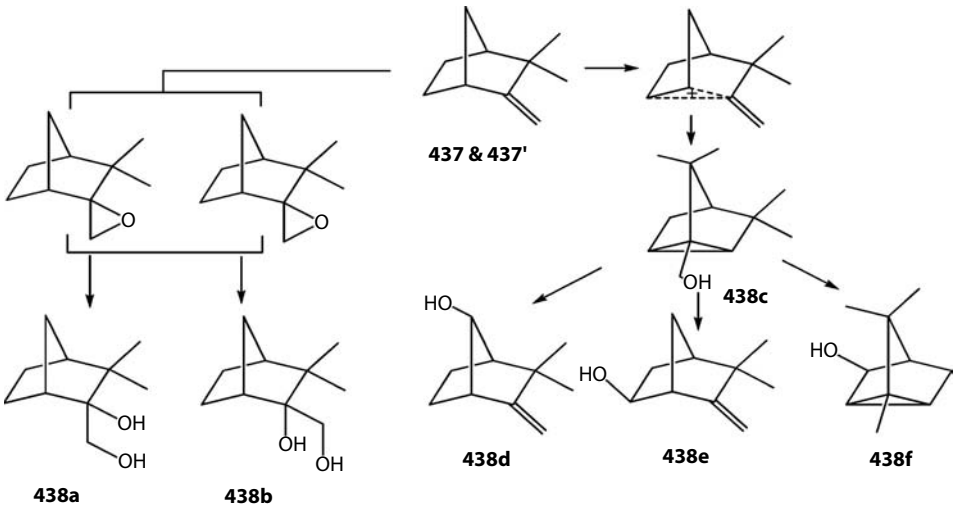
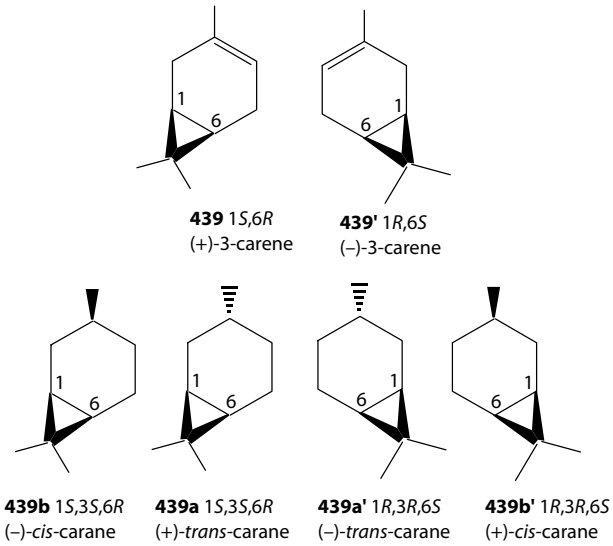


FIGURE 14.165 Biotransformation of (±)-camphene (**437** and **437′**) by rabbits. (Modified from Ishida, T. et al., 1979. *J. Pharm. Sci.*, 68: 928–930.)

14.4.1.4 3-Carene and Carane



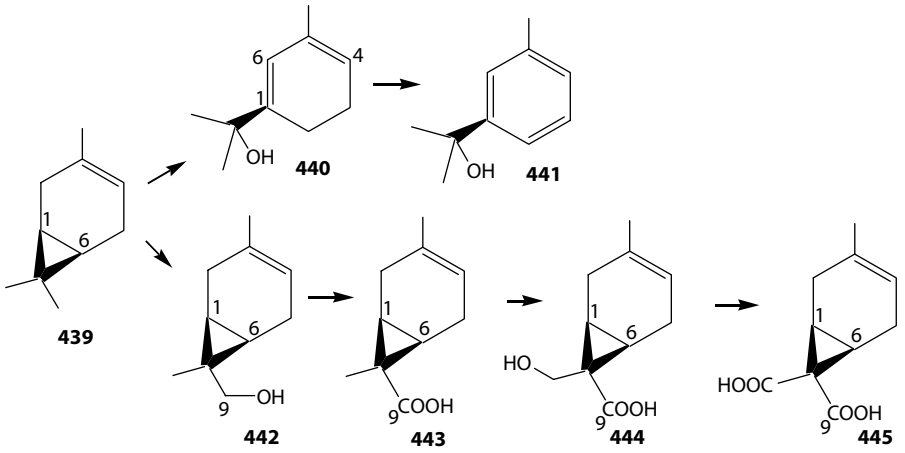


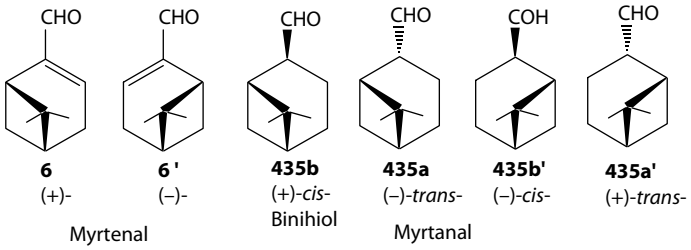
FIGURE 14.166 Metabolic pathways of (+)-3-carene (**439**) by rabbit (Modified from Ishida, T. et al., 1981b. *J. Pharm. Sci*., 70: 406–415). 3-(+)-Carene (**439**) was converted by *Aspergillus niger* NC 1M612 to give either hydroxylated compounds of 3-carene-2-one or 3-carene-5-one, which was not fully identified. (Modified from Noma, Y. et al., 2002. *Book of Abstracts of the 33rd ISEO*, p. 142) (Figure 14.167).

(+)-3-Carene (**439**) was biotransformed by rabbits to give *m*-mentha-4,6-dien-8-ol (**440**) (71.6%) as the main metabolite together with its aromatized *m*-cymen-8-ol (**441**). The position of C-5 in the substrate is thought to be more easily hydroxylated than C-2 by enzymatic systems in the rabbit liver. In addition to ring opening compound, 3-carene-9-ol (**442**), 3-carene-9-carboxylic acid (**443**), 3-carene-9,10-dicarboxilic acid (**445**), chamic acid, and 3-caren-10-ol-9-carboxylic acid (**444**) were formed. The formation of such compounds is explained by stereoselective hydroxylation and carboxylation of *gem*-dimethyl group (Ishida et al., 1981b) (Figure 14.166). In case of (–)-*cis*-carane (**446**), two C-9 and C-10 methyl groups were oxidized to give dicarboxylic acid (**447**) (Ishida et al., 1981b) (Figure 14.166).

3-(+)-Carene (**439**) was converted by *Aspergillus niger* NC 1M612 to give either hydroxylated compounds of 3-carene-2-one or 3-carene-5-one, which was not fully identified (Noma et al., 2002) (Figure 14.167).

14.4.2 Bicyclic Monoterpene Aldehyde

14.4.2.1 Myrtenal and Myrtanal



Euglena gracilis Z biotransformed (–)-myrtenal (**6′**) to give (–)-myrtenol (**5′**) as the major product and (–)-myetenoic acid (**7′**) as the minor product. However, further hydrogenation of (–)-myrtenol (**5′**) to *trans*- and *cis*-myrtanol (**8a** and **8b**) did not occur even at a concentration less than ca. 50 mg/L. (*S*)-*Trans* and (*R*)-*cis*-myrtanal (**435a′** and **435b′**) were also transformed to *trans*- and *cis*-myrtanol (**8a′** and **8b′**) as the major products and (*S*)-*trans*- and (*R*)-*cis*-myrtanoic acid (**436a′** and **436b′**) as the minor products, respectively (Noma et al., 1991a) (Figure 14.168).

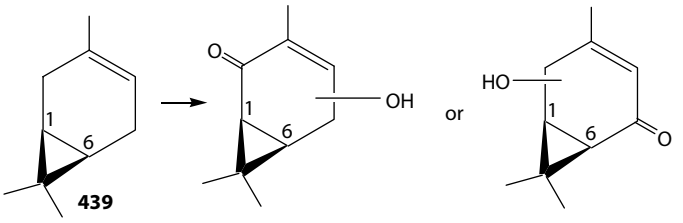


FIGURE 14.167 Metabolic pathways of (+)-3-Carene (**439**) by *Aspergillus niger* NC 1M612. (Modified from Noma, Y. et al., 2002. *Book of Abstracts of the 33rd ISEO*, p. 142.)

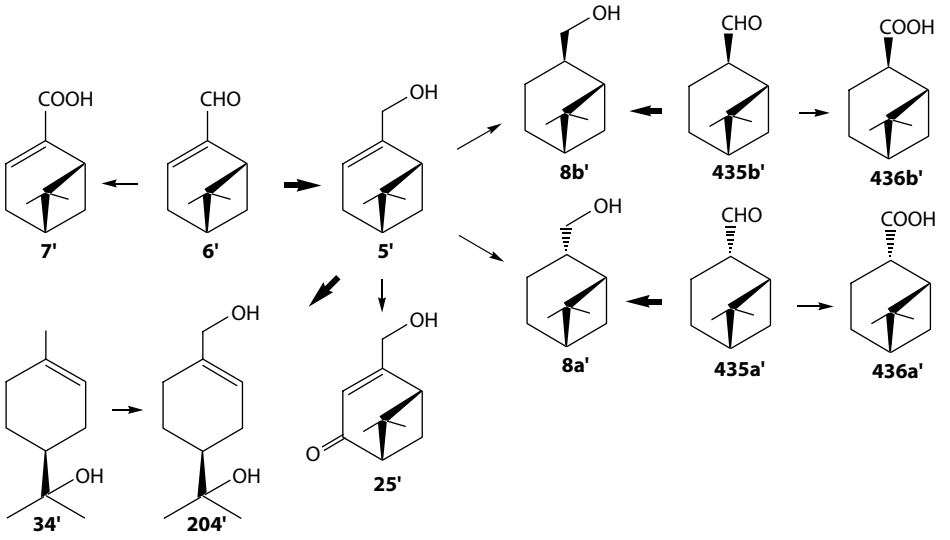


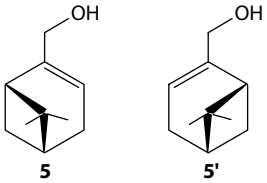
FIGURE 14.168 Biotransformation of (–)-myrtenal (**6′**) and (+)-*trans*- (**435a′**) and (–)-*cis*-myrtanal (**435b′**) by microorganisms. (Modified from Noma, Y. et al., 1991a. *Phytochem.*, 30: 1147–1151; Noma, Y. and Y. Asakawa, 2005b. *Proc. 49th TEAC*, pp. 78–80; Noma, Y. and Y. Asakawa, 2006b. *Book of Abstracts of the 37th ISEO*, p. 144.)

In case of *Aspergillus niger* TBUYN-2, *Aspergillus sojae*, and *Aspergillus usami*, (–)-myrtenol (**5′**) was further metabolized to 7-hydroxyverbenone (**25′**) as a minor product together with (–)-oleuropeyl alcohol (**204′**) as a major product (**279, 280**). (–)-Oleuropeyl alcohol (**204′**) is also formed from (–)-α-terpineol (**34**) by *Aspergillus niger* TBUYN-2 (Noma et al., 2001) (Figure 14.168).

Rabbits metabolized myrtenal (**6′**) to myrtenic acid (**7′**) as the major metabolite and myrtanol (**8a′** or **8b′**) as the minor metabolite (Ishida et al., 1981b) (Figure 14.168).

14.4.3 Bicyclic Monoterpene Alcohol

14.4.3.1 Myrtenol



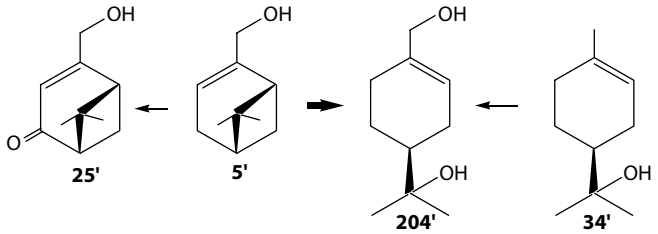


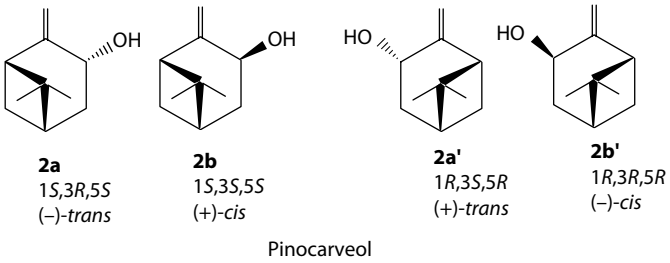
FIGURE 14.169 Biotransformation of (–)-myrtenol (**5′**) and (–)-α-terpineol (**34′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005b. *Proc. 49th TEAC*, pp. 78–80.)

(–)-Myrtenol (**5′**) was biotransformed mainly to (–)-oleuropeyl alcohol (**204′**), which was formed from (–)-α-terpineol (**34′**) as a major product by *Aspergillus niger*, TBUYN-2. In case of *Aspergillus sojae* IFO 4389 and *Aspergillus usami* IFO 4338, (–)-myrtenol (**5′**) was metabolized to 7-hydroxyverbenone (**25′**) as a minor product together with (–)-oleuropeyl alcohol (**204′**) as a major product (Noma and Asakawa, 2005b) (Figure 14.169).

14.4.3.2 Myrtanol

Spodoptera litura converted (–)-*trans*-myrtanol (**8a**) and its enantiomer (**8a′**) to give the corresponding myrtanic acid (**436** and **436′**) (Miyazawa et al., 1997b) (Figure 14.170).

14.4.3.3 Pinocarveol



(+)-*trans*-Pinocarveol (**2a′**) was biotransformed by *Aspergillus niger* TBUYN-2 to the following two pathways. Namely, (+)-*trans*-pinocarveol (**2a′**) was metabolized via (+)-pinocarvone (**3′**),

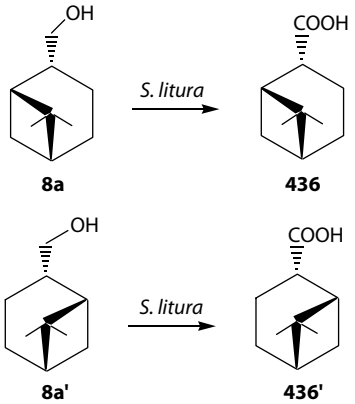
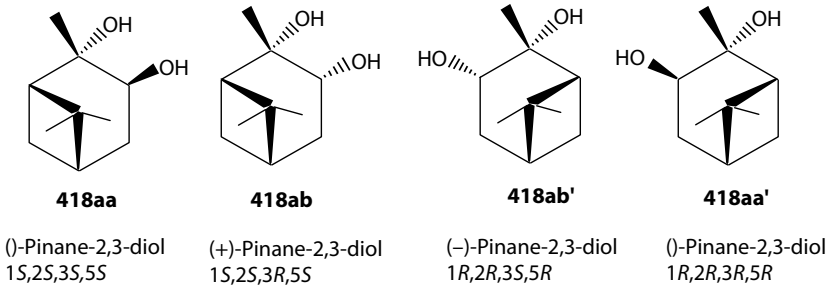


FIGURE 14.170 Biotransformation of (–)-*trans*-myrtanol (**8a**) and its enantiomer (**8a′**) by *Spodptera litura*. (Modified from Miyazawa, M. et al., 1997b. *Proc. 41st TEAC*, pp. 389–390.)

(–)-3-isopinanone (**413′**), and (+)-2α-hydroxy-3-pinanone (**414′**) to (+)-2α,5-dihydroxy-3-pinanone (**415′**) (pathway 1). Furthermore, (+)-*trans*-pinocarveol (**2a′**) was metabolized to epoxide followed by rearrangement reaction to give 6β-hydroxyfenchol (**349ba′**) and 6β,7-dihydroxyfenchol (**412ba′**) (Noma and Asakawa, 2005a) (Figure 14.171). *Spodoptera litura* converted (+)-*trans*-pinocarveol (**2a′**) to (+)-pinocarvone (**3′**) as a major product (Miyazawa et al., 1995c) (Figure 14.171).

14.4.3.4 Pinane-2,3-diol



This results led us to study the biotransformation of (–)-pinane-2,3-diol (**418ab′**) and (+)-pinane-2,3-diol (**418ab**) by *Aspergillus niger* TBUYN-2. (–)-Pinane-2,3-diol (**418ab′**) was easily biotransformed to give (–)-pinane-2,3,5-triol (**419ab′**) and (+)-2,5-dihydroxy-3-pinanone (**415a′**) as the major products and (+)-2-hydroxy-3-pinanone (**414a′**) as the minor product.

On the other hand, (+)-pinane-2,3-diol (**418ab**) was also biotransformed easily to give (+)-pinane-2,3,5-triol (**419ab**) and (–)-2,5-dihydroxy-3-pinanone (**415a**) as the major products and (–)-2-hydroxy-3-pinanone (**414a**) as the minor product (Noma et al., 2003) (Figure 14.172). *Glomerella cingulata* transformed (–)-pinane-2,3-diol (**418ab′**) to a small amount of (+)-2α-hydroxy-3-pinanone (**414ab′**, 5%) (Kamino and Miyazawa, 2005), whereas (+)-pinane-2,3-diol (**418ab**) was transformed to a small amount of (–)-2α-hydroxy-3-pinanone (**414ab**, 10%) and (–)-3-acetoxy-2α-pinanol (**433ab-Ac**, 30%) (Kamino et al., 2004) (Figure 14.172).

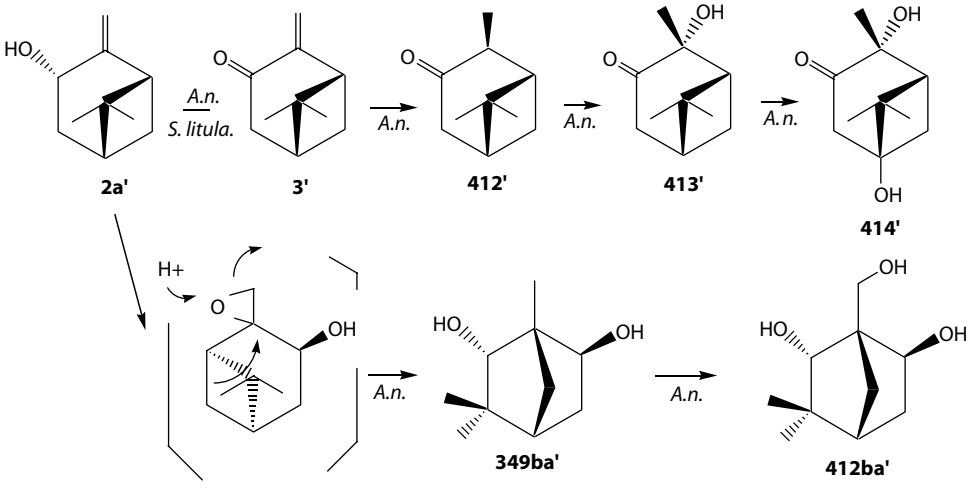


FIGURE 14.171 Biotransformation of (+)-*trans*-pinocarveol (**2a′**) by *Aspergillus niger* TBUYN-2 and *Spodptera litura*. (Modified from Miyazawa, M. et al., 1995c. *Proc. 39th TEAC*, pp. 360–361; Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

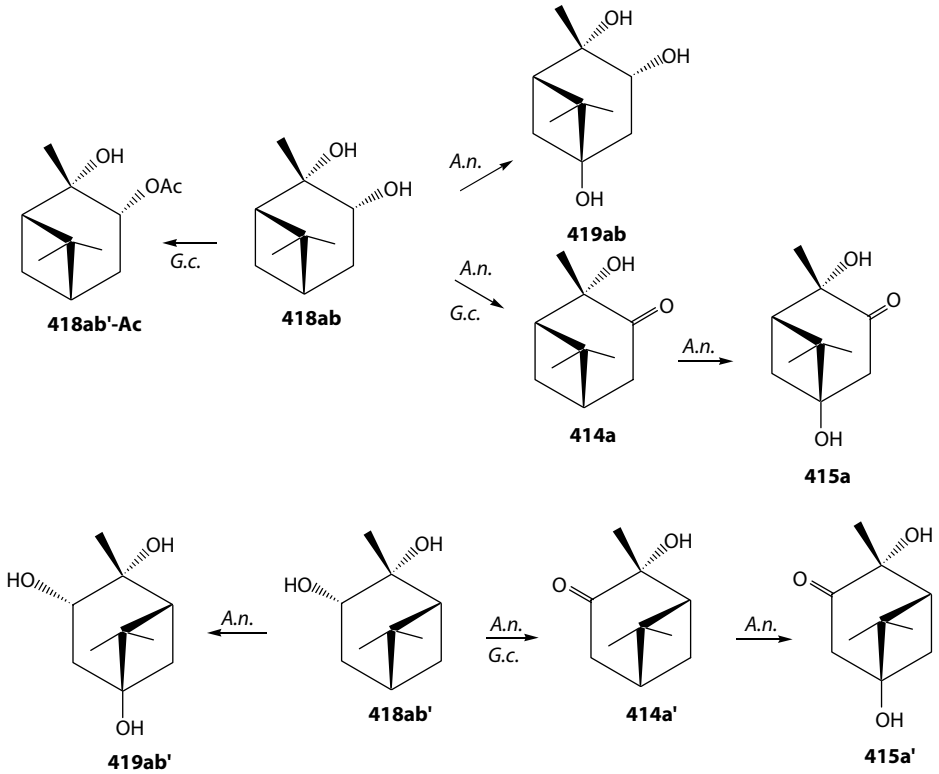
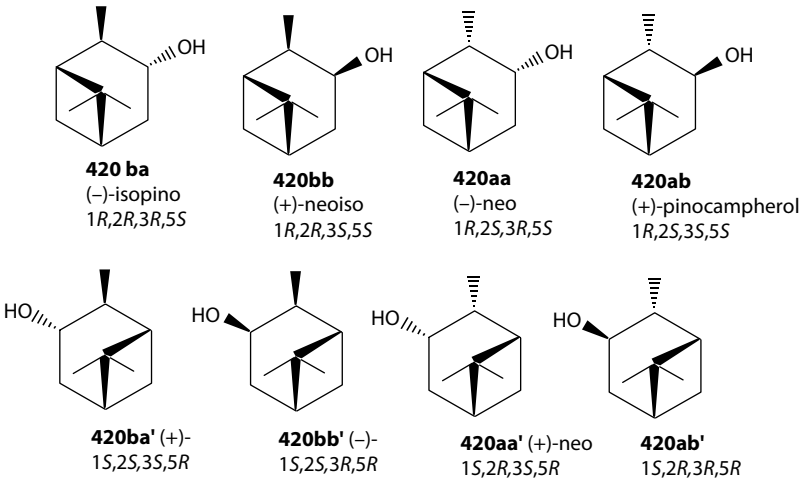


FIGURE 14.172 Biotransformation of (+)-pinane-2,3-diol (**418ab′**) and (–)-pinane-2,3-diol (**418ab′**) by *Aspergillus niger* TBUYN-2(**276**)] and *Glomerella cingulata*. (Modified from Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93; Kamino, F. et al., 2004. *Proc. 48th TEAC*, pp. 383–384; Kamino, F. and M. Miyazawa, 2005. *Proc. 49th TEAC*, pp. 395–396.)

14.4.3.5 Isopinocampheol (3-Pinanol)



*14.4.3.5.1 Chemical Structure of (–)-Isopinocampheol (**420ba**) and (+)-Isopinocampheol (**420ba′**)*

Biotransformation of isopinocampheol (3-pinanol) with 100 bacterial and fungal strains yielded 1-, 2-, 4-, 5-, 7-, 8-, and 9-hydroxyisopinocampheol besides three rearranged monoterpenes, one

of them bearing the novel isocarene skeleton. A pronounced enantioselectivity between (–)-(**420ba**) and (+)-isopinocampheol (**420ba′**) was observed. The phylogenetic position of the individual strains could be seen in their ability to form the products from (+)-isopinocampheol (**420ba′**). The formation of 1,3-dihydroxypinane (**421ba′**) is a domain of bacteria, while 3,5-(**415ba′**) or 3,6-dihydroxypinane (**428baa′**) was mainly formed by fungi, especially those of the phylum *Zygomycotina*. The activity of *Basidiomycotina* towards oxidation of isopinocampheol was rather low. Such informations can be used in a more effective selection of strains for screening (Wolf-Rainer, 1994) (Figure 14.173).

(+)-Isopinocampheol (**420ba′**) was metabolized to 4β-hydroxy-(+)-isopino-campheol (**424′**), 2β-hydroxy-(+)-isopinocampherol acetate (**425ba′-Ac**), and 2α-methyl,3-(2-methyl-2-hydroxypropyl)-cyclopenta-1β-ol (**432′**) (Wolf-Rainer, 1994) (Figure 14.174).

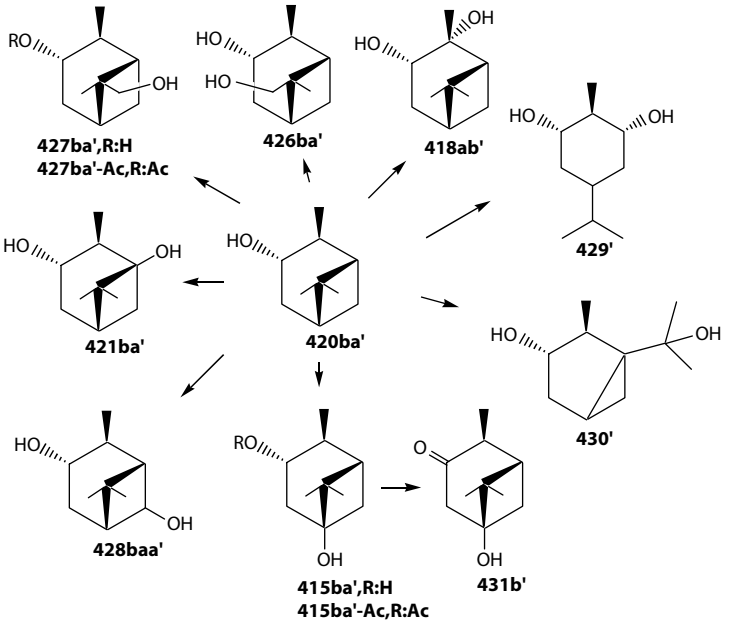


FIGURE 14.173 Metabolic pathways of (+)-isopinocampheol (**420ba′**) by microorganisms. (Modified from Wolf-Rainer, A., 1994. *Naturforsch*., 49c: 553–560.)

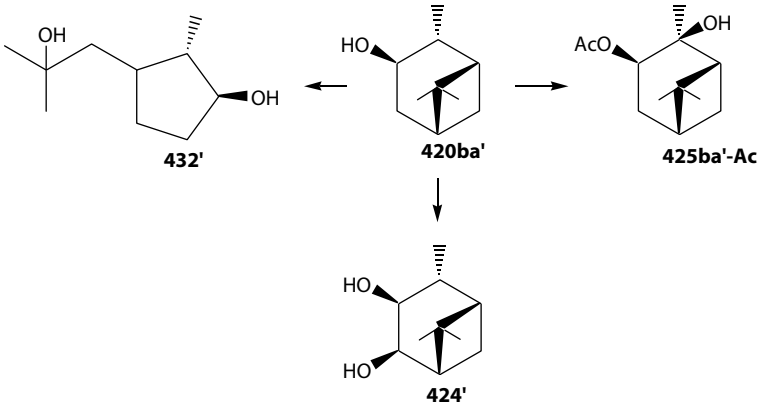


FIGURE 14.174 Metabolic pathways of (+)-isopinocampheol (**420ba′**) by microorganisms. (Modified from Wolf-Rainer, A., 1994. *Naturforsch*., 49c: 553–560.)

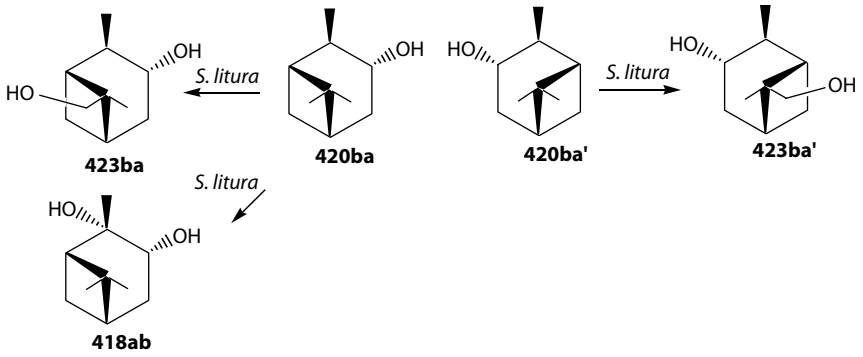


FIGURE 14.175 Biotransformation of (−)- (**420ba**) and (+)-isopinocampheol (**420ba′**) by *Spodptera litura*. (Modified from Miyazawa, M. et al., 1997c. *Phytochemistry*, 45: 945–950.)

(−)-Isopinocampheol (**420ba**) was converted by *Spodoptera litura* to give (1*R*,2*S*,3*R*,5*S*)-pinane-2,3-diol (**418ba**) and (−)-pinane-3,9-diol (**423ba**), whereas (+)-isopinocampheol (**420ba′**) was converted to (+)-pinane-3,9-diol (**423ba′**) (Miyazawa et al., 1997c) (Figure 14.175).

(−)-Isopinocampheol (**420ba**) was biotransformed by *Aspergillus niger* TBUYN-2 to give (+)-(1*S*,2*S*,3*S*,5*R*)-pinane-3,5-diol (**422ba**, 6.6%), (−)-(1*R*,2*R*,3*R*,5*S*)-pinane-1,3-diol (**421ba**, 11.8%), and pinane-2,3-diol (**418ba**, 6.6%), whereas (+)-isopinocampheol (**420ba′**) was biotransformed by *Aspergillus niger* TBUYN-2 to give (+)-(1*S*,2*S*,3*S*,5*R*)-pinane-3,5-diol (**422ba′**, 6.3%) and (−)-(1*R*,2*R*,3*R*,5*S*)-pinane-1,3,-diol (**421ba′**, 8.6%) (Noma et al., 2009) (Figure 14.176). On the other hand, *Glomerella cingulata* converted (−)- (**420ba**) and (+)-isopinocampheol (**420ba′**) mainly to (1*R*,2*R*,3*S*,4*S*,5*R*)-3,4-pinanediol (**484ba**) and (1*S*,2*S*,3*S*,5*R*,6*R*)-3,6-pinanediol (**485ba′**), respectively, together with (**418ba**), (**422ba**), (**422ba′**), and (**486ba′**) as minor products (Miyazawa et al., 1997c) (Figure 14.176). Some similarities exist between the main metabolites with *Glomerella cingulata* and *Rhizoctonia solani* (Miyazawa et al., 1997c) (Figure 14.176).

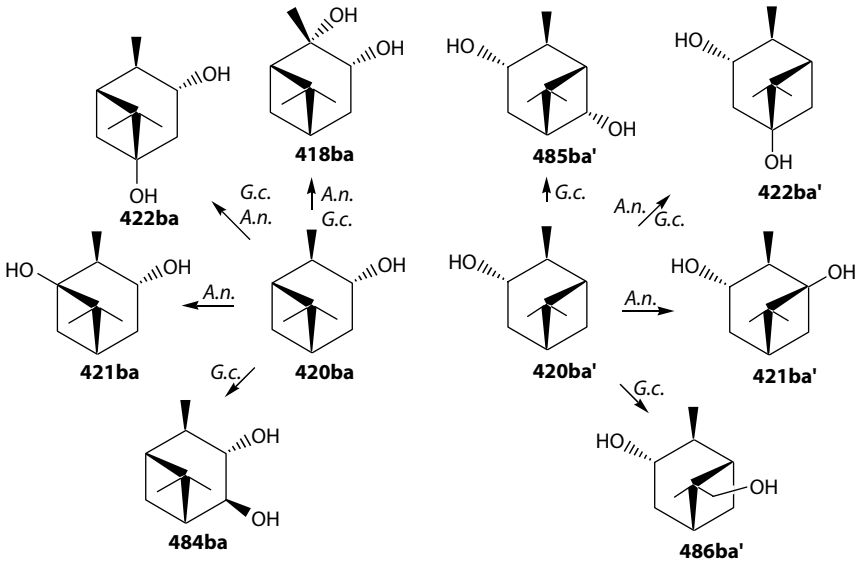
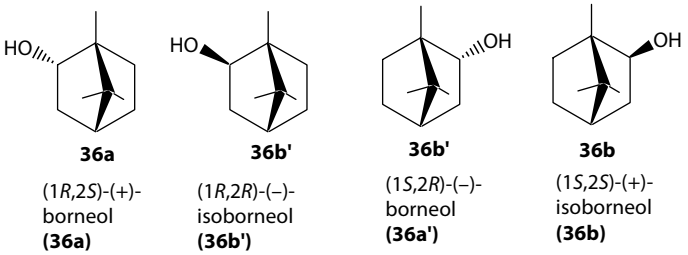


FIGURE 14.176 Biotransformation of (−)- (**420ba**) and (+)-isopinocampheol (**420ba′**) by *Aspergillus niger* TBUYN-2 and *Glomerella cingulata*. (Modified from Miyazawa, M. et al., 1997c. *Phytochemistry*, 45: 945–950; Noma, Y. et al., 2009. unpublished data.)

14.4.3.6 Borneol and Isoborneol



(–)-Borneol (**36a′**) was biotransformed by *Pseudomonas pseudomonallei* strain H to give (–)-camphor (**37′**), 6-hydroxycamphor (**228′**), and 2,6-diketocamphor (**229′**) (Hayashi et al., 1969) (Figure 14.177).

Euglena gracilis Z. showed enantio- and diastereoselectivity in the biotransformation of (+)- (**36a**), (–)- (**36a′**), and (±)-racemic borneols (equal mixture of **36a** and **36a′**) and (+)- (**36b**), (–)- (**36b′**), and (±)-isoborneols (equal mixture of **36b** and **36b′**). The enantio- and diastereoselective dehydrogenation for (–)-borneol (**36a′**) was carried out to give (–)-camphor (**37′**) at *ca.* 50% yield (Noma et al., 1992d; Noma and Asakawa, 1998). The conversion ratio was always *ca.* 50% even at different kinds of concentration of (–)-borneol (**36a′**). When (–)-camphor (**37′**) was used as a substrate, it was also converted to (–)-borneol (**36a′**) in 22% yield for 14 days. Furthermore, (+)-camphor (**37**) was also reduced to (+)-borneol (**36a**) in 4% and 18% yield for 7 and 14 days, respectively (Noma et al., 1992d, Noma and Asakawa, 1998) (Figure 14.178).

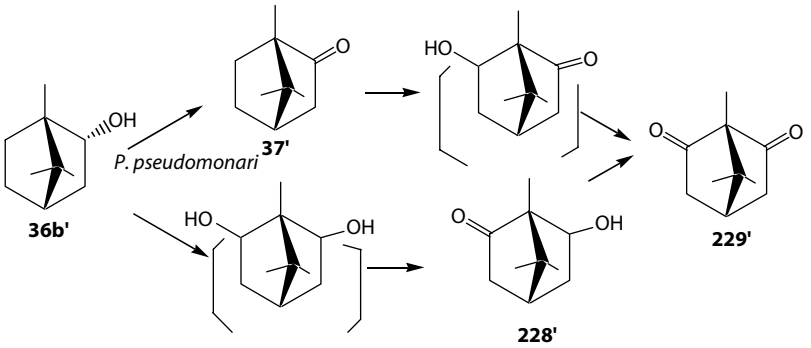


FIGURE 14.177 Biotransformation of (–)-borneol (**36a′**) by *Pseudomonas pseudomonallei* strain. (Modified from Hayashi, T. et al., 1969. *J. Agric. Chem. Soc. Jpn*., 43: 583–587.)

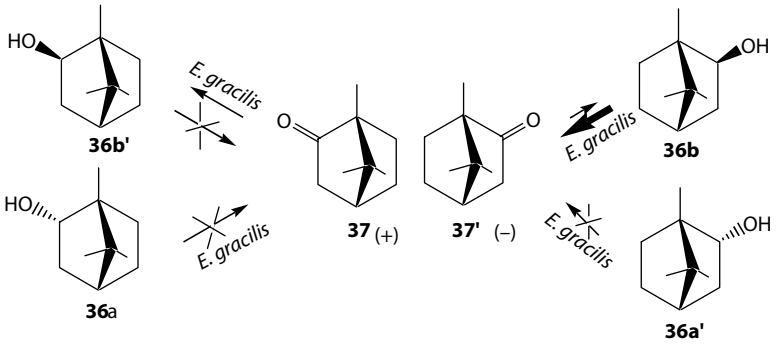


FIGURE 14.178 Enantio- and diastereoselectivity in the biotransformation of (+)- (**36a**) and (–)-borneols (**36a′**) by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1992d. *Proc. 36th TEAC*, pp. 199–201; Noma, Y. and Y. Asakawa, 1998. *Biotechnology in Agriculture and Forestry, Vol. 41. Medicinal and Aromatic Plants X*, Y.P.S. Bajaj, ed., pp. 194–237. Berlin Heidelberg: Springer.)

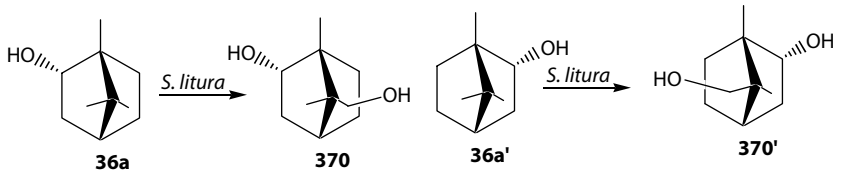
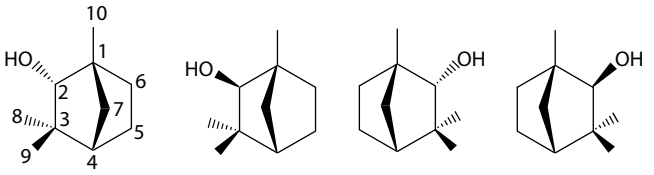


FIGURE 14.179 Biotransformation of (+)- (**36a**) and (–)-borneols (**36a′**) by *Spodptera litura*. (Modified from Miyamoto, Y. and M. Miyazawa, 2001. *Proc. 45th TEAC*, pp. 377–378.)

(+)- (**36a**) and (–)-Borneols (**36a′**) were biotransformed by *Spodoptera litura* to (+)- (**370a**) and (–)-bornane-2,8-diols (**370a′**), respectively (Miyamoto and Miyazawa, 2001) (Figure 14.179).

14.4.3.7 Fenchol and Fenchyl Acetate



(1*R*,2*R*,4*S*)-(+)-Fenchol (**11a**) was converted by *Aspergillus niger* TBUYN-2 and *Aspergillus cellulosae* IFO 4040 to give (–)-fenchone (**12**), (+)-6β-hydroxyfenchol (**349ab**), (+)-5β-hydroxyfenchol (**350ab**) and 5α-hydroxyfenchol (**350aa**) (Noma and Asakawa, 2005a) (Figure 14.180).

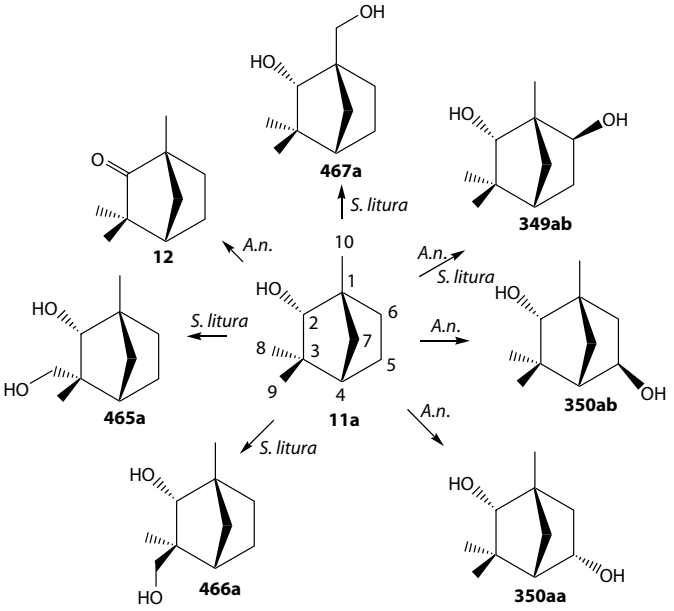


FIGURE 14.180 Biotransformation of (+)-fenchol (**11a**) by *Aspergillus niger* TBUYN-2, *Aspergillus cellulosae* IFO 4040, and the larvae of common cutworm, *Spodptera litura*. (Modified from Miyazawa, M. and Y. Miyamoto, 2004. *Tetrahadron*, 60: 3091–3096; Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

The larvae of common cutworm, *Spodoptera litura*, converted (+)-fenchol (**11a**) to (+)-10-hydroxyfenchol (**467a**), (+)-8-hydroxyfenchol (**465a**), (+)-6β-hydroxyfenchol (**349ab**), and (−)-9-hydroxyfenchol (**466a**) (Miyazawa and Miyamoto, 2004) (Figure 14.180).

(+)-*trans*-Pinocarveol (**2**), which was formed from (−)-β-pinene (**1**), was metabolized by *Aspergillus niger* TBUYN-2 to 6β-hydroxy- (+)-fenchol (**349ab**) and 6β,7-dihydroxy-(+)-fenchol (**412ba′**). (−)-Fenchone (**12**) was also metabolized to 6α-hydroxy- (**13b**) and 6β-hydroxy- (−)-fenchone (**13a**). (+)-Fenchol (**11**) was metabolized to 6β-hydroxy-(+)-fenchol (**349ab**) by *Aspergillus niger* TBUYN-2. Relationship of the metabolisms of (+)-*trans*-pinocarveol (**2**), (−)-fenchone (**12**), and (+)-fenchol (**11**) by *Aspergillus niger* TBUYN-2 is shown in Figure 14.181 (Noma and Asakawa 2005a).

(+)-α-Fencyl acetate (**11a-Ac**) was metabolized by *Glomerella cingulata* to give (+)-5-β-hydroxy-α-fencyl acetate (**350a-Ac**, 50%) as the major metabolite and (+)-fenchol (**11a**, 20%) as the minor metabolite (Miyazato and Miyazawa 1999). On the other hand, (−)-α-fencyl acetate (**11a′-Ac**) was metabolized to (−)-5-β-hydroxy-α-fencyl acetate (**350a′-Ac**, 70%) and (−)-fenchol (**11a′**, 10%) as the minor metabolite by *Glomerella cingulata* (Miyazato and Miyazawa, 1999) (Figure 14.182).

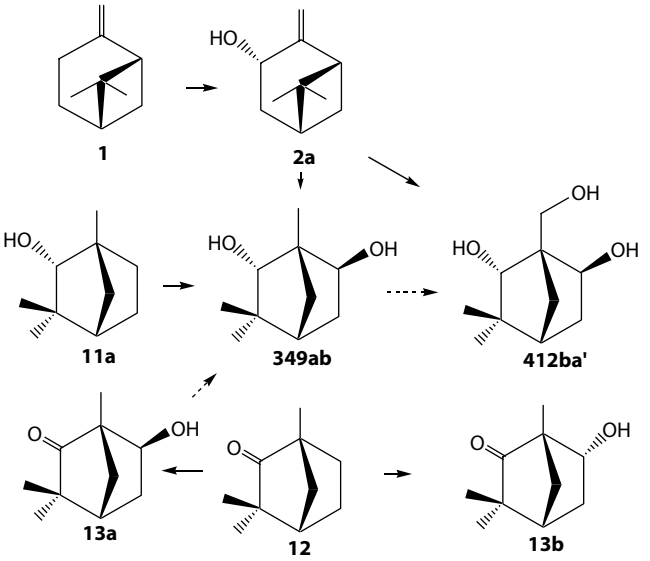


FIGURE 14.181 Metabolism of (+)-*trans*-pinocarveol (**2**), (−)-fenchone (**12**), and (+)-fenchol (**11**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

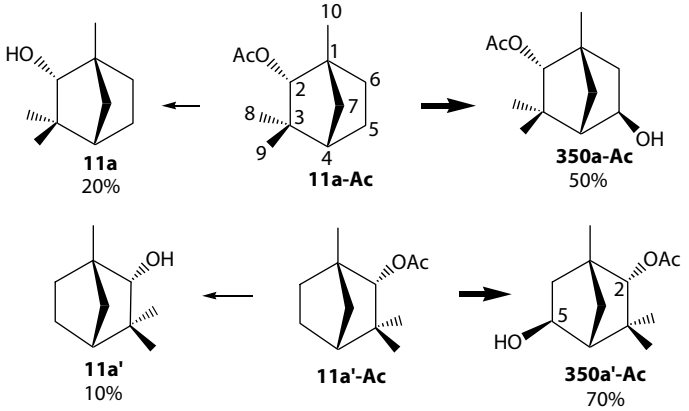
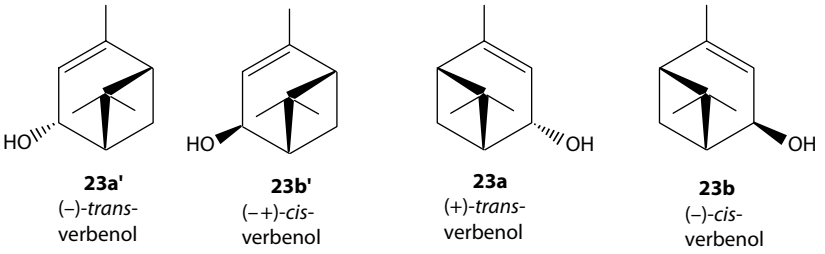


FIGURE 14.182 Biotransformation of (+)- (**11a-Ac**) and (−)-α-fencyl acetate (**11a′-Ac**) by *Glomerella cingulata*. (Modified from Miyazato, Y. and M. Miyazawa, 1999. *Proc. 43rd TEAC*, pp. 213–214.)

14.4.3.8 Verbenol



(–)-*trans*-Verbenol (**23a′**) was biotransformed by *Spodoptera litura* to give 10-hydroxyverbenol (**451a′**). Furthermore, (–)-verbenone (**24′**) was also biotransformed in the same manner to give 10-hydroxyverbenone (**25′**) (Yamanaka and Miyazawa, 1999) (Figure 14.183).

14.4.3.9 Nopol and Nopol Benzyl Ether

Biotransformation of (–)-nopol (**452′**) was carried out at 30°C for 7 days at the concentration of 100 mg/200 mL medium by *Aspergillusniger* TBUYN-2, *Aspergillus sojae* IFO 4389, and *Aspergillus usami* IFO 4338. (–)-Nopol (**452′**) was incubated with *Aspergillus niger* TBUYN-2 to give 7-hydroxymethyl-1-*p*-menthen-8-ol (**453′**). In cases of *Aspergillus sojae* IFO 4389 and *Aspergillus usami* IFO 4338, (–)-nopol (**452′**) was metabolized to 3-oxonopol (**454′**) as a minor product together with 7-hydroxymethyl-1-*p*-menthen-8-ol (**453′**) as a major product (Noma and Asakawa, 2005b; 2006c) (Figure 14.184).

Biotransformation of (–)-nopol benzyl ether (**455′**) was carried out at 30°C for 8–13 days at the concentration of 277 mg/200 mL medium by *Aspergillus niger* TBUYN-2, *Aspergillus sojae* IFO 4389, and *Aspergillus usami* IFO 4338. (–)-Nopol benzyl ether (**455′**) was biotransformed by *Aspergillus niger* TBUYN-2 to give 4-oxonopl-2′, 4′-dihydroxy benzyl ether (**456′**), and (–)-oxonopol (**454′**). 7-Hydroxymethyl-1-*p*-menthen-8-ol benzyl ether (**457′**) was not formed at all (Figure 14.185).

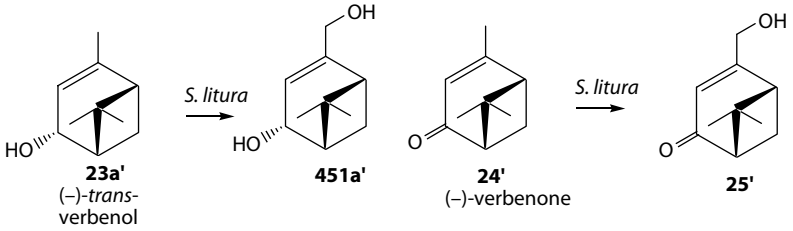


FIGURE 14.183 Metabolism of (–)-*trans*-verbenol (**23a′**) and (–)-verbenone (**24′**) by *Spodptera litura*. (Modified from Yamanaka, T. and M. Miyazawa, 1999. *Proc. 43rd TEAC*, pp. 391–392.)

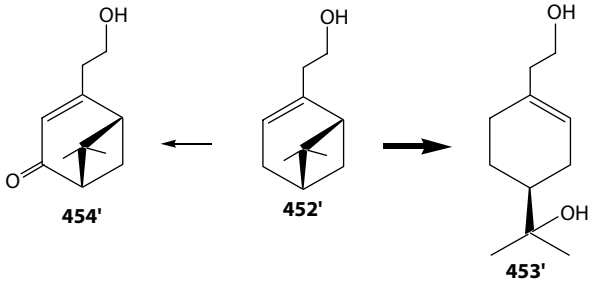


FIGURE 14.184 Biotransformation of (–)-nopol (**452′**) by *Aspergillus niger,* TBUYN-2, *Aspergillus sojae* IFO 4389 and *Aspergillus usami* IFO 4338. (Modified from Noma, Y. and Y. Asakawa, 2005b. *Proc. 49th TEAC*, pp. 78–80; Noma, Y. and Y. Asakawa, 2006c. *Proc. 50th TEAC*, pp. 434–436.)

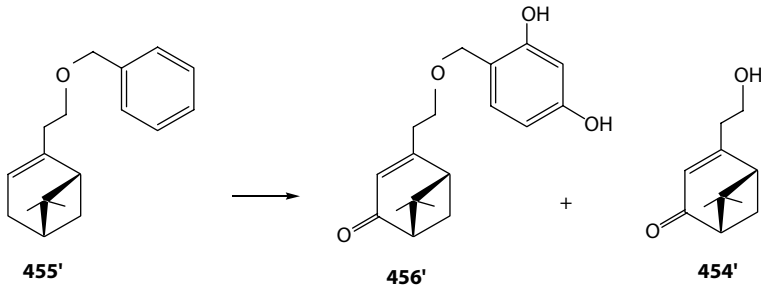


FIGURE 14.185 Biotransformation of (–)-Nopol benzyl ether (**455′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2006b. *Book of Abstracts of the 37th ISEO*, p. 144; Noma, Y. and Y. Asakawa, 2006c. *Proc. 50th TEAC*, pp. 434–436.)

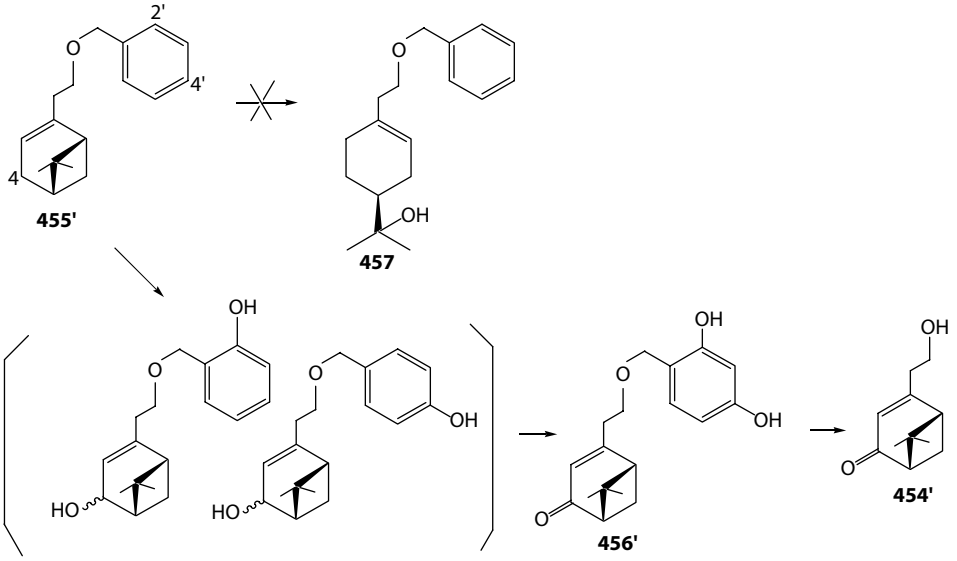


FIGURE 14.186 Proposed metabolic pathways of (–)-nopol benzyl ether (**455′**) by microorganisms. (Modified from Noma, Y. and Y. Asakawa, 2006b. *Book of Abstracts of the 37th ISEO*, p. 144; Noma, Y. and Y. Asakawa, 2006c. *Proc. 50th TEAC*, pp. 434–436.)

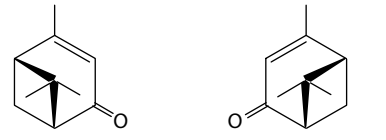
4-Oxonopol-2',4'-dihydroxybenzyl ether (**456′**) shows strong antioxidative activity (IC_{50} 30.23 μM). Antioxidative activity of 4-oxonopol-2',4'-dihydroxybenzyl ether (**456′**) is the same as that of butyl hydroxyl anisol (BHA) (Noma and Asakawa, 2006b,c).

Citrus pathogenic fungi, *Aspergillus niger* Tiegh (CBAYN) also transformed (–)-nopol (**452′**) to (–)-oxonopol (**454′**) and 4-oxonopol-2',4'-dihydroxybenzyl ether (**456′**) (Noma and Asakawa, 2006b,c) (Figure 14.186).

14.4.4 Bicyclic Monoterpene Ketones

14.4.4.1 α-, β-Unsaturated Ketone

14.4.4.1.1 Verbenone



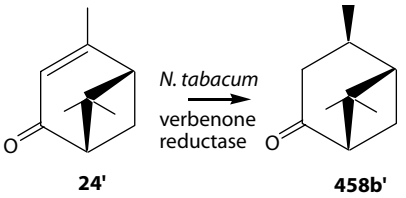


FIGURE 14.187 Hydrogenation of (–)-verbenone (**24′**) to (–)-isoverbanone (**458b′**) by verbenone reductase of *Nicotiana tabacum*. (Modified from Suga, T. and T. Hirata, 1990. *Phytochemistry*, 29: 2393–2406; Shimoda, K. et al., 1996. *J. Chem. Soc., Perkin Trans.* 1, 355–358; Shimoda, K. et al., 1998. *Phytochem.*, 49: 49–53; Shimoda, K. et al., 2002. *Bull. Chem. Soc. Jpn*., 75: 813–816; Hirata, T. et al., 2000. *Chem. Lett*., 29: 850–851.)

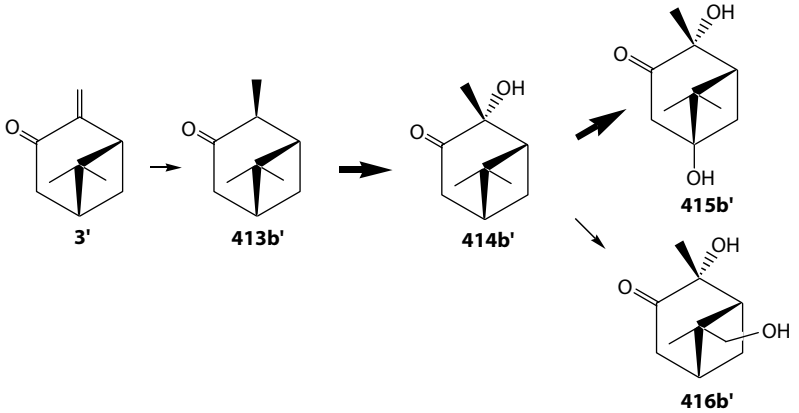
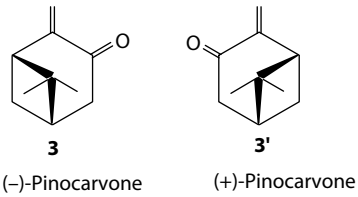


FIGURE 14.188 Biotransformation of (+)-pinocarvone (**3′**) by *Aspergillusniger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

(–)-Verbenone (**24′**) was hydrogenated by reductase of *Nicotiana tabacum* to give (–)-isoverbanone (**458b′**) (Suga and Hirata, 1990; Shimoda et al., 1996, 1998, 2002; Hirata et al., 2000) (Figure 14.187).

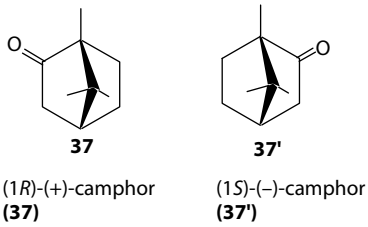
14.4.4.1.2 Pinocarvone



Aspergillus niger TBUYN-2 transformed (+)-pinocarvone (**3′**) to give (–)-isopinocamphone (**413b′**), 2α-hydroxy-3-pinanone (**414b′**), 2α, 5-dihydroxy-3-pinanone (**415b′**) together with small amounts of 2α, 10-dihydroxy-3-pinanone (**416b′**) (Noma and Asakawa, 2005a) (Figure 14.188).

14.4.4.2 Saturated Ketone

14.4.4.2.1 Camphor



(+)- (**37**) and (–)-Camphor (**37′**) are found widely in nature, of which (+)-camphor (**37**) is more abundant. It is the main component of oils obtained from the camphor tree *Cinnamomum camphora* (Bauer et al., 1990). The hydroxylation of (+)-camphor (**37**) by *Pseudomonas putida* C_1 was described (Abraham et al., 1988). The substrate was hydroxylated exclusively in its 5-exo- (**235b**) and 6-exo- (**228b**) positions.

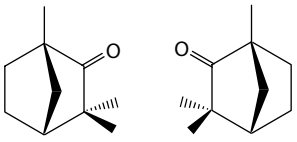
Although only limited success was achieved in understanding the catabolic pathways of (+)-camphor (**37**), key roles for methylene group hydroxylation and biological Baeyer–Villiger monooxygenases in ring cleavage strategies were established (Trudgill, 1990). A degradation pathway of (+)-camphor (**37**) by *Pseudomonas putida* ATCC 17453 and *Mycobacaterium rhodochorus* T_1 was proposed (Trudgill, 1990).

The metabolic pathway of (+)-camphor (**37**) by microorganisms is shown in Figure 14.189. (+)-Camphor (**37**) is metabolized to 3-hydroxy- (**243**), 5-hydroxy- (**235**), 6-hydroxy- (**228**), and 9-hydroxycamphor (**225**) and 1,2- campholide (**237**). 6-Hydroxycamphor (**228**) is degradatively metabolized to 6-oxocamphor (**229**) and 4-carboxymethyl-2,3,3-trimethylcyclopentanone (**230**), 4-carboxymethyl-3,5,5-trimethyltetrahydro-2-pyrone (**231**), isohydroxy-camphoric acid (**232**), isoketocamphoric acid (**233**), and 3,4,4-trimethyl-5-oxo-*trans*-2-hexenoic acid (**234**), whereas 1,2-campholide (**237**) is also degradatively metabolized to 6-hydroxy-1,2-campholide (**238**), 6-oxo-1, 2-campholide (**239**), and 5-carboxymethyl-3,4,4-trimethyl-2-cyclopentenone (**240**), 6-carboxymethyl-4,5,5-trimethyl-5,6-dihydro-2-pyrone (**241**) and 5-carboxymethyl-3,4,4-trimethyl-2-heptene-1,7-dioic acid (**242**). 5-Hydroxycamphor (**235**) is metabolized to 6-hydroxy-1,2-campholide (**238**), 5-oxo-camphor (**236**), and 6-oxo-1,2-campholide (**239**). 3-Hydroxycamphor (**243**) is also metabolized to camphorquinone (**244**) and 2-hydroxyepicamphor (**245**) (Bradshaw et al., 1959; Conrad et al., 1961, 1965a, 1965b; Gunsalus et al., 1965; Chapman et al., 1966; Hartline and Gunsalus, 1971; Oritani and Yamashita, 1974) (Figure 14.189).

Human CYP 2A6 converted (+)-camphor (**37**) and (–)-camphor (**37′**) to 6-*endo*-hydroxycamphor (**228a**) and 5-*exo*-hydroxycamphor (**235b**), while rat CYP 2B1 did 5-*endo*- (**235a**), 5-*exo*- (**235b**) and 6-*endo*-hydroxycamphor (**228a**) and 8-hydroxycamphor (**225**) (Gyoubu and Miyazawa 2006) (Figure 14.190).

(+)-Camphor (**37**) was glycosylated by *Eucalyptus perriniana* suspension cells to (+)-camphor monoglycoside (3 new, 11.7%) (Hamada et al., 2002) (Figure 14.191).

14.4.4.2.2 Fenchone



12 (+)- **12'** (–)-

(+)-Fenchone (**12**) was incubated with *Corynebacterium* sp. (Chapman et al., 1965) and *Absidia orchidis* (Pfrunder and Tamm, 1969a) give 6β-hydroxy- (**13a**) and 5β-hydroxyfenchones (**14a**) (Figure 14.191). On the other hand, *Aspergillus niger* biotransformed (+)-fenchone (**12**) to (+)-6α-(**13b**) and (+)-5α-hydroxyfenchones (**14b**) (Miyazawa et al., 1990a, 1990b) and 5-oxofenchone (**15**), 9-formylfenchone (**17b**), and 9-carboxyfenchone (**18b**) (Miyazawa et al., 1990a, 1990b) (Figure 14.192).

Furthermore, *Aspergillus niger* biotransformed (–)-fenchone (**12′**) to 5α-hydroxy- (**14b′**) and 6α-hydroxyfenchones (**13b′**) (Yamamoto et al., 1984) (Figure 14.193).

(+)- and (–)-Fenchone (**12** and **12′**) were converted to 6β-hydroxy- (**13a**, **13a′**), 6α-hydroxyfenchone (**13b**, **13b′**), and 10 hydroxyfenchone (**4**, **4′**) by P-450. Of the 11 recombinant human P450 enzymes tested, CYP2A6, CYP2B6 catalyzed oxidation of (+)- (**12**) and (–)-fenchone (**12′**) (Gyoubu and Miyazawa, 2005) (Figure 14.194).

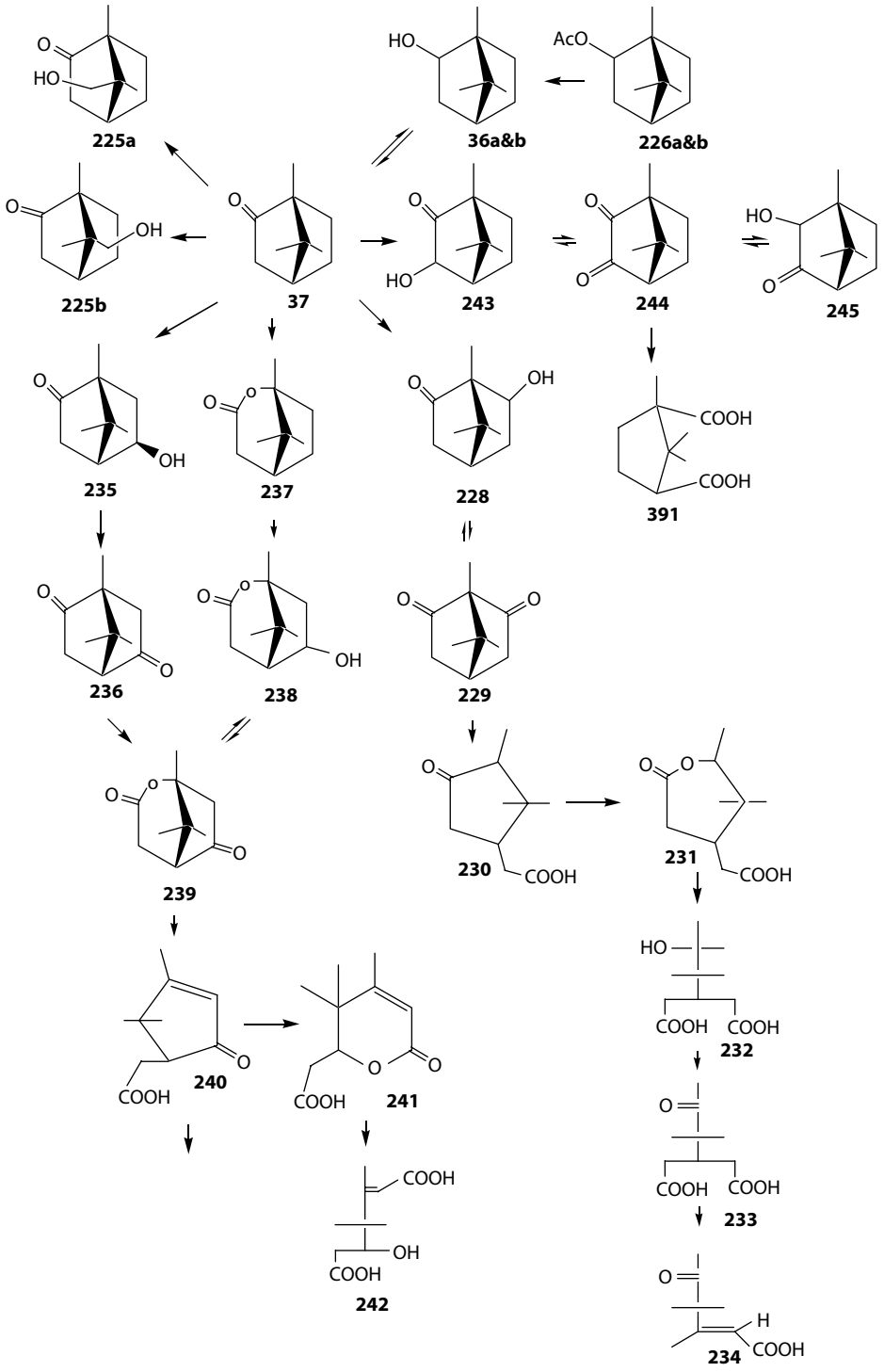


FIGURE 14.189 Metabolic pathways of (+)-camphor (**37**) by *Pseudomonas putida* and *Corynebacterium diphtheroides*. (Modified from Bradshaw, W.H. et al., 1959. *J. Am. Chem. Soc*., 81: 5507; Conrad, H.E. et al., 1961. *Biochem. Biophys. Res. Commun*., 6: 293–297; Conrad, H.E. et al., 1965a. *J. Biol. Chem*., 240: 495–503; Conrad, H.E. et al., 1965b. *J. Biol. Chem*., 240: 4029–4037; Gunsalus, I.C. et al., 1965. *Biochem. Biophys. Res. Commun*., 18: 924–931; Chapman, P.J. et al., 1966. *J. Am. Chem. Soc*., 88: 618–619; Hartline, R.A. and I.C. Gunsalus, 1971. *J. Bacteriol*., 106: 468–478; Oritani, T. and K. Yamashita, 1974. *Agric. Biol. Chem*., 38: 1961–1964.)

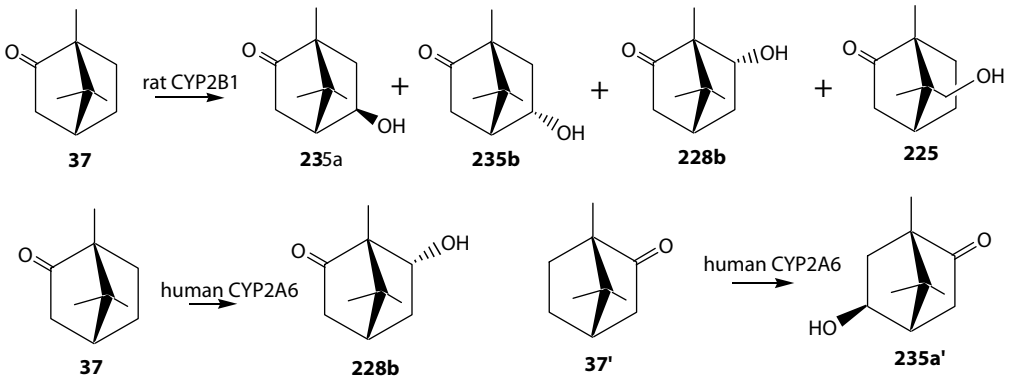


FIGURE 14.190 Biotransformation of (+)-camphor (**37**) by rat P450 enzyme (above) and (+)- (**37**) and (−)-camphor (**37′**) by human P450 enzymes.

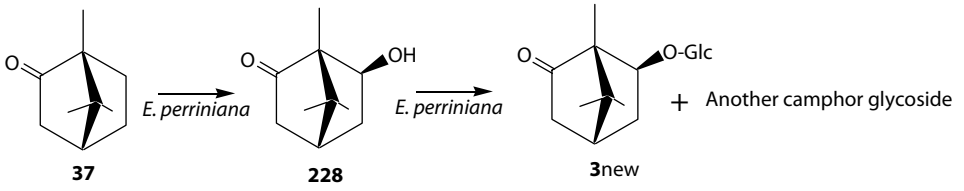


FIGURE 14.191 Biotransformation of (+)-camphor (**37**) by *Eucalyptus perriniana* suspension cell.

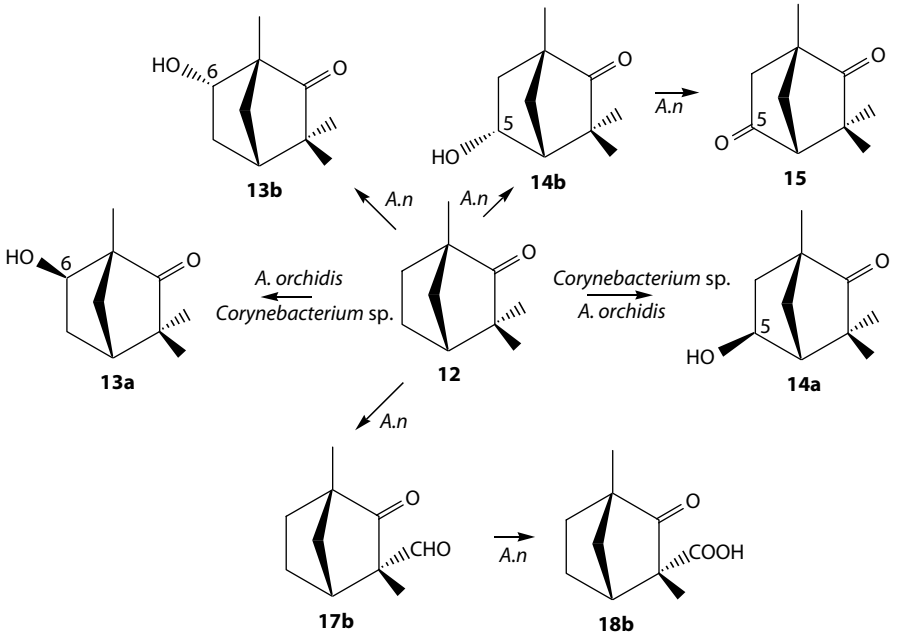


FIGURE 14.192 Metabolic pathways of (+)-fenchone (**12**) by *Corynebacterium* sp., *A. orchidis* and *Aspergillus niger* TBUYN-2. (Modified from Chapman, P.J. et al., 1965. *Biochem. Biophys. Res. Commun*., 20: 104–108; Pfrunder, B. and Ch. Tamm, 1969a. *Helv. Chim. Acta*., 52: 1643–1654; Miyazawa, M. et al., 1990a. *Chem. Express*, 5: 237–240; Miyazawa, M. et al., 1990b. *Chem. Express*, 5: 407–410.)

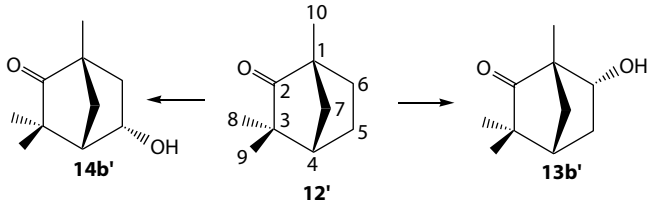


FIGURE 14.193 Metabolic pathways of (–)-fenchone (**12′**) by *Aspergillus niger* TBUYN-2. (Modified from Yamamoto, K. et al., 1984. *Proc. 28th TEAC*, pp. 168–170.)

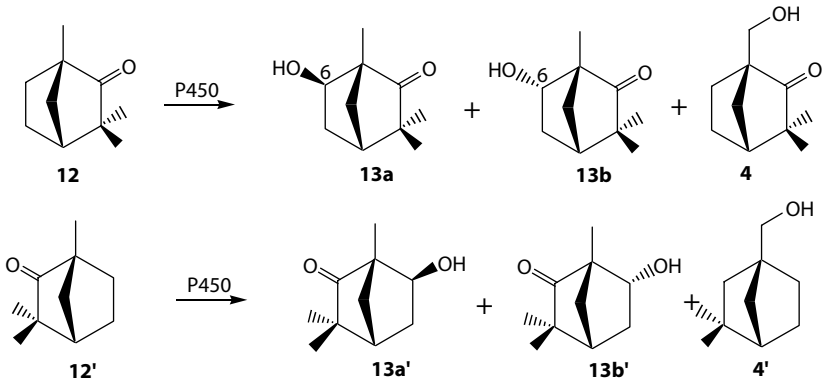
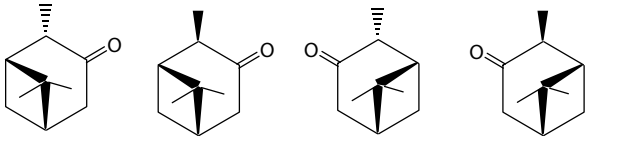


FIGURE 14.194 Biotransformation of (+)-fenchone (**12**) and (–)-fenchone (**12′**) by P-450 enzymes. (Modified from Gyoubu, K. and M. Miyazawa, 2005. *Proc. 49th TEAC*, pp. 420–422.)

14.4.4.2.3 3-Pinanone (Pinocamphone and Isopinocamphone)



413a 1*R*2*S*5*S* (+)-pino-camphone

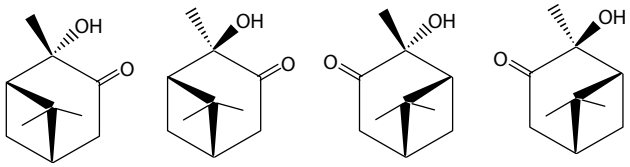
413b 1*R*,2*R*,5*S* (+)-isopino-camphone

413a' 1*S*2*R*5*R* (–)-pino-camphone

413b' 1*S*2*S*5*R* (–)-isopino-camphone

(+)- (**413**) and (–)-Isopinocamphone (**413′**) were biotransformed by *Aspergillus niger* to give (–)-(**414**) and (+)-2-hydroxy-3-pinanone (**414′**) as the main products, respectively, which inhibit strongly germination of lettuce seeds, and (–)- (**415**) and (+)-2,5-dihydroxy-3-pinanone (**415′**) as the minor components, respectively (Noma et al., 2003, 2004) (Figure 14.195).

14.4.4.2.4 2-Hydroxy-3-Pinanone



(1*S*,2*R*,5*S*) (–)-2-OH-3-pinanone **414a**

(1*S*,2*R*,5*S*) (+)-2-OH-3-pinanone **414b**

(1*R*,2*R*,5*R*) (+)-2-OH-3-pinanone **414a'**

(1*R*,2*S*,5*R*) (+)-2-OH-3-pinanone **414b'**

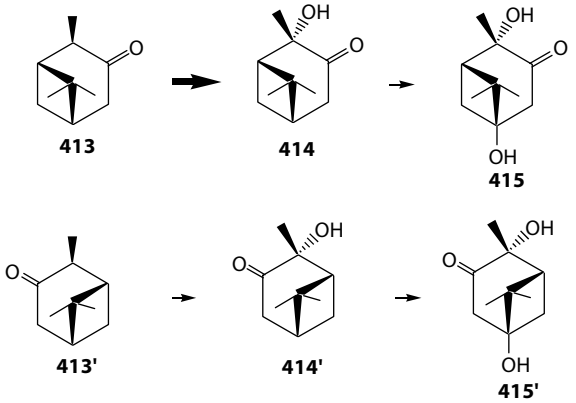


FIGURE 14.195 Biotransformation of (+)-isopinocamphone (**413b**) and (−)-isopinocamphone (**413b′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93; Noma, Y. et al., 2004. *Proc. 48th TEAC*, pp. 390–392.)

(−)-2α-Hydroxy-3-pinanone (**414**) was incubated with *Aspergillus niger* TBUYN-2 to give (−)-2α, 5-dihydroxy-3-pinanone (**415**) predominantly, whereas the fungus converted (+)-2α-hydroxy-3-pinanone (**414′**) mainly to 2α, 5-dihydroxy-3-pinanone (**415′**), 2α,9-dihydroxy-3-pinanone (**416′**), and (−)-pinane-2α,3α,5-triol (**419ba′**) (Noma et al., 2003, 2004) (Figure 14.196).

Aspergillus niger TBUYN-2 metabolized β-pinene (**1**), isopinocamphone (**414b**), 2α-hydroxy-3-pinanone (**414a**), and pinane-2,3-diol (**419ab**) as shown in Figure 14.197. On the other hand, *Aspergillus niger* TBUYN-2 and *Botrytis cinerea* metabolized β-pinene (**1′**), isopinocamphone (**414b′**), 2α-hydroxy-3-pinanone (**414a′**), and pinane-2,3-diol (**419ab′**) as shown in Figure 14.198. Relationship of the metabolism of β-pinene (**1**, **1′**), isopinocamphone (**414b, 414b′**), 2α-hydroxy-3-pinanone (**414a**, **414a′**), and pinane-2,3-diol (**419ab**, **419ab′**) in *Aspergillus niger* TBUYN-2 and *Botrytis cinerea* is shown in Figures 14.197 and 14.198.

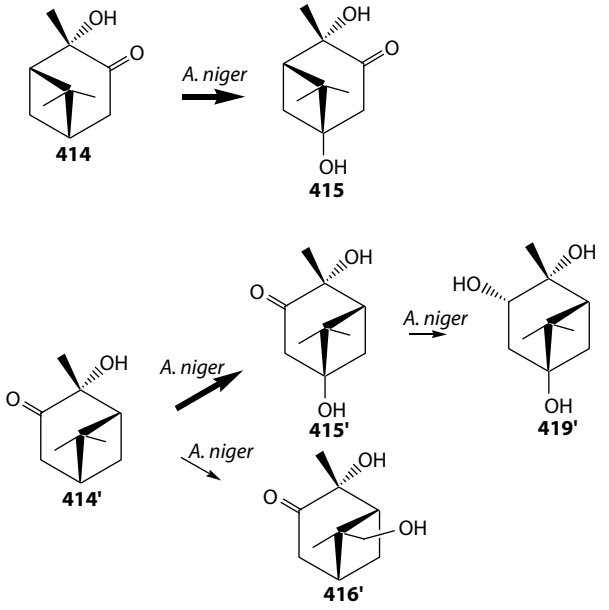


FIGURE 14.196 Biotransformation of (−)- (**414**) and (+)-2-hydroxy-3-pinanone (**414′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93; Noma, Y. et al., 2004. *Proc. 48th TEAC*, pp. 390–392.)

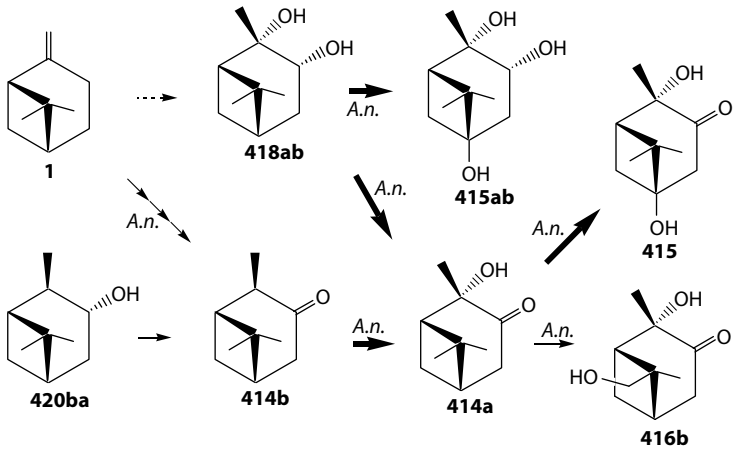


FIGURE 14.197 Relationship of the metabolism of β-pinene (**1**), isopinocamphone (**414b**), 2α-hydroxy-3-pinanone (**414a**), and pinane-2,3-diol (**419ab**) in *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93; Noma, Y. et al., 2004. *Proc. 48th TEAC*, pp. 390–392.)

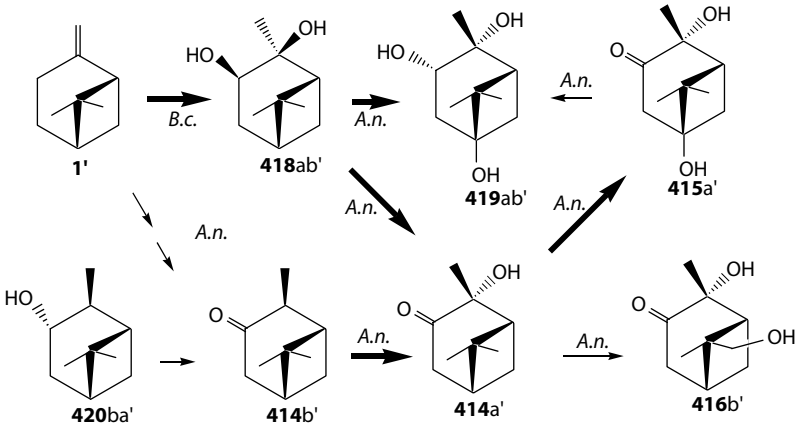


FIGURE 14.198 Relationship of the metabolism of β-pinene (**1′**), isopinocamphone (**414b′**), 2α-hydroxy-3-pinanone (**414a′**), and pinane-2,3-diol (**419ab′**) in *Aspergillus niger* TBUYN-2 and *Botrytis cinerea*. (Modified from Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93; Noma, Y. et al., 2004. *Proc. 48th TEAC*, pp. 390–392.)

14.4.4.2.4.1 Mosquitocidal and Knock-Down Activity Knock-down and mortality activity toward mosquito, *Culex quinequefasciatus*, was carried out for the metabolites of (+)- (**418ab**) and (−)-pinane-2,3-diols (**418ab′**) and (+)- and (−)-2-hydroxy-3-pinanones (**414** and **414′**) by Dr. Radhika Samarasekera, Industrial Technology Institute, Sri Lanka. (−)-2-Hydroxy-3-pinanone (**414′**) showed the mosquito knock-down activity and the mosqutocidal activity at the concentration of 1% and 2% (Table 14.16).

14.4.4.2.4.2 Antimicrobial Activity The microorganisms were refreshed in Mueller Hilton Broth (Merck) at 35–37°C, and inoculated on Mueller Hinton Agar (Mast Diagnostics, Merseyside, UK) media for preparation of inoculum. *Escherichia coli* (NRRL B-3008), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter aerogenes* (NRRL 3567), *Salmonella typhimurium* (NRRL B-4420), *Staphylococcus epidermidis (*ATCC 12228), Methicillin-resistant *Staphylococcus aureus* (MRSA, Clinical isolate, Osmangazi University, Faculty of Medicine, Eskisehir, Turkey), and *Candida albicans* (Clinical Isolate, Osmangazi University, Faculty of Medicine, Eskisehir, Turkey)

TABLE 14.16
Knock-down and Mortality Activity Toward Mosquito[a]

Compounds	Knock-Down (%)	Mortality (%)
(+)-2,5-Dihydroxy-3-pinanone (**415**, 2%)	27	20
(−)-2,5-Dihydroxy-3-pinanone (**415′**, 2%)	NT	7
(+)-2-Hydroxy-3-pinanone (**414**, 2%)	40	33
(−)-2-Hydroxy-3-pinanone (**414′**, 2%)	100	40
(−)-2-Hydroxy-3-pinanone (**414′**, 1%)	53	7
(+)-Pinane-2,3,5-triol (**419**, 2%)	NT	NT
(−)-Pinane-2,3,5-triol (**419**, 2%)	13	NT
(+)-Pinane-2,3-diol (**418**, 2%)	NT	NT
(−)-Pinane-2,3-diol (**418′**, 2%)	NT	NT

[a] The results are against Culex quinequefasciatus.

were used as pathogen test microorganisms. Microdilution broth susceptibility assay (*R1*, *R2*) was used for the antimicrobial evaluation of the samples. Stock solutions were prepared in DMSO (Carlo-Erba). Dilution series were prepared from 2 mg/mL in sterile distilled water in micro-test tubes from where they were transferred to 96-well micro-titer plates. Overnight grown bacterial and candial suspensions in double strength Mueller–Hilton broth (Merck) was standardized to approximately 10^8 CFU/mL using McFarland No:0.5 (10^6 CFU/mL for *Candida albicans*). A volume of 100 µL of each bacterial suspension was then added to each well. The last row containing only the serial dilutions of samples without microorganism was used as negative control. Sterile distilled water, medium, and microorganisms served as a positive growth control. After incubation at 37°C for a 24 h the first well without turbidity was determined as the minimal inhibition concentration (MIC), chloramphenicol (Sigma), ampicillin (sigma), and ketoconazole (Sigma) were used as standard antimicrobial agents (Koneman et al., 1997; Amsterdam, 1997) (Table 14.17).

TABLE 14.17
Biological Activity of Pinane-2,3,5-Triol (419 and 419′), 2,5-Dihydroxy-3-Pinanone (415 and 415′), and 7-Hydroxymethyl-1-*p*-menthene-8-ol (453′) Toward MRSA

	MIC (mg/mL)							
	Compounds					Control		
Microorganisms	419	415′	415	419′	453′	ST1	ST2	ST3
Escherichia coli	0.5	0.5	0.25	0.5	0.25	0.007	0.0039	Nt
Pseudomonas aeruginosa	0.5	0.125	0.125	0.25	0.25	0.002	0.0078	Nt
Enterobacter aerogenes	0.5	0.5	0.25	0.5	1.00	0.007	0.0019	Nt
Salmonella typhimurium	0.25	0.125	0.125	0.25	0.25	0.01	0.0019	Nt
Candida albicans	0.5	0.125	0.125	0.25	1.00	Nt	Nt	0.0625
Staphylococcus epidermidis	0.5	0.5	0.25	0.5	1.00	0.002	0.0009	Nt
MRSA	0.25	0.125	0.125	0.25	0.125	0.5	0.031	Nt

Source: Iscan (2005, unpublished data).
MRSA, methicillin-resistant Staphylococcus aureus; Nt, not tested; ST1, ampicillin-Na (Sigma); ST2, chloramphenicol (Sigma); ST3, ketoconazole (sigma).

14.5 SUMMARY

14.5.1 Metabolic Pathways of Monoterpenoids by Microorganisms

About 50 years are over since the hydroxylation of α-pinene (**4**) was reported by *Aspergillus niger* in 1960 (Bhattacharyya et al., 1960). During these years many investigators have studied the biotransformation of a number of monoterpenoids by using various kinds of microorganisms. Now we summarize the microbiological transformation of monoterpenoids according to the literatures listed in the references including the metabolic pathways (Figures 14.199 through 14.206) for the further development of the investigation on microbiological transformation of terpenoids.

Metabolic pathways of β-pinene (**1**), α-pinene (**4**), fenchol (**11**), fenchone (**12**), thujone (**28**), carvotanacetone (**47**), and sobrerol (**43**) are summarized in Figure 14.199. In general, β-pinene (**1**) is metabolized by six pathways. At first, β-pinene (**1**) is metabolized via α-pinene (**4**) to many metabolites such as myrtenol (**5**) (Shukla et al., 1968; Shukla and Bhattacharyya, 1968), verbenol (**23**) (Bhattacharyya et al., 1960; Prema and Bhattacharyya, 1962), and thujone (**28**) (Gibbon and Pirt, 1971). Myrtenol (**5**) is further metabolized to myrtenal (**6**) and myrtenic acid (**7**). Verbenol (**23**) is further metabolized to verbenone (**24**), 7-hydroxyverbenone (**25**), 7-hydroxyverbanone (**26**), and 7-formyl verbanone (**27**). Thujone (**28**) is further metabolized to thujoyl alcohol (**29**), 1-hydroxythujone (**30**), and 1,3-dihydroxythujone (**31**). Secondly, β-pinene (**1**) is metabolized to pinocarveol (**2**) and pinocarvone (**3**) (Ganapathy and Bhattacharyya, unpublished data). Pinocarvone (**3**) is further metabolized to isopinocamphone (**413**), which is further hydroxylated to give 2-hydroxy-3-pinanone (**414**). Compound **414** is further metabolized to give pinane-2,3-diol (**419**), 2,5- dihydroxy- (**415**), and 2,9-dihydroxy-3-pinanone (**416**). Thirdly, β-pinene (**1**) is metabolized to α-fenchol (**11**) and fenchone (**12**) (Dhavlikar et al., 1974), which are further metabolized to 6-hydroxy- (**13**) and 5-hydroxyfenchone (**14**), 5-oxofenchone (**15**), fenchone-9-al (**17**), fenchone-9-oic acid (**18**) via 9-hydroxyfenchone (**16**), 2,3-fencholide (**21**), and 1,2-fencholide (**22**) (Pfrunder and Tamm, 1969a, 1969b; Yamamoto, et al., 1984; Christensen and Tuthill, 1985; Miyazawa et al., 1990a, 1969b). Fenchol (**12**) is also metabolized to 9-hydroxyfenchol (**466**) and 7-hydroxyfenchol (**467**), 6-hydroxyfenchol (**349**), and 6,7-dihydroxyfenchol (**412**). Fourthly, β-pinene (**1**) is metabolized via fenchoquinone (**19**) to 2-hydroxyfenchone (**20**) (Pfrunder and Tamm 1969b; Gibbon et al., 1972). Fifthly, β-pinene (**1**) is metabolized to α-terpineol (**34**) via pinyl cation (**32**) and 1-*p*-menthene-8-cation (**33**) (Hosler, 1969; Hayashi et al., 1972; Saeki and Hashimoto, 1968, 1971). α-Terpineol (**34**) is metabolized to 8,9-epoxy-1-*p*-menthanol (**58**) via diepoxide (**57**), terpine hydrate (**60**), and oleuropeic acid (**204**) (Shukla et al., 1968; Shukla and Bhattacharyya, 1968; Hosler 1969; Hungund et al., 1970; Hayashi et al., 1972; Saeki and Hashimoto, 1968, 1971). As shown in Figure 14.202, oleuropeic acid (**204**) is formed from linalool (**206**) and α-terpineol (**34**) via **204**, **205**, and **213** as intermediates (Shukla et al., 1968; Shukla and Bhattacharyya, 1968; Hungund et al., 1970) and degradatively metabolized to perillic acid (**82**), 2-hydroxy-8-*p*-menthen-7-oic acid (**84**), 2-oxo-8-*p*-menthen-7-oic acid (**84**), 2-oxo-8-*p*-menthen-1-oic acid (**85**), and β-isopropyl pimelic acid (**86**) (Shukla et al., 1968; Shukla and Bhattacharyya, 1968; Hungund et al., 1970). Oleuropeic acid (**204**) is also formed from β-pinene (**1**) via α-terpineol (**34**) as the intermediate (Noma et al., 2001). Oleuropeic acid (**204**) is also formed from myrtenol (**5**) by rearrangement reaction by *Aspergillus niger* TBUYN-2 (Noma and Asakawa 2005b). Finally, β-pinene (**1**) is metabolized to borneol (**36**) and camphor (**37**) via two cations (**32** and **35**) and to 1-*p*-menthene (**62**) via two cations (**33** and **59**) (Shukla and Bhattacharyya, 1968). 1-*p*-Menthene (**62**) is metabolized to phellandric acid (**65**) via phellandrol (**63**) and phellandral (**64**), which is further degradatively metabolized through **246**–**251** and **89** to water and carbon dioxide as shown in Figure 14.204 (Shukla et al., 1968). Phellandral (**64**) is easily reduced to give phellandrol (**63**) by *Euglena* sp. and *Dunaliella* sp. (Noma et al., 1984, 1986, 1991a, 1991b; 1992d). Furthermore, 1-*p*-menthene (**62**) is metabolized to 1-*p*-menthen-2-ol (**46**) and *p*-menthane-1,2-diol (**54**) as shown in Figure 14.204. Perillic acid (**82**) is easily formed from perillandehyde (**78**) and perillyl alcohol (**74**) (Figure 14.19) (Swamy et al., 1965; Dhavalikar and

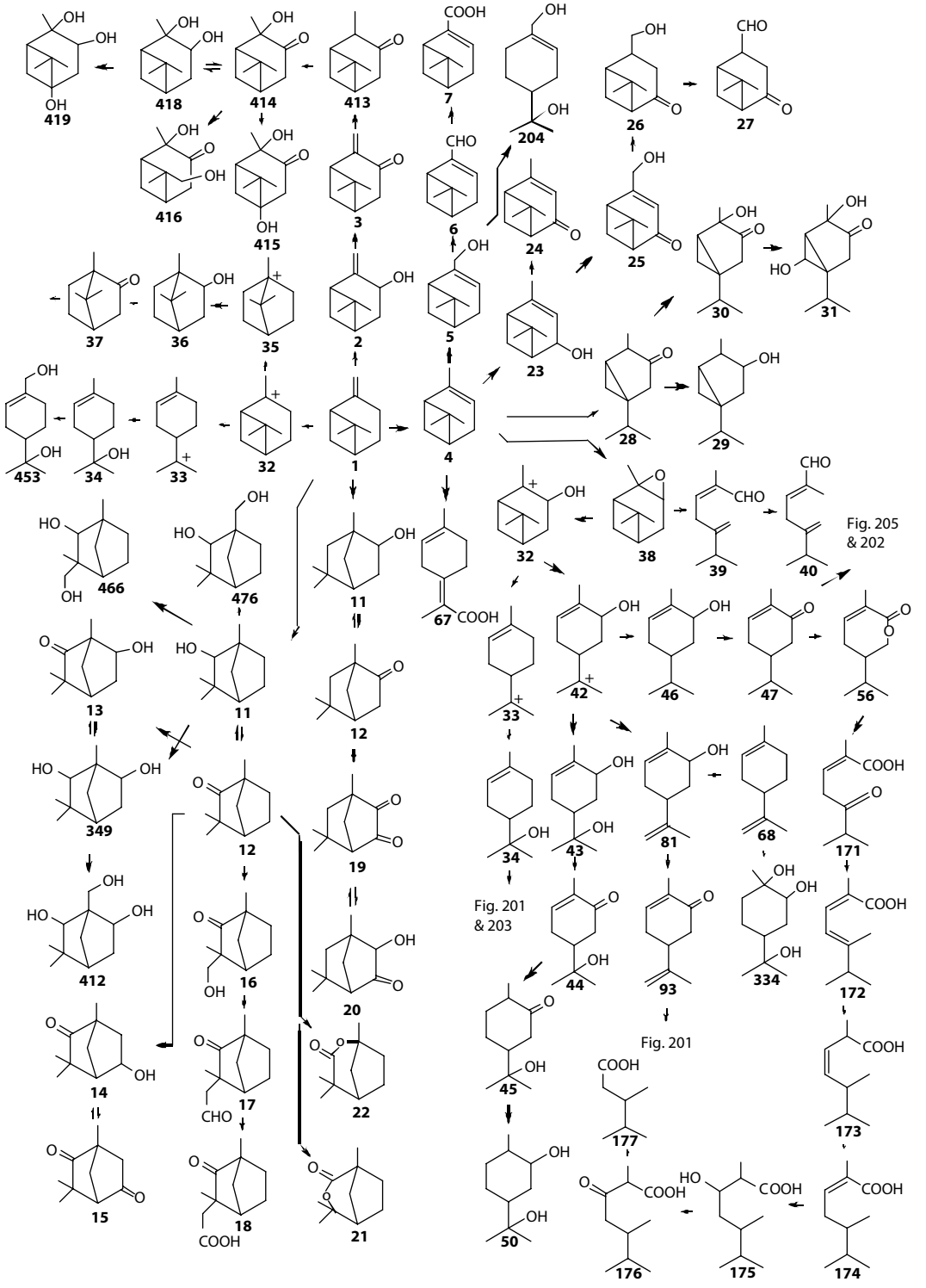


FIGURE 14.199 Metabolic pathways of β-pinene (**1**), α-pinene (**4**), fenchone (**9**), thujone (**28**), and carvotanacetone (**44**) by microorganisms.

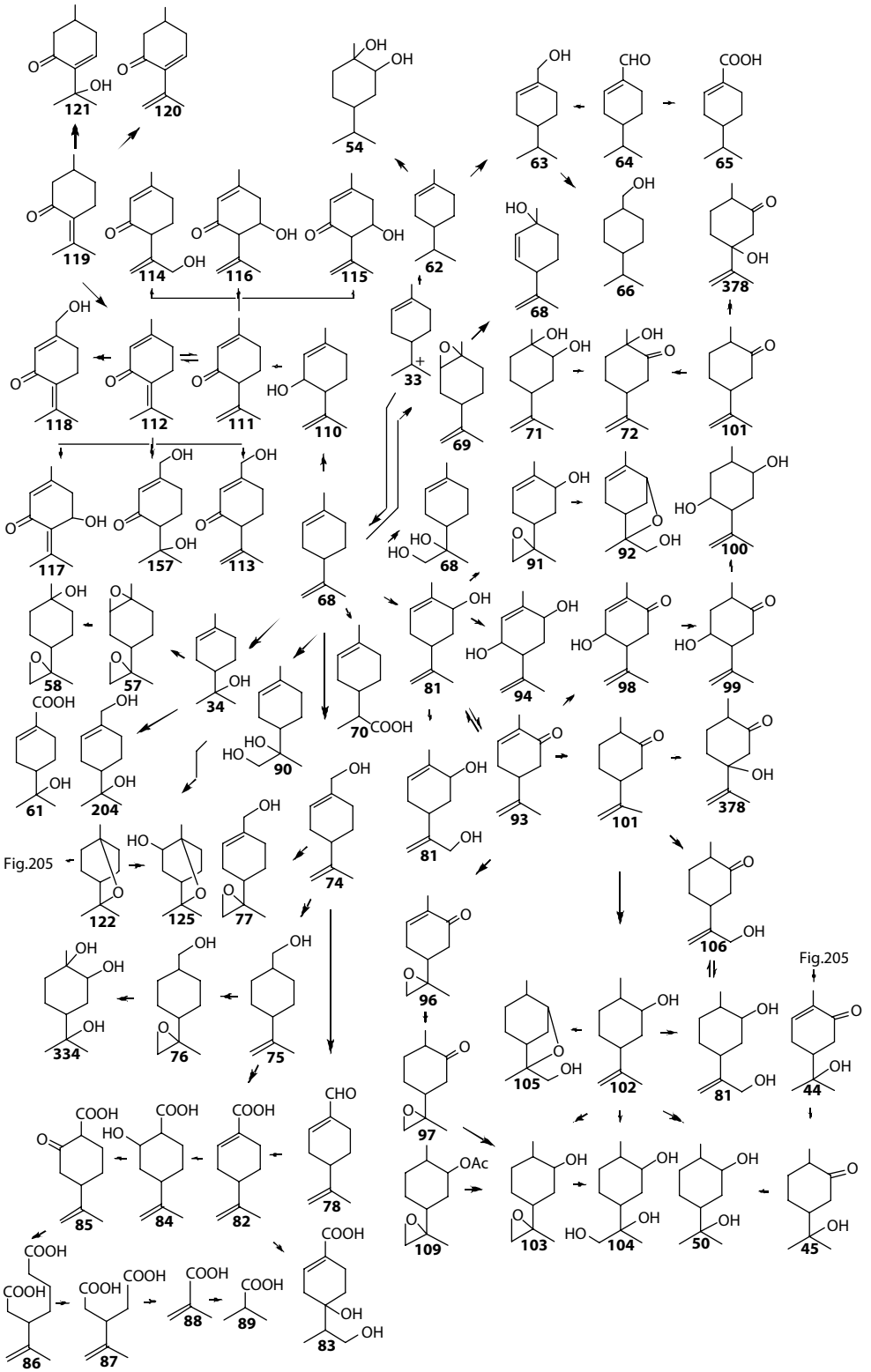


FIGURE 14.200 Metabolic pathways of limonene (**68**), perillyl alcohol (**74**), carvone (**93**), isopiperitenone (**111**), and piperitenone (**112**) by microorganisms.

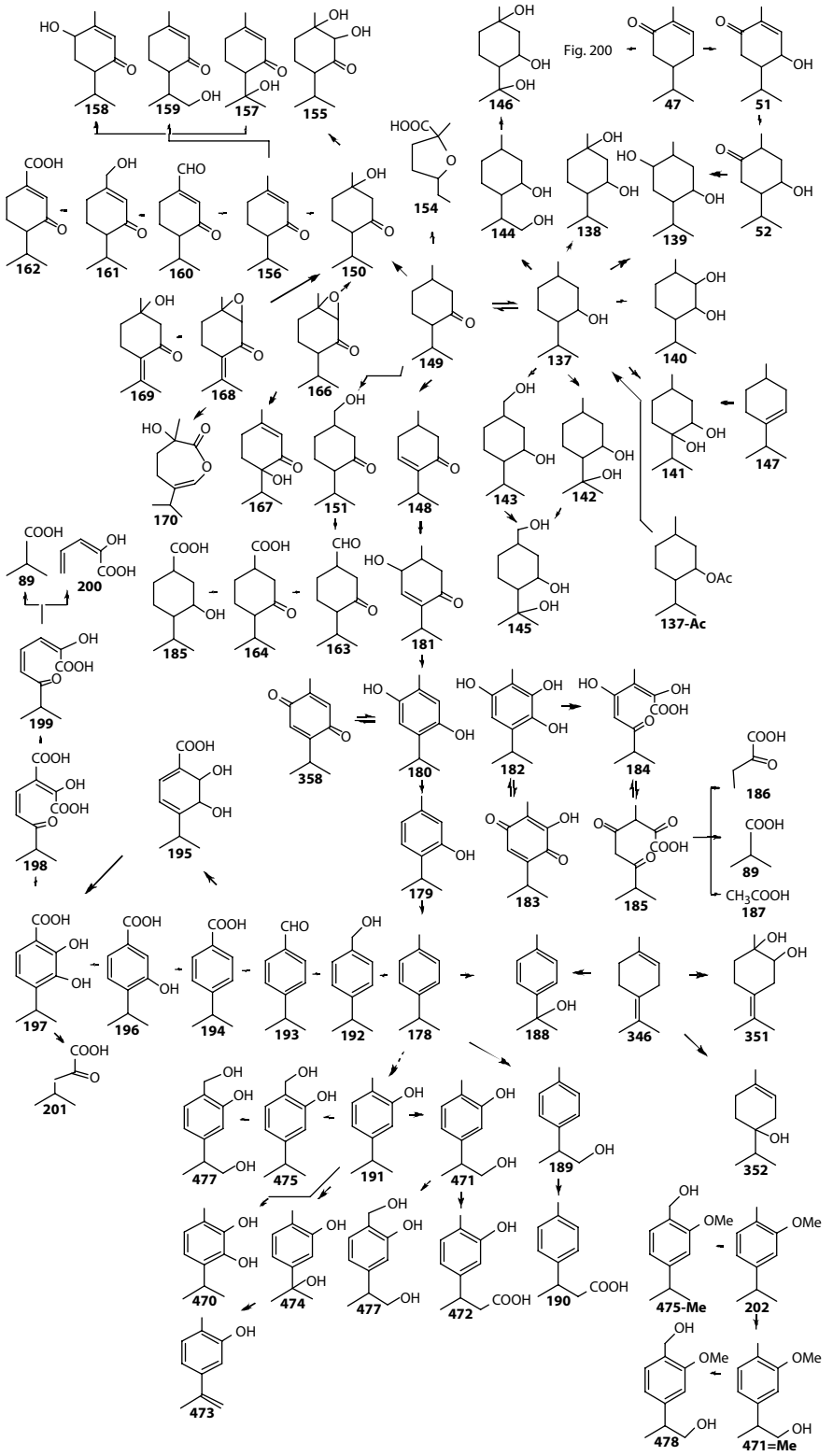


FIGURE 14.201 Metabolic pathways of menthol (**137**), menthone (**149**), *p*-cymene (**178**), thymol (**179**), carvacrol methyl ether (**201**), and carvotanacetone (**47**) by microorganisms and rabbit.

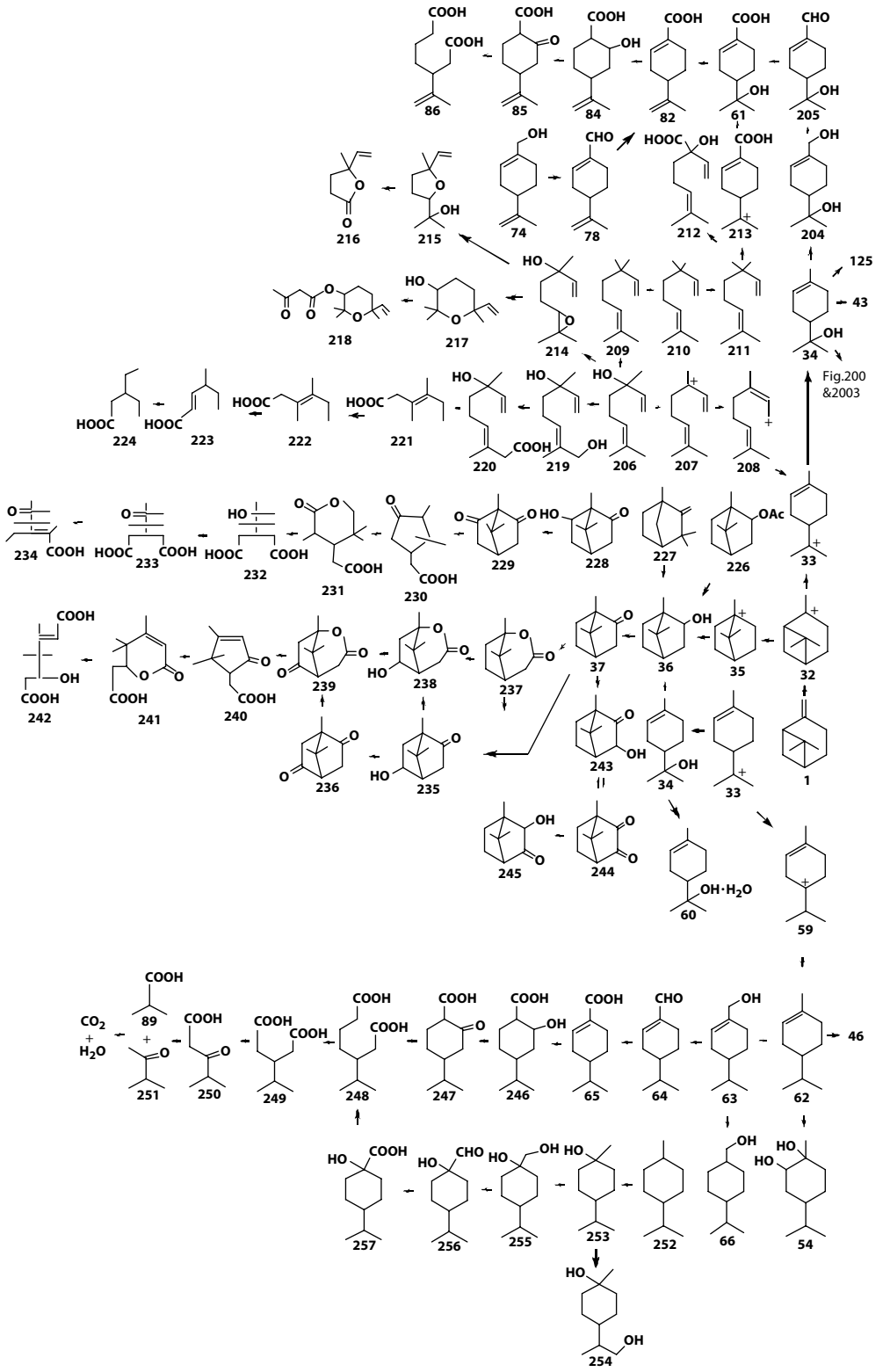


FIGURE 14.202 Metabolic pathway of borneol (**36**), camphor (**37**), phellandral (**64**), linalool (**206**), and *p*-menthane (**252**) by microorganisms.

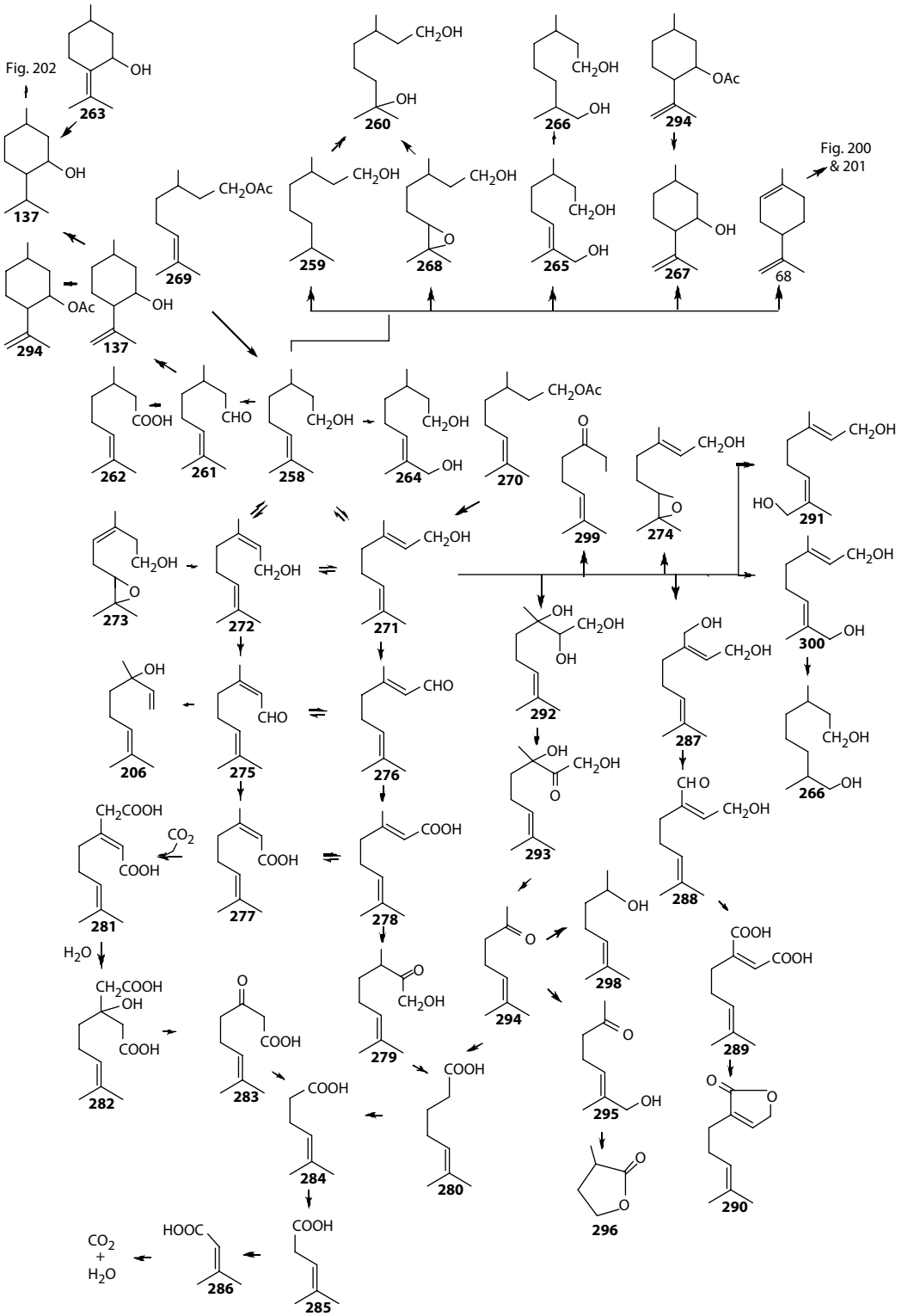


FIGURE 14.203 Metabolic pathways of citronellal (**258**), geraniol (**271**), nerol (**272**), and citral (**275** and **276**) by microorganisms.

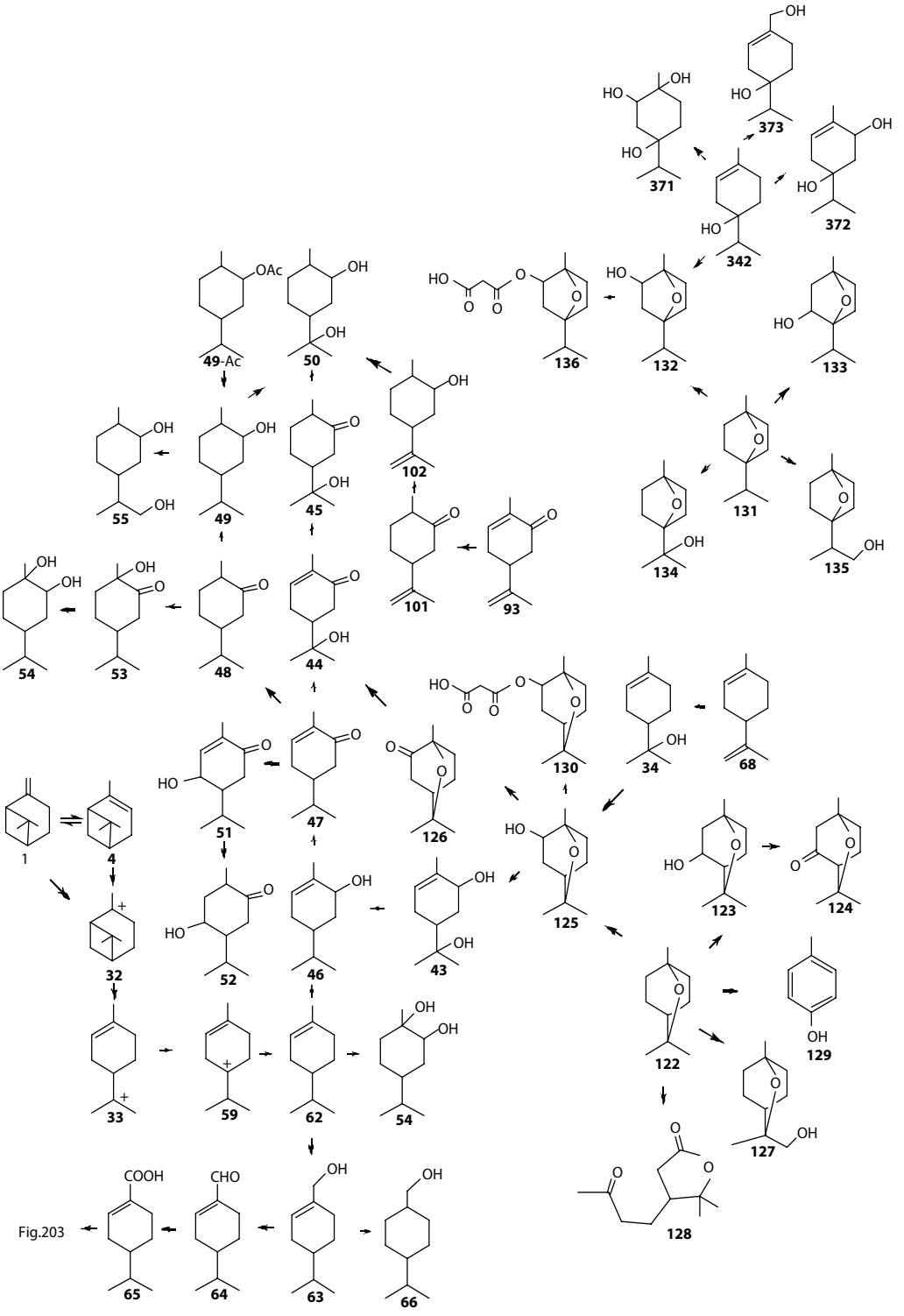


FIGURE 14.204 Metabolic pathways of 1,8-cineole (**122**), 1,4-cineole (**131**), phellandrene (**62**), and carvotanacetone (**47**) by microorganisms.

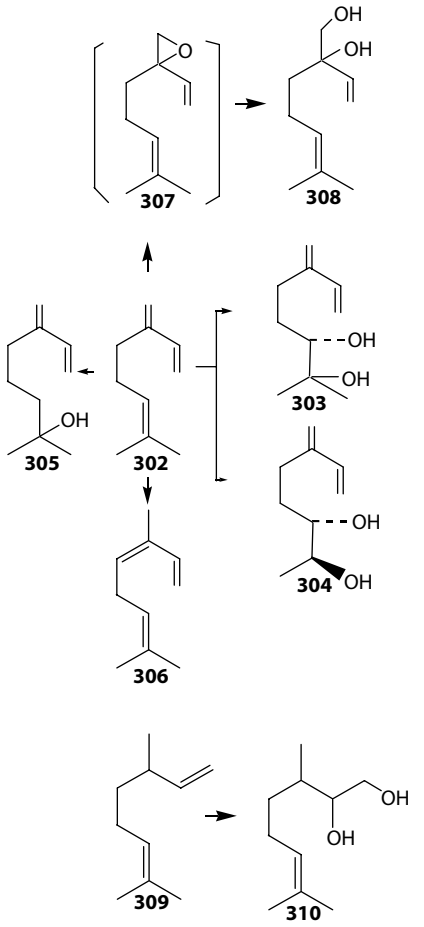


FIGURE 14.205 Metabolic pathways of myrcene (**302**) and citronellene (**309**) by rat and microorganisms.

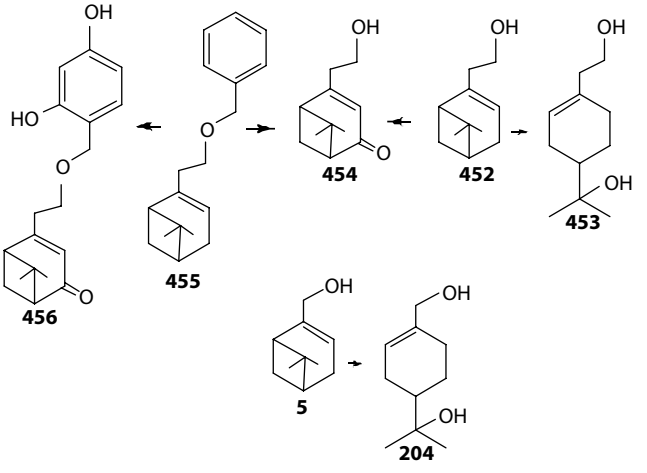


FIGURE 14.206 Metabolic pathways of nopol (**452**) and nopol benzyl ether (**455**) by microorganisms.

Bhattacharyya, 1966; Dhavalikar et al., 1966; Ballal et al., 1967; Kayahara et al., 1973; Shima et al., 1972). α-Terpineol (**34**) is also formed from linalool (**206**). α-Pinene (**4**) is metabolized by five pathways as follows: firstly, α-pinene (**4**) is metabolized to myrtenol (**5**), myrtenal (**6**), and myratenoic acid (**7**) (Shukla et al., 1968; Shukla and Bhattacharyya, 1968; Hungund et al., 1970; Ganapathy and Bhattacharyya, unpublished results). Myrtenal (**6**) is easily reduced to myrtenol (**5**) by *Euglena* and *Dunaliella* spp., (Noma et al., 1991a, 1991b; 1992d). Myrtanol (**8**) is metabolized to 3-hydroxy- (**9**) and 4-hydroxymyrtanol (**10**) (Miyazawa et al., 1994b). Secondly, α-pinene (**4**) is metabolized to verbenol (**23**), verbenone (**24**), 7-hydroxyverbenone (**25**), and verbanone-7-al (**27**) (Bhattacharyya et al., 1960; Prema and Bhattacharyya, 1962; Miyazawa et al., 1991d). Thirdly, α-pinene (**4**) is metabolized to thujone (**28**), thujoyl alcohol (**29**), 1-hydroxy- (**30**), and 1,3-dihydroxythujone (**31**) (Gibbon and Pirt, 1971; Miyazawa et al., 1992a; Noma, 2000). Fourthly, α-pinene (**4**) is metabolized to sobrerol (**43**) and carvotanacetol (**46**, 1-*p*-menthen-2-ol) via α-pinene epoxide (**38**) and two cations (**41** and **42**). Sobrerol (**43**) is further metabolized to 8-hydroxycarvotanacetone (**44**, carvonhydrate), 8-hydrocarvomenthone (**45**), and *p*-menthane-2,8-diol (50) (Prema and Bhattacharyya, 1962; Noma, 2007). In the metabolism of sobrerol (**43**), 8-hydroxycarvotanacetone (**44**), and 8-hydroxycarvomenthone (**45**) by *Aspergillus niger* TBUYN-2, the formation of *p*-menthane-2,8-diol (**50**) is very high enantio- and diastereoselective in the reduction of 8-hydroxycarvomenthone (Noma, 2007). 8-Hydroxycarvotanacetone (**44**) is a common metabolite from sobrerol (**43**) and carvotanacetone (**47**). Namely, carvotanacetone (**47**) is metabolized to carvomenthone (**48**), carvomenthol (**49**), 8-hydroxycarvomenthol (**50**), 5-hydroxycarvotanacetone (**51**), 8-hydroxycarvotanacetone (**44**), 5-hydroxycarvomenthone (**52**), and 2,3-lactone (**56**) (Gibbon and Pirt, 1971; Gibbon et al., 1972, Noma et al., 1974a; 1985c; 1988b). Carvomenthone (**48**) is metabolized to **45**, 8-hydroxycarvomenthol (**50**), 1-hydroxycarvomenthone (**53**), and *p*-menthane-1,2-diol (**54**) (Noma et al., 1985b, 1988b). Compound **52** is metabolized to 6-hydroxymenthol (**139**), which is the common metabolite of menthol (**137**) (see Figure 14.201). Carvomenthol (**49**) is metabolized to 8-hydroxycarvomenthol (**50**) and *p*-menthane-2, 9-diol (**55**). Finally, α-pinene (**4**) to borneol (**36**) and camphor (**37**) via **32** and **35** and to phellandrene (**62**) via **32** and two cations (**33** and **59**) as mentioned in the metabolism of β-pinene (**1**). Carvotanacetone (**47**) is also metabolized degradatively to 3,4-dimethylvaleric acid (**177**) via **56** and **158**-**163** as shown in Figure 201 (Gibbon and Pirt, 1971; Gibbon et al., 1972). α-Pinene (**4**) is also metabolized to 2-(4-methyl-3-cyclohexenylidene)-propionic acid (**67**) (Figure 14.199).

Metabolic pathways of limonene (**68**), perillyl alcohol (**74**), carvone (**93**), isopiperitenone (**111**), and piperitenone (**112**) are summarized in Figure 14.199. Limonene (**68**) is metabolized by eight pathways. Namely, limonene (**68**) is converted into α-terpineol (**34**) (Savithiry et al., 1997), limonene-1,2-epoxide (**69**), 1-*p*-menthene-9-oic acid (**70**), perillyl alcohol (**74**), 1-p-menthene-8,9-diol (**79**), isopiperitenol (**110**), *p*-mentha-1,8-diene-4-ol (**80**, 4-terpineol), and carveol (**81**) (Dhavalikar and Bhattacharyya, 1966; Dhavalikar et al., 1966; Bowen, 1975; Miyazawa et al., 1983; Van der Werf et al., 1997; Savithrry et al., 1997; Van der Werf and de Bont, 1998a, 1998b; Noma et al., 1982, 1992d). Dihdyrocarvone (**101**), limonene-1,2-diol (**71**), 1-hydroxy-8-*p*-menthene-2-one (**72**), and *p*-mentha-2,8-diene-1-ol (**73**) are formed from limonene (**68**) via limonene epoxide (**69**) as intermediate. Limonene (**68**) is also metabolized via carveol (**78**), limonene-1,2-diol (**71**), carvone (**93**), 1-*p*-menthene-6,9-diol (**95**), 8,9-dihydroxy-1-*p*-menthene (**90**), α-terpineol (**34**), 2α-hydroxy-1,8-cineole (**125**), and *p*-menthane-1,2,8-triol (**334**). Bottrospicatol (**92**) and 5-hydroxycarveol (**94**) are formed from *cis*-carveol by *Streptomyces bottropensis* SY-2-1 (Noma et al., 1982; Nishimura et al., 1983a; Noma and Nishimura, 1992; Noma and Asakawa, 1992). Carveyl acetate and carveyl propionate (both are shown as **106**) are hydrolyzed enantio- and diastereoselectively to carveol (**78**) (Oritani and Yamashita, 1980; Noma, 2000). Carvone (**93**) is metabolized through four pathways as follows: firstly, carvone (**93**) is reduced to carveol (**81**) (Noma, 1980). Secondly, it is epoxidized to carvone-8,9-epoxide (**96**), which is further metabolized to dihydrocarvone-8,9-epoxide (97), dihydrocarveol-8,9-epoxide (**103**), and menthane-2,8,9-triol (**104**) (Noma, 2000; Noma et al., 1980; Noma and Nishimura, 1982). Thirdly, **93** is hydroxylated to 5-hydroxycarvone (**98**), 5-hydroxydihydrocarvone

(**99**), and 5-hydroxydihydrocarveol (**100**) (Noma and Nishimura, 1982). Dihydrocarvone (**101**) is metabolized to 8-*p*-menthene-1,2-diol (**71**) via l-hydroxydihydrocarvone (**72**), 10-hydroxydihydrocarvone (**106**), and dihydrocarveol (**102**), which is metabolized to 10-hydroxydihydrocarveol (**107**), *p*-menthane-2,8-diol (**50**), dihydrocarveol-8,9-epoxide (**100**), *p*-menthane-2,8,9-triol (**104**), and dihydrobottrospicatol (**105**) (Noma et al., 1985a, 1985b). In the biotransformation of (+)-carvone by plant pathogenic fungi, *Aspergillus niger* Tiegh, isodihydrocarvone (**101**) was metabolized to 4-hydroxyisodihydrocarvone (**378**) and 1-hydroxyisodihydrocarvone (**72**) (Noma and Asakawa, 2008). 8,9-Epoxydihydrocarveyl acetate (**109**) is hydrolyzed to 8,9-epoxydihydrocarveol (**103**). Perillyl alcohol (**74**) is metabolized through three pathways to shisool (**75**), shisool-8,9-epoxide (**76**), perillyl alcohol-8,9-epoxide (**77**), perilladehyde (**78**), perillic acid (**82**), and 4,9-dihydroxy-1-*p*-menthen-7-oic acid (**83**). Perillic acid (**82**) is metabolized degradatively to **84**–**89** as shown in Figure 14.200 (Swamy et al., 1965; Dhavalikar and Bhattacharyya, 1966; Dhavalikar et al., 1966; Ballal et al., 1967; Shukla et al., 1968; Shukala and Bhattacharyya, 1968; Hungund et al., 1977; Kayahara et al., 1973; Shima et al., 1972). Isopiperitenol (**110**) is reduced to isopiperitenone (**111**), which is metabolized to 3-hydroxy- (**115**), 4-hydroxy- (**116**), 7-hydroxy- (**113**) and 10-hydroxy-isopiperitenone (**114**), and piperitenone (**112**). Compounds isopiperitenone (**111**) and piperitenone (**112**) are isomerized to each other (Noma et al., 1992c). Furthermore, piperitenone (**112**) is metabolized to 8-hydroxypiperitone (**157**), 5-hydroxy- (**117**) and 7-hydroxypiperitenone (**118**). Pulegone (**119**) is metabolized to **112**, 8-hydroxymenthenone (**121**), and 8,9-dehydromenthenone (**120**).

Metabolic pathways of menthol (**137**), menthone (**149**), thymol (**179**), and carvacryol methyl ether (**202**) are summarized in Figure 14.201. Menthol (**137**) is generally hydroxylated to give 1-hydroxy- (**138**), 2-hydroxy- (**140**), 4-hydroxy- (**141**), 6-hydroxy- (**139**), 7-hydroxy- (**143**), 8-hydroxy- (**142**), and 9-hydroxymenthol (**144**) and 1,8-dihydroxy- (**146**) and 7,8-dihydroxymenthol (**148**) (Asakawa et al., 1991; Takahashi et al., 1994; Van der Werf et al., 1997). Racemic menthyl acetate and menthylchloroacetate are hydrolyzed asymmetrically by an esterase of microorganisms (Brit Patent, 1970; Moroe et al., 1971; Watanabe and Inagaki, 1977a, 1977b). Menthone (**149**) is reductively metabolized to **137** and oxidatively metabolized to 3,7-dimethyl-6-hydroxyoctanoic acid (**152**), 3,7-dimethyl-6-oxooctanoic acid (**153**), 2-methyl-2,5-oxidoheptenoic acid (**154**), 1-hydroxymenthone (**150**), piperitone (**156**), 7-hydroxymenthone (**151**), menthone-7-al (**163**), menthone-7-oic acid (**164**), and 7-carboxylmenthol (**165**) (Sawamura et al., 1974). Compound **156** is metabolized to menthone-1,2-diol (**155**) (Miyazawa et al., 1991e, 1992d,e). Compound **148** is metabolized to 6-hydroxy- (**158**), 8-hydroxy- (**157**), and 9-hydroxypiperitone (**159**), piperitone-7-al (**160**), 7-hydroxypiperitone (**161**), and piperitone-7-oic acid (**162**) (Lassak et al., 1973). Compound **149** is also formed from menthenone (**148**) by hydrogenation (Mukherjee et al., 1973), which is metabolized to 6-hydroxymenthenone (**181**, 6-hydroxy-4-*p*-menthen-3-one). 6-hydroxymenthenone (**181**) is also formed from thymol (**179**) via 6-hydroxythymol (**180**). 6-Hydroxythymol (**180**) is degradatively metabolized through **182**–**185** to **186**, **187**, and **89** (Mukherjee et al., 1974). Piperitone oxide (**166**) is metabolized to 1-hydroxymenthone (**150**) and 4-hydroxypiperitone (**167**) (Lassak et al., 1973; Miyazawa et al., 1991e). Piperitenone oxide (**168**) is metabolized to 1-hydroxymenthone (**150**), 1-hydroxypulegone (**169**), and 2,3-seco-p-menthalacetone-3-en-1-ol (**170**) (Lassak et al., 1973; Miyazawa et al., 1991e). *p*-Cymene (**178**) is metabolized to 8-hydroxy- (**188**) and 9-hydroxy-p-cymene (**189**), 2- (4-methylphenyl)-propanic acid (**190**), thymol (**179**), and cumin alcohol (**192**), which is further converted degradatively to *p*-cumin aldehyde (**193**), cumic acid (**194**), *cis*-2,3-dihydroxy-2,3-dihydro-*p*-cumic acid (**195**), 2,3-dihydroxy-*p*-cumic acid (**197**), **198**–**200,** and **89** as shown in Figure 14.3 (Chamberlain and Dagley, 1968; DeFrank and Ribbons, 1977a, 1977b; Hudlicky et al., 1999; Noma, 2000). Compound **197** is also metabolized to 4-methyl-2-oxopentanoic acid (**201**) (DeFrank and Ribbons, 1977a). Compound **193** is easily metabolized to 192 and **194** (Noma et al., 1991a, 1992). Carvacrol methyl ether (**202**) is easily metabolized to 7-hydroxycarvacrol methyl ether (**203**) (Noma, 2000).

Metabolic pathways of borneol (**36**), camphor (**37**), phellandral (**64**), linalool (**206**), and *p*-menthane (**252**) are summarized in Figure 14.202. Borneol (**36**) is formed from β-pinene (**1**), α-pinene

(**4**), **34**, bornyl acetate (**226**), and camphene (**229**) and it is metabolized to **36**, 3-hydroxy- (**243**), 5-hydroxy- (**235**), 6-hydroxy- (**228**), and 9-hydroxycamphor (**225**), and 1,2-campholide (**23**). Compound **228** is degradatively metabolized to 6-oxocamphor (**229**) and **230**–**234**, whereas **237** is also degradatively metabolized to 6-hydroxy-1,2-campholide (**238**), 6-oxo-1,2-campholide (**239**), and **240**–**242**. 5-Hydroxycamphor (**235**) is metabolized to **238**, 5-oxocamphor (**236**), and 6-oxo-1,2-campholide (**239**). Compound **243** is also metabolized to camphorquinone (**244**) and 2-hydroxyepicamphor (**245**) (Bradshaw et al., 1959; Conrad et al., 1961, 1965a, 1965b; Gunsalus et al., 1965; Chapman et al., 1966; Hartline and Gunsalus, 1971; Oritani and Yamashita, 1974). 1-p-Menthene (**62**) is formed **1** and **4** via three cations (**32**, **33**, and **59**) and metabolized to phellandrol (**63**) (Noma et al., 1991a) and *p*-menthane-1,2-diol (**54**). Compound **63** is metabolized to phellandral (**64**) and 7-hydroxy-*p*-menthane (**66**). Compound **64** is furthermore metabolized degradatively to CO_2 and water via phellandric acid (**65**), **246**–**251**, and **89** (Dhavalikar and Bhattacharyya, 1966; Dhavalikar et al., 1966; Bahhal et al., 1967; Shukla et al., 1968; Shukla and Bhattacharyya, 1968; Hungund et al., 1970). Compound **64** is also easily reduced to phellandrol (**63**) (Noma et al., 1991a, 1992a). *p*-Menthane (**252**) is metabolized via 1-hydroxy-p-menthane (**253**) to *p*-menthane-1,9-diol (**254**) and *p*-menthane-1,7-diol (**255**) (Tukamoto et al., 1974, 1975; Noma et al., 1990). Compound **255** is degradatively metabolized via **256**–**248** to CO_2 and water through the degradation pathway of phellandric acid (**65**, **246**–**251**, and **89**) as mentioned above. Linalool (**206**) is metabolized to α-terpineol (**34**), camphor (**37**), oleuropeic acid (**61**), 2-methyl-6-hydroxy-6-carboxy-2,7-octadiene (**211**), 2-methyl-6-hydroxy-6-carboxy-7-octene (**199**), 5-methyl-5-vinyltetrahydro-2-furanol (**215**), 5-methyl-5-vinyltetrahydro-2-furanone (**216**), and malonyl ester (**218**). 1-Hydroxylinalool (**219**) is metabolized degradatively to 2,6-dimethyl-6-hydroxy-*trans*-2,7-octadienoic acid (**220**), 4-methyl-*trans*-3,5-hexadienoic acid (**221**), 4-methyl-*trans*-3,5-hexadienoic acid (**222**), 4-methyl-*trans*-2-hexenoic acid (**223**), and isobutyric acid (**224**). Compound **206** is furthermore metabolized via **213** to **61**, **82**, and **84**–**86** as shown in Figure 14.2 (Mizutani et al., 1971; Murakami et al., 1973; Rama Devi and Bhattacharyya, 1977a, 1977b; Rama Devi et al., 1977; Madyastha et al., 1977; David and Veschambre, 1985; Miyazawa et al., 1994a, 1994b).

Metabolic pathways of citronellol (**258**), citronellal (**261**), geraniol (**271**), nerol (**272**), citral [neral (**275**) and geranial (**276**)], and myrcene (**302**) are summarized in Figure 14.203 (Seubert and Fass, 1964; Hayashi et al., 1968; Rama Devi and Bhattacharyya, 1977a, 1977b). Geraniol (**271**) is formed from citronellol (**258**), nerol (**272**), linalool (**206**), and geranyl acetate (**270**) and metabolized through 10 pathways. Namely, compound **271** is hydrogenated to give citronellol (**258**), which is metabolized to 2,8-dihydroxy-2,6-dimethyl octane (**260**) via 6,7-epoxycitronellol (**268**), isopulegol (**267**), limonene (**68**), 3,7-dimethyloctane-1,8-diol (**266**) via 3,7-dimethyl-6-octene-1,8-diol (**265**), **267**, citronellal (**261**), dihydrocitronellol (**259**), and nerol (**272**). Citronellyl acetate (**269**) and isopulegyl acetate (**301**) are hydrolyzed to citronellol (**258**) and isopulegol (**267**), respectively. Compound **261** is metabolized via pulegol (**263**) and isopulegol (**267**) to menthol (**137**). Compound **271** and **272** are isomerized to each other. Compound **272** is metabolized to **271**, **258**, citronellic acid (**262**), nerol-6-,7-epoxide (**273**), and neral (**275**). Compound **272** is metabolized to neric acid (**277**). Compounds **275** and **276** are isomerized to each other. Compound **276** is completely decomposed to CO_2 and water via geranic acid (**278**), 2,6-dimethyl-8-hydroxy-7-oxo-2-octene (**279**), 6-methyl-5-heptenoic acid (**280**), 7-methyl-3-oxo-6-octenoic acid (**283**), 6-methyl-5-heptenoic acid (**284**), 4-methyl-3-heptenoic acid (**284**), 4-methyl-3-pentenoic acid (**285**), and 3-methyl-2-butenoic acid (**286**). Furthermore, compound **271** is metabolized via 3-hydroxymethyl-2,6-octadiene-1-ol (**287**), 3-formyl-2,6-octadiene-1-ol (**288**), and 3-carboxy-2,6-octadiene-1-ol (**289**) to 3- (4-methyl-3-pentenyl)-3-butenolide (**290**). Geraniol (**271**) is also metabolized to 3,7-dimethyl-2,3-dihydroxy-6-octen-1-ol (**292**), 3,7-dimethyl-2-oxo-3-hydroxy-6-octen-1-ol (**293**), 2-methyl-6-oxo-2-heptene (**294**), 6-methyl-5-hepten-2-ol (**298**), 2-methyl-2-heptene-6-one-1-ol (**295**), and 2-methyl-γ-butyrolactone (**296**). Furthermore, **271** is metabolized to 7-methyl-3-oxo-6-octanoic acid (**299**), 7-hydroxymethyl-3-methyl-2,6-octadien-1-ol (**291**), 6,7-epoxygeraniol (**274**), 3,7-dimethyl-2,6-octadiene-1,8-diol (**300**), and 3,7-dimethyloctane-1,8-diol (**266**).

Metabolic pathways of 1,8-cineole (**122**), 1,4-cineole (**131**), phellandrene (**62**), carvotanacetone (**47**), and carvone (**93**) by micororganisms are summarized in Figure 14.204.

1,8-Cineole (**112**) is biotransformed to 2-hydroxy- (**125**), 3-hydroxy- (**123**), and 9-hydroxy-1,8-cineole (**127**), 2-oxo- (**126**) and 3-oxo-1,8-cineole (**124**), lactone [**128**, (*R*)-5,5-dimethyl-4-(3′-oxobutyl)-4,5-dihydrofuran-2-(3H)-one] and *p*-hydroxytoluene (**129**) (MacRae et al., 1979, Nishimura et al., 1982; Noma and Sakai, 1984). 2-Hydroxy-1,8-cineole (**125**) is further converted into 2-oxo-1,8-cineole (**126**), 1,8-cineole-2-malonyl ester (**130**), sobrerol (**43**), and 8-hydroxycarvotanacetone (**44**) (Miyazawa et al., 1995b). 2-Hydroxy-1,8-cineole (**125**) and 2-oxo-1,8-cineole (**126**) are also biodegradated to sobrerol (**43**) and 8-hydroxycarvotanacetone (**44**), respectively. 2-Hydroxy-1,8-cineole (**125**) was esterified to give malonyl ester (**130**). 2-Hydroxy-1,8-cineole (**125**) was formed from limonene (**68**) by Citrus pathogenic fungi, *Penicillium digitatum* (Noma and Asakawa 2007b). 1,4-Cineole (**131**) is metabolized to 2-hydroxy- (**132**), 3-hydroxy- (**133**), 8-hydroxy- (**134**), and 9-hydroxy-1,4-cineole (**135**). Compound **132** is also eaterified to malonyl ester (**136**) as well as **125** (Miyazawa et al., 1995b). Terpinen-4-ol (342) is metabolized to 2-hydroxy-1,4-cineole (**132**), 2-hydroxy- (**372**) and 7-hydroxyterpinene-4-ol (**342**), and p-mentane-1,2,4-triol (**371**) (Abraham et al., 1986; Noma and Asakawa, 2007a; Kumagae and Miyazawa, 1999). Phellandrene (**62**) is metabolized to carvotanacetol (**46**) and phellandrol (**63**). Carvotanacetol (**46**) is further metabolized through the metabolism of carvotanacetone (**47**). Phelandrol (**63**) is also metabolized to give phellandral (**64**), phellandric acid (**65**), and 7-hydroxy-p-menthane (**66**). Phellandric acid (**65**) is completely degradated to carbon dioxide and water as shown in Figure 14.202.

Metabolic pathways of myrcene (**302**) and citronellene (**309**) by microorganisms and insects are summarized in Figure 14.205. β-Myrcene (**302**) was metabolized with *Diplodia gossypina* ATCC 10936 (Abragam et al., 1985) to the diol (**303**) and a side-product (**304**). β-Myrcene (**302**) was metabolized with *Ganoderma applanatum*, *Pleurotus flabellatus*, and *Pleurotus sajor-caju* to myrcenol (**305**) (2-methyl-6-methylene-7-octen-2-ol) and **306** (Busmann and Berger, 1994).

β-Myrcene (**302**) was converted by common cutworm larvae, *Spodoptera litura*, to give myrcene-3, (10)-glycol (**308**) via myrcene-3,(10)-epoxide (**307**) (Miyazawa et al., 1998). Citronellene (**309**) was metabolized by cutworm *Spodoptera litura* to give 3,7-dimethyl-6-octene-1,2-diol (**310**) (Takechi and Miyazawa, 2005). Myrcene (**302**) is metabolized to two kinds of diols (**303** and **304**), myrcenol (**305**), and ocimene (**306**) (Seubert and Fass, 1964; Abraham et al., 1985). Citronellene (**309**) was metabolized to (**310**) by *Spodoptera litura* (Takeuchi and Miyazawa, 2005).

Metabolic pathways of nopol (**452**) and nopol benzyl ether (**455**) by microorganisms are summarized in Figure 14.206. Nopol (**452**) is metabolized mainly to 7-hydroxyethyl-α-terpineol (453) by rearrangement reaction and 3-oxoverbenone (454) as minor metabolite by *Aspergillus* spp. including *Aspergillus niger* TBUYN-2 (Noma and Asakawa, 2006b,c). Myrtenol (**5**) is also metabolized to oleoropeic alcohol (**204**) by rearrangement reaction. However, nopol benzyl ether (**455**) was easily metabolized to 3-oxoverbenone (**454**) and 3-oxonopol-2′,4′-dihydroxybenzylether (**456**) as main metabolites without rearrangement reaction (Noma and Asakawa 2006c).

14.5.2 Microbial Transformation of Terpenoids as Unit Reaction

Microbiological oxidation and reduction patterns of terpenoids and related compounds by fungi belonging to *Aspergillus* spp. containing *Aspergillus niger* TBUYN-2 and *Euglena gracilis Z* are summarized in Tables 14.18 and 14.19, respectively. Dehydrogenation of secondary alcohols to ketones, hydroxylation of both nonallylic and allylic carbons, oxidation of olefins to form diols and triols via epoxides, reduction of both saturated and α,β-unsaturated ketones and hydrogenation of olefin conjugated with the carbonyl group were the characteristic features in the biotransformation of terpenoids and related compounds by *Aspergillus* spp.

Compound names: **1**, β-pinene; **2**, pinocarveol; **3**, pinocarvone; **4**, α-pinene; **5**, myrtenol; **6**, myrtenal; **7**, myrtenoic acid; **8**, myrtanol; **9**, 3-hydroxymyrtenol; **10**, 4-hydroxymyrtanol; **11**, α-fenchol; **12**, fenchone; **13**, 6-hydroxyfenchone; **14**, 5-hydroxyfenchone; **15**, 5-oxofenchone; **16**,

TABLE 14.18
Microbiological Oxidation and Reduction Patterns of Monoterpenoids by *Aspergillus niger* TBUYN-2

	Microbiological Oxidation	
Oxidation of alcohols	Oxidation of primary alcohols to aldehydes and acids	
	Oxidation of secondary alcohols to ketones	(−)-*trans*-Carveol (**81a′**), (+)-*trans*-carveol (**81a**), (−)-*cis*-carveol (**81b′**), (+)-*cis*-carveol (**81b**), 2α-hydroxy-1,8-cineole (**125b**), 3α-hydroxy-1,8-cineole (**123b**), 3β-hydroxy-1,8-cineole (**123**a)
Oxidation of aldehydes to acids		
Hydroxylation	Hydroxylation of nonallylic carbon	(−)-Isodihydrocarvone (**101c′**), (−)-carvotanacetone (**47′**), (+)-carvotanacetone (**47**), *cis*-*p*-menthane (**252**), 1α-hydroxy-*p*-menthane (**253**), 1,8-cineole (**122**), 1,4-cineole (**131**), (+)-fenchone (**12**), (−)-fenchone (**12′**), (−)-menthol (**137b′**), (+)-Menthol (**137b**), (−)-neomenthol (**137a**), (+)-neomenthol (**137a**), (+)-isomenthol (**137c**)
	Hydroxylation of allylic carbon	(−)-Isodihydrocarvone (**101b**), (+)-neodihydrocarveol (**102a′**), (−)-dihydrocarveol (**102b′**), (+)-dihydrocarveol (**102b**), (+)-limonene (**68**), (−)-limonene (**68′**)
Oxidation of olefins	Formation of epoxides and oxides	
	Formation of diols	(+)-Neodihydrocarveol (**102a′**), (+)-dihydrocarveol (**102b**), (−)-dihydrocarveol (**102b′**), (+)-limonene (**68**), (−)-limonene (**68′**)
	Formation of triols	(+)-Neodihydrocarveol (**102a′**)
Lactonization		
	Microbiological Reduction	
Reduction of aldehydes to alcohols		
Reduction of ketones to alcohols	Reduction of saturated ketones	(+)-Dihydrocarvone (**101a′**), (−)-isodihydrocarvone (**101b**), (+)-carvomenthone (**48a′**), (−)-isocarvomenthone (**48b**)
	Reduction of α,β-unsaturated ketones	
Hydrogenation of olefins	Hydrogenation of olefin conjugated with carbonyl group	(−)-Carvone (**93′**), (+)-carvone (**93**), (−)-carvotanacetone (**47′**), (+)-carvotanacetone (**47**)
	Hydrogenation of olefin not conjugated with a carbonyl group	

TABLE 14.19
Microbiological Oxidation, Reduction, and Another Reaction Patterns of Monoterpenoids by *Euglena gracilis Z*

	Microbiological Oxidation	
Oxidation of alcohols	Oxidation of primary alcohols to aldehydes and acids	
	Oxidation of secondary alcohols to ketones	(−)-*trans*-Carveol (**81a′**), (+)-*cis*-carveol (**81b**), (+)-isoborneol (**36b**) *Diastereo- and enantioselective dehydrogenation is observed in carveol, borneol, and isoborneol
Oxidation of aldehydes to acids		Myrtenal (**6**), myrtanal, (−)-perillaldehyde (**78**), *trans*- and *cis*-1,2-dihydroperillaldehydes (**261a** and **261b**), (−)-phellandral (**64**), *trans*- and *cis*-tetrahydroperillaldehydes, cuminaldehyde (**193**), (+)- and (−)-citronellal (**261** and **261′**) *Acids were obtained as minor products
Hydroxylation	Hydroxylation of nonallylic carbon	
	Hydroxylation of allylic carbon	(+)-Limonene (**68**), (−)-limonene (**68′**)
Oxidation of olefins	Formation of epoxides and oxides	
	Formation of diols	
	Formation of triols	(+)-and (−)-Neodihydrocarveol (**102a′** and **a**), (−)- and (+)-dihydrocarveol (**102b′** and **b**), (+)- and (−)-isodihydrocarveol (**102c′** and **c**), (+)- and (−)-neoisodihydrocarveol (**102d′** and **d**)
Lactonization		
	Microbiological Reduction	
Reduction of aldehydes to alcohols	Reduction of terpene aldehydes to terpene alcohols	Myrtenal (**6**), myrtanal, (−)-perillaldehyde (**78**), *trans*- and *cis*-1,2-dihydroperillaldehydes (**261a** and **261b**), phellandral (**64**), *trans*- and *cis*-1,2-dihydroperillaldehydes (**261a** and **261b**), *trans*- and *cis*-tetrahydroperillaldehydes, cuminaldehyde (**193**), citral (**275** and **276**), (+)- (**261**) and (−)-citronellal (**261′**)
	Reduction of aromatic and related aldehydes to alcohols	
	Reduction of aliphatic aldehydes to alcohols	
Reduction of ketones to alcohols	Reduction of saturated ketones	(+)-Dihydrocarvone (**101a′**), (−)-isodihydrocarvone (**101b**), (+)-carvomenthone (**48a′**), (−)-isocarvomenthone (**48b**), (+)-dihydrocarvone-8,9-epoxides (**97a′**), (+)-isodihydrocarvone-8,9-epoxides (**97b′**), (−)-dihydrocarvone-8,9-epoxides (**97a**)
	Reduction of α,β-unsaturated ketones	
Hydrogenation of olefins	Hydrogenation of olefin conjugated with carbonyl group	(−)-Carvone (**93′**), (+)-carvone (**93**), (−)-carvotanacetone (**47′**), (+)-carvotanacetone (**47**), (−)-carvone-8,9-epoxides (**96′**), (+)-carvone-8,9-epoxides (**96**)
	Hydrogenation of olefin not conjugated with a carbonyl group	

continued

TABLE 14.19 (continued)
Microbiological Oxidation, Reduction, and Another Reaction Patterns of Monoterpenoids by *Euglena gracilis Z*

	Hydrolysis	
Hydrolysis	Hydrolysis of ester	(+)-*trans*- and *cis*-Carveyl acetates (**108a** and **b**), (−)-*cis*-carveyl acetate (**108b′**), (−)-*cis*-carveyl propionate, geranyl acetate (**270**)
	Hydration	
Hydration	Hydration of C=C bond in isopropenyl group to tertiary alcohol	(+)-Neodihydrocarveol (**102a′**), (−)-dihydrocarveol (**102b′**), (+)-isodihydrocarveol (**102c′**), (+)-neoisodihydrocarveol (**102d′**) (−)-neodihydrocarveol (**102a**), (+)-dihydrocarveol (**102b**), (−)-isodihydrocarveol (**102c**), (−)-neoisodihydrocarveol (**102d**), *trans*- and *cis*-shisools (**75a** and **75b**)
	Isomerization	
Isomerization		Geraniol (**271**), nerol (**272**)

9-hydroxyfenchone; **17**, fenchone-9-al; **18**, fenchone-9-oic acid; **19**, fenchoquinone; **20**, 2-hydroxyfenchone; **21**, 2,3-fencholide; **22**, 1,2-fencholide; **23**, verbenol; **24**, verbenone; **25**, 7-hydroxyverbenone; **26**, 7-hydroxyverbenone; **27**, verbanone-4-al; **28**, thujone; **29**, thujoyl alcohol; **30**, 1-hydroxythujone; **31**, 1,3-dihydroxythujone; **32**, pinyl cation; **33**, 1-*p*-menthene-8-cation; **34**, α-terpineol; **35**, bornyl cation; **36**, borneol; **37**, camphor; **38**, α-pinene epoxide; **39**, isonovalal; **40**, novalal; **41**, 2-hydroxypinyl cation; **42**, 6-hydroxy-1-*p*-menthene-8-cation; **43**, *trans*-sobrerol; **44**, 8-hydroxycarvotanacetone; (carvonehydrate); **45**, 8-hydraocarvomenthone; **46**, 1-*p*-menthen-2-ol; **47**, carvotanacetone; **48**, carvomenthone; **49**, carvomenthol; **50**, 8-hydroxycarvomenthol; **51**, 5-hydroxycarvotanacetone; **52**, 5-hydroxycarvomenthone; **53**, 1-hydroxycarvomenthone; **54**, *p*-menthane-1,2-diol; **55**, *p*-menthane-2,9-diol; **56**, 2,3-lactone; **57**, diepoxide; **58**, 8,9-epoxy-1-*p*-menthanol; **59**, 1-*p*-menthene-4-cation; **60**, terpine hydrate; **61**, oleuropeic acid (8-hydroxyperillic acid); **62**, 1-*p*-menthene; **63**, phellandrol; **64**, phellandral; **65**, phellandric acid; **66**, 7-hydroxy-*p*-menthane; **67**, 2-(4-methyl-3-cyclohexenylidene)-propionic acid; **68**, limonene; **69**, limonene-1,2-epoxide; **70**, 1-*p*-menthene-9-oic acid; **71**, limonene-1,2-diol; **72**, 1-hydroxy-8-*p*-menthene-2-one; **73**, 1-hydroxy-*p*-menth-2,8-diene; **74**, perillyl alcohol; **75**, shisool; **76**, shisool-8,9-epoxide; **77**, perillyl alcohol-8,9-epoxide; **78**, perillandehyde; **79**, 1-p-menthene-8,9-diol; **80**, 4-hydroxy-*p*-menth-1,8-diene (4-terpineol); **81**, carveol; **82**, perillic acid; **83**, 4,9-dihydroxy-1-*p*-menthene-7-oic acid; **84**, 2-hydroxy-8-*p*-menthen-7-oic acid; **85**, 2-oxo-8-*p*-menthen-7-oic acid; **86**, β-isopropyl pimelic acid; **87**, isopropenylglutaric acid; **88**, isobutenoic acid; **89**, isobutyric acid; **90**, 1-*p*-menthene-8,9-diol; **91**, carveol-8,9-epoxide; **92**, bottrospicatol; **93**, carvone; **94**, 5-hydroxycarveol; **95**, 1-*p*-menthene-6,9-diol; **96**, carvone-8,9-epoxide; **97**, dihydrocarvone-8,9-epoxide; **98**, 5-hydroxycarvone; **99**, 5-hydroxydihydrocarvone; **100**, 5-hydroxydihydrocarveol; **101**, dihydrocarvone; **102**, dihydrocarveol; **103**, dihydrocarveol-8,9-epoxide; **104**, *p*-menthane-2,8,9-triol; **105**, dihydrobottrospicatol; **106**, 10-hydroxydihydrocarvone; **107**, 10-hydroxydihydrocarveol; **108**, carveyl acetate and carveyl propionate; **109**, 8,9-epoxydihydrocarveyl acetate; **110**, isopiperitenol; **111**, isopiperitenone; **112**, piperitenone; **113**, 7-hydroxyisopiperitenone; **114**, 10-hydroxyisopiperitenone; **115**, 4-hydroxyisopiperitenone; **116**, 5-hydroxyisopiperitenone; **117**, 5-hydroxypiperitenone; **118**, 7-hydroxypiperitenone; **119**, pulegone; **120**, 8,9-dehydromenthenone; **121**, 8-hydroxymenthenone; **122**, 1,8-cineole; **123**, 3-hydroxy1,8-cineole; **124**, 3-oxo-1,8-cineole; **125**, 2-hydroxy-1,8-cineole; **126**, 2-oxo-1,8-cineole;

127, 9-hydroxy-1,8-cineole; **128**, lactone (*R*)-5,5-dimethyl-4-(3′-oxobutyl)-4,5-dihydrofuran-2-(3H)-one; **129**, *p*-hydroxytoluene; **130**, 1,8-cineole-2-malonyl ester; **131**, 1,4-cineole; **132**, 2-hydroxy-1,4-cineole; **133**, 3-hydroxy-1,4-cineole; **134**, 8-hydroxy-1,4-cineole; **135**, 9-hydroxy-1, 4-cineole; **136**, 1,4-cineole-2-malonyl ester; **137**, menthol; **138**, 1-hydroxymenthol; **139**, 6-hydroxymenthol; **140**, 2-hydroxymenthol; **141**, 4-hydroxymenthol; **142**, 8-hydroxymenthol; **143**, 7-hydroxymenthol; **144**, 9-hydroxymenthol; **145**, 7,8-dihydroxymenthol; **146**, 1,8-dihydroxymenthol; **147**, 3-*p*-menthene; **148**, menthenone; **149**, menthone; **150**, 1-hydroxymenthone; **151**, 7-hydroxymenthone; **152**, 3,7-dimethyl-6-hydroxyoctanoic acid; **153**, 3,7-dimethyl-6-oxoactanoic acid; **154**, 2-methyl-2,5-oxidoheptenoic acid; **155**, menthone-1,2-diol; **156**, piperitone; **157**, 8-hydroxypiperitone; **158**, 6-hydroxypiperitone; **159**, 9-hydroxypiperitone; **160**, piperitone-7-al; **161**, 7-hydroxypiperitone; **162**, piperitone-7-oic acid; **163**, menthone-7-al; **164**, menthone-7-oic acid; **165**, 7-carboxylmenthol; **166**, piperitone oxide; **167**, 4-hydroxypiperitone; **168**, piperitenone oxide; **169**, 1-hydroxypulegone; **170**, 2,3-seco-*p*-menthylacetone-3-en-1-ol; **171**, 2-methyl-5-isopropyl-2,5-hexadienoic acid; **172**, 2,5,6-trimethyl-2,4-heptadienoic acid; **173**, 2,5,6-trimethyl-3-heptenoic acid; **174**, 2,5,6-trimethyl-2-heptenoic acid; **175**, 3-hydroxy-2,5,6-trimethyl-3-heptanoic acid; **176**, 3-oxo-2,5,6-trimethyl-3-heptanoic acid; **177**, 3,4-dimethylvaleric acid; **178**, *p*-cymene; **179**, thymol; **180**, 6-hydroxythymol **181**, 6-hydroxymenthenone, 6-hydroxy-4-*p*-menthen-3-one; **182**, 3-hydroxythymo1,4-quinol; **183**, 2-hydroxythymoquionone; **184**, 2,4-dimethyl-6-oxo-3,7-dimethyl-2,4-octadienoic acid; **185**, 2,4,6-trioxo-3,7-dimethyl octanoic acid; **186**, 2-oxobutanoic acid; **187**, acetic acid; **188**, 8-hydroxy-*p*-cymene; **189**, 9-hydroxy-*p*-cymene; **190**, 2-(4-methylphenyl)-propanoic acid; **191**, carvacrol; **192**, cumin alcohol; **193**, *p*-cumin aldehyde; **194**, cumic acid; **195**, *cis*-2,3-dihydroxy-2, 3-dihydro-*p*-cumic acid; **196**, 3-hydroxycumic acid; **197**, 2,3-dihydroxy-*p*-cumic acid; **198**, 2-hydroxy-6-oxo-7-methyl-2,4-octadien-1,3-dioic acid; **199**, 2-methyl-6-hydroxy-6-carboxy-7-octene; **201**, 4-methyl-2-oxopentanoic acid; **202**, carvacrol methyl ether; **203**, 7-hydroxycarvacrol methyl ether; **204**, 8-hydroxyperillyl alcohol; **205**, 8-hydroxyperillaldehyde; **206**, linalool; **207**, linalyl-6-cation; **208**, linalyl-8-cation; **209**, 6-hydroxymethyl linalool; **210**, linalool-6-al; **211**, 2-methyl-6-hydroxy-6-carboxy-2,7-octadiene; **212**, 2-methyl-6-hydroxy-7-octen-6-oic acid; **213**, phellandric acid-8-cation; **214**, 2,3-epoxylinalool; **215**, 5-methyl-5-vinyltetrahydro-2-furanol; **216**, 5-methyl-5-vinyltetrahydro-2-furanone; **217**, 2,2,6-trimethyl-3-hydroxy-6-vinyltetrahydropyrane; **218**, malonyl ester; **219**, 1-hydroxylinalool (3,7-dimethyl-1,6-octadiene-8-ol); **220**, 2,6-dimethyl-6-hydroxy-*trans*-2,7-octadienoic acid; **221**, 4-methyl-*trans*-3,5-hexadienoic acid; **222**, 4-methyl-trans-3,5-hexadienoic acid; **223**, 4-methyl-*trans*-2-hexenoic acid; **224**, isobutyric acid; **225**, 9-hydroxycamphor; **226**, bornyl acetate; **228**, 6-hydroxycamphor; **229**, 6-oxocamphor; **230**, 4-carboxymethyl-2,3,3-trimethylcyclopentanone; **231**, 4-carboxymethyl-3,5,5-trimethyltetrahydro-2-pyrone; **232**, isohydroxycamphoric acid; **233**, isoketocamphoric acid; **234**, 3,4,4-trimethyl-5-oxo-trans-2-hexenoic acid; **235**, 5-hydroxycamphor; **236**, 5-oxocamphor; **237**, **238**, 6-hydroxy-1, 2-campholide; **239**, 6-oxo-1,2-campholide; **240**, 5-carboxymethyl-3,4,4-trimethyl-2-cyclopentenone; **241**, 6-carboxymethyl-4,5,5-trimethyl-5,6-dihydro-2-pyrone; **242**, 5-hydroxy-3,4,4-trimethyl-2-heptene-1,7-dioic acid; **243**, 3-hydroxycamphor; **244**, camphorquinone; **245**, 2-hydroxyepicamphor; **246**, 2-hydroxy-*p*-menthan-7-oic acid; **247**, 2-oxo-*p*-menthan-7-oic acid; **248**, 3-isopropylheptane-1,7-dioic acid; **249**, 3-isopropylpentane-1,5-dioic acid; **250**, 4-methyl-3-oxopentanoic acid; **251**, methyl-isopropyl ketone; **252**, *p*-menthane; **253**, 1-hydroxy-*p*-menthane; **254**, *p*-menthane-1,9-diol; **255**, *p*-menthane-1,7-diol; **256**, 1-hydroxy-*p*-menthene-7-al; **257**, 1-hydroxy-*p*-menthene-7-oic acid; **258**, citronellol; **259**, dihydrocitronellol; **260**, 2,8-dihydroxy-2,6-dimethyl octane; **261**, citronellal; **262**, citronellic acid; **263**, pulegol; **264**, 7-hydroyxmethyl-6-octene-3-ol; **265**, 3,7-dimethyl-6-octane-1,8-diol; **266**, 3,7-dimethyloctane-1,8-diol; **267**, isopulegol; **268**, 6,7-epoxycitronellol; **269**, citronellyl acetate; **270**, geranyl acetate; **271**, geraniol; **272**, nerol **273**, nerol-6,7-epoxide; **274**, 6,7-epoxygeraniol; **275**, neral; **276**, geranial; **277**, neric acid; **278**, geranic acid; **279**, 2,6-dimethyl-8-hydroxy-7-oxo-2-octene; **280**, 6-methyl-5-heptenoic acid; **281**, 7-methyl-3-carboxymethyl-2,6-octadiene-1-oic acid; **282**, 7-methyl-3-hydroxy-3-carboxymethyl-6-octen-1-oic acid; **283**, 7-methyl-3-oxo-6-octenoic acid; **284**, 6-methyl-5-heptenoic acid; **284**, 4-methyl-3-heptenoic acid; **285**, 4-methyl-3-pentenoic acid; **286**, 3-methyl-2-butenoic acid; **287**, 3-hydroxymethyl-2,6-octadiene-1-ol; **288**, 3-formyl-2,6-

octadiene-1-ol; **289**, 3-carboxy-2,6-octadiene-1-ol; **290**, 3-(4-methyl-3-pentenyl)-3-butenolide; **291**, 7-hydroxymethyl-3-methyl-2,6-octadien-1-ol; **292**, 3,7-dimethyl-2,3-dihydroxy-6-octen-1-ol; **293**, 3,7-dimethyl-2-oxo-6-octene-1,3-diol; **294**, 6-methyl-5-hepten-2-one; **295**, 6-methyl-7-hydroxy-5-heptene-2-one; **296**, 2-methyl-γ-butyrolactone; **297**, 6-methyl-5-heptenoic acid; **298**, 6-methyl-5-hepten-2-ol; **299**, 7-methyl-3-oxo-6-octanoic acid; **300**, 3,7-dimethyl-2,6-octadiene-1,8-diol; **301**, isopulegyl acetate; **302**, myrcene; **303**, 2-methyl-6-methylene-7-octene-2,3-diol; **304**, 6-methylene-7-octene-2,3-diol; **305**, myrcenol; **306**, ocimene; **307**, myrcene-3,(10)-epoxide; **308**, myrcene-3,(10)-glycol; **309**, (−)-citronellene; **309′**, (+)-citronellene; **310**, (3*R*)-3,7-dimethyl-6-octene-1,2-diol; **310′**, (3*S*)-3,7-dimethyl-6-octene-1,2-diol; **311**, (*E*)-3,7-dimethyl-5-octene-1,7-diol; **312**, *trans*-rose oxide; **313**, *cis*-rose oxide; **314**, (2*Z*,5*E*)-3,7-dimethyl-2,5-octadiene-1,7-diol; **315**, (Z)-3,7-dimethyl-2,7-octadiene-1,6-diol; **316**, (2*E*,6*Z*)-2,6-dimethyl-2,6-octadiene-1,8-diol; **317**, a cyclization product; **318**, (2*E*,5*E*)-3,7-dimethyl-2,5-octadiene-1,7-diol; **319**, (*E*)-3,7-dimethyl-2,7-octadiene-1,6-diol; **320**, 8-hydroxynerol; **321**, 10-hydroxynerol; **322**, 1-hydroxy-3,7-dimethyl-2*E*,6*E*-octadienal; **323**, 1-hydroxy-3,7-dimethyl-2*E*,6*E*-octadienoic acid; **324**, 3,9-epoxy-*p*-menth-1-ene; **325**, tetrahydrolinalool; **326**, 3,7-dimethyloctane-3,5-diol; **327**, 3,7-dimethyloctane-3,7-diol; **328**, 3,7-dimethyl-octane-3,8-diol; **329**, dihydromyrcenol; **330**, 1,2-epoxydihydromyrcenol; **331**, 3β-hydroxydihydromyrcerol; **332**, dihydromyrcenyl acetate; **333**, 1,2-dihydroxydihydromyrcenyl acetate; **334**, (+)-*p*-menthane-1-β,2α,8-triol; **335**, α-pinene-1,2-epoxide; **336**, 3-carene; **337**, 3-carene-1,2-epoxide; **338**, (1*R*)-*trans*-isolimonene; **338**, (1*R*,4*R*)-*p*-menth-2-ene-8,9-diol; **339**, (1*R*,4*R*)-*p*-menth-2-ene-8,9-diol; **340**, α-Terpinene; **341**, α-terpinene-7-oic acid; **342**, (−)-terpinen-4-ol; **343**, *p*-menthane-1,2,4-triol; **344**, γ-terpinene; **345**, γ-terpinene-7-oic acid; **346**, terpinolene; **347**, (1*R*)-8-hydroxy-3-*p*-menthen-2-one; **348**, (1*R*)-1,8-dihydroxy-3-*p*-menthen-2-one; **349**, 6β-hydroxyfenchol; **350**, 5β-hydroxyfenchol (a,5β,b,5α); **351**, terpinolene-1,2-*trans*-diol; **352**, terpinolene-4,8-diol; **353**, terpinolene-9-ol; **354**, terpinolene-10-ol; **355**, α-phellandrene; **356**, α-phellandrene-7-oic acid; **357**, terpinolene-7-oic acid; **358**, thymoquinone; **359**, 1,2-dihydroperillaldehyde; **360**, 1,2-dihydroperillic acid; **361**, 8-hydroxy-1,2-dihydroperillyl alcohol; **362**, tetrahydroperillaldehyde (a *trans*, b *cis*); **363**, tetrahydroperillic acid (a *trans*, b *cis*); **364**, (−)-menthol monoglucoside; **365**, (+)-menthol diglucoside; **366**, (+)-isopulegol; **367**, 7-hydroxy-(+)-isopulegol; **368**, 10-hydroxy-(+)-isopulegol; **369**, 1,2-epoxy-α-terpineol; **370**, bornane-2,8-diol; **371**, *p*-menthane-1α,2β,4β-triol; **372**, 1-*p*-menthene-4β,6-diol; **373**, 1-*p*-menthene-4a,7-diol; **374**, (+)-bottrospicatal; **375**, 1-*p*-menthene-2β,8,9-triol; **376**, (−)-perillyl alcohol monoglucoside; **377**, (−)-perillyl alcohol diglucoside; **378**, 4α-hydroxy-(−)-isodihydrocarvone; **379**, 2-methyl-2-cyclohexenone; **380**, 2-cyclohexenone; **381**, 3-methyl-2-cyclohexenone; **382**, 2-methylcyclohexanone; **383**, 2-methylcyclohexanol (a, *trans* b, *cis*); **384**, 4-hydroxycarvone; **385**, 2-ethyl-2-cyclohexenone; **386**, 2-ethylcyclohexenone (a1*R*) (b1*S*); **387**, 1-hydroxypulegone; **388**, 5-hydroxypulegone; **389**, 8-hydroxymenthone; **390**, 10-hydroxy-(−)-carvone; **391**, 1,5,5-trimethylcyclopentane-1,4-dicarboxylic acid; **392**, 4β-hydroxy-(−)-menthone; **393**, 1α,4β-dihydroxy-(−)-menthone; **394**, 7-hydroxy-9-carboxymenthone; **395**, 7-hydroxy-1,8-cineole; **396**, methyl ester of 2α-hydroxy-1,8-cineole; **397**, ethyl ester of 2α-hydroxy-1,8-cineole; **398**, *n*-propyl ester of 2α-hydroxy-1,8-cineole; **399**, *n*-butyl ester of 2α-hydroxy-1,8-cineole; **400**, isopropyl ester of 2α-hydroxy-1,8-cineole; **401**, tertiary butyl ester of 2α-hydroxy-1,8-cineole; **402**, methylisopropyl ester of 2α-hydroxy-1,8-cineole; **403**, methyl tertiary butyl ester of 2α-hydroxy-1,8-cineole; **404**, 2α-hydroxy-1,8-cineole monoglucoside (404 and 404'); **405**, 2α-hydroxy-1,8-cineole diglucoside; **406**, *p*-menthane-1,4-diol; **407**, 1-*p*-menthene-4β,6-diol; **408**, (−)-pinane-2α,3α-diol; **409**, (−)-6β-hydroxypinene; **410,** (−)-4α,5-dihydroxypinene; **411**, (−)-4α-hydroxypinen-6-one; **412**, 6β,7-dihydroxyfenchol; **413**, 3-oxo-pinane; **414**, 2α-hydroxy-3-pinanone; **415**, 2α, 5-dihydroxy-3-pinanone; **416**, 2α,10-dihydroxy-3-pinanone; **417**, *trans*-3-pinanol; **418**, pinane-2α,3α-diol; **419**, pinane-2α, 3α, 5-triol; **420**, isopinocampheol (3-pinanol); **421**, pinane-1,3α-diol; **422**, pinane-3α,5-diol; **423**, pinane-3β,9-diol; **424**, pinane-3β,4β,-diol; **425**, **426**, pinane-3α,4β-diol; **427**, pinane-3α,9-diol; **428**, pinane-3α,6-diol; **429**, *p*-menthane-2α,9-diol; **430**, 2-methyl-3α-hydroxy-1-hydroxyisopropyl cyclohexane propane; **431**, 5-hydroxy-3-pinanone; **432**, 2α-methyl,3-(2-methyl-2-hydroxypropyl)-cyclopenta-1β-ol; **433**, 3-acetoxy-2α-pinanol; **434**, 8-hydroxy-α-pinene;**435**, **436**, myrtanoic acid; **437**, camphene; **438**, camphene gylcol; **439**, (+)-3-carene; **440**, *m*-mentha-4,6-dien-8-ol; **441**,

m-cymen-8-ol; **442**, 3-carene-9-ol; **443**, 3-carene-9-carboxylic acid; **444**, 3-caren-10-ol-9-carboxylic acid; **445**, 3-carene-9,10-dicarboxylic acid; **446**, (–)-*cis*-carane; **447**, dicarboxylic acid of (–)-*cis*-carane; **448**, (–)-6β-hydroxypinene; **449**, (–)-4α,5-dihydroxypinene; **450**, (–)-4α-hydroxypinen-6-one; **451**, 10-hydroxyverbenol; **452**, (–)-nopol; **453**, 7-hydroxymethyl-1-*p*-menthen-8-ol; **454**, 3-oxonopol; **455**, nopol benzyl ether; **456**, 4-oxonopl-2′,4′-dihydroxybenzyl ether; **457**, 7-hydroxymethyl-1-*p*-menthen-8-ol benzyl ether; **458**, piperitenol; **459**, thymol methyl ether; **460**, 7-hydroxythymol methyl ether; **461**, 9-hydroxythymol methyl ether; **462**, 1,8-cineol-9-oic acid; **463**, 4-hydroxyphellandric acid; **464**, 4-hydroxydihydrophellandric acid; **465**, (+)-8-hydroxyfenchol; **466**, (–)-9-hydroxyfenchol; **467**, (+)-10-hydroxyfenchol, **468**, 4α-hydroxy-6-oxo-α-pinene; **469**, dihydrolinalyl acetate; **470**, 3-hydroxycarvacrol; **471**, 9-hydroxycarvacrol; **472**, carvacrol-9-oic acid; **473**, 8,9-dehydrocarvacrol; **474**, 8-hydroxycarvacrol; **475**, 7-hydroxycarvacrol; **476**, carvacrol-7-oic acid; **477**, 8,9-dihydroxycarvacrol; **478**, 7,9-dihydroxycarvacrol methyl ether; **479**, 7-hydroxythymol; **480**, 9-hydroxythymol; **481**, thymol-7-oic acid; **482**, 7,9-dihydroxythymol; **483**, thymol-9-oic acid; **484,** (1*R*,2*R*,3*S*,4*S*,5*R*)-3,4-pinanediol.

REFERENCES

Abraham, W.-R., H.-A. Arfmann, B. Stumpf, P. Washausen, and K. Kieslich, 1988. Microbial transformations of some terpenoids and natural compounds. In: *Bioflavour'87. Analysis—Biochemistry—Biotechnology*, P. Schreier, ed., pp. 399–414. Berlin: Walter de Gruyter and Co.

Abraham, W.-R., H.M.R. Hoffmann, K. Kieslich, G. Reng, and B. Stumpf, 1985. Microbial transformation of some monoterpenes and sesquiterpenoids. In: *Enzymes in Organic Synthesis*, R. Porter and S. Clark, Ciba Foundation Symposium 111, pp. 146–160. London: Pitman Press.

Abraham, W.-R., K. Kieslich, H. Reng, and B. Stumpf, 1984. Formation and production of 1,2-trans-glycols from various monoterpenes with 1-menthene skeleton by microbial transformations with *Diplodia gossypina*. In: *3rd European Congr. on Biotechnology*, Vol. 1, pp. 245–248. Verlag Chemie, Weinheim.

Abraham, W.-R., B. Stumpf, and H.-A. Arfmann, 1990. Chiral intermediates by microbial epoxidations. *J. Essent. Oil Res.*, 2: 251–257.

Abraham, W.-R., B. Stumpf, and K. Kieslich, 1986. Microbial transformation of terpenoids with 1-*p*-menthene skeleton. *Appl. Microbiol. Biotechnol.*, 24: 24–30.

Amsterdam, D. 1997. Susceptibility Testing of Antimicrobials in Liquid Media. In: *Antibiotics in Laboratory Medicine*, V. Lorian, ed., 4th ed. Maryland, USA: Williams & Wilkins, Maple Press.

Asakawa, Y., H. Takahashi, M. Toyota, Y. and Noma, 1991. Biotransformation of monoterpenoids, (–)- and (+)-menthols, terpionolene and carvotanacetone by *Aspergillus* species. *Phytochemistry*, 30: 3981–3987.

Asakawa, Y., M. Toyota, T. Ishida, T. Takemoto, 1983. Metabolites in rabbit urine after terpenoid administration. *Proc. 27th TEAC*, pp. 254–256.

Atta-ur-Rahman, M. Yaqoob, A. Farooq, S. Anjum, F. Asif, and M.I. Choudhary, 1998. Fungal transformation of (1*R*,2*S*,5*R*)-(–)-menthol by *Cephalosporium aphidicola*. *J. Nat. Prod.*, 61: 1340–1342.

Ausgulen, L.T., E. Solheim, and R.R. Scheline, 1987. Metabolism in rats of *p*-cymene derivatives: Carvacrol and thymol. *Pharmacol. Toxicol.*, 61: 98–102.

Babcka, J., J. Volf, J. Czchec, and P. Lebeda, 1956. Patent 56-9686b.

Ballal, N.R., P.K. Bhattacharyya, and P.N. Rangachari, 1967. Microbiological transformation of terpenes. Part XIV. Purification and properties of perillyl alcohol dehydrogenase. *Indian J. Biochem.*, 5:1–6.

Bauer, K., D. Garbe, and H. Surburg (eds.), 1990. *Common Fragrance and Flavor Materials: Preparation, Properties and Uses.* 2nd revised ed., 218pp. New York: VCH Publishers.Best, D.J. and K.J. Davis, 1988. *Soap, perfumery and Cosmetics* 4: 47.

Best, D.J., N.C. Floyd, A. Magalhaes, A. Burfield, and P.M. Rhodes, 1987. Initial enzymatic steps in the degradation of alpha-pinene by *Pseudomonas fluorescens* Ncimb 11671. *Biocatal. Biotransform.*, 1: 147–159.

Bhattacharyya, P.K. and K. Ganapathy, 1965. Microbial transformation of terpenes. VI. Studies on the mechanism of some fungal hydroxylation reactions with the aid of model systems. *Indian J. Biochem.*, 2: 137–145.

Bhattacharyya, P.K., B.R. Prema, B.D. Kulkarni, and S.K. Pradhan, 1960. Microbiological transformation of terpenes: Hydroxylation of α-pinene. *Nature*, 187: 689–690.

Bock, G., I. Benda, and P. Schreier, 1986. Biotransformation of linalool by *Botrytis cinerea***.** *J. Food Sci.*, 51: 659–662.

Bock, G., I. Benda, and P. Schreier, 1988. Microbial transformation of geraniol and nerol by *Botrytis cinerea*. *Appl. Microbiol. Biotechnol.*, 27: 351–357.

Bouwmester, H.J., J.A.R. Davies, and H. Toxopeus, 1995. Enantiomeric composition of carvone, limonene, and carveols in seeds of dill and annual and biennial caraway varieties. *J. Agric. Food Chem.*, 43: 3057–3064.

Bowen, E.R., 1975. Potential by-products from microbial transformation of *d*-limonene, *Proc. Fla. State Hortic. Soc.*, 88: 304–308.

Bradshaw, W.H., H.E. Conrad, E.J. Corey, I.C. Gunsalus, and D. Lednicer, 1959. Microbiological degradation of (+)-camphor. *J. Am. Chem. Soc*., 81: 5507.

Brit Patent, 1970, No. 1,187,320.

Brunerie, P., I. Benda, G. Bock, and P. Schreier, 1987a. Bioconversion of citronellol by *Botrytis cinerea. Appl. Microbiol. Biotechnol.*, 27: 6–10.

Brunerie, P., I. Benda, G. Bock, and P. Schreier, 1987b. Biotransformation of citral by *Botrytis cinerea*. *Z. Naturforsch.*, 42C: 1097–1100.

Brunerie, P., I. Benda, G. Bock, and P. Schreier, 1988. Bioconversion of monoterpene alcohols and citral by *Botrytis cinerea*. In: *Bioflavour'87. Analysis—Biochemistry—Biotechnology*, P. Schreier, ed., pp. 435–444. Berlin: Walter de Gruyter and Co.

Busmann, D. and R.G. Berger, 1994. Conversion of myrcene by submerged cultured basidiomycetes. *J. Biotechol.*, 37: 39–43.

Cadwallander, K.R., R.J. Braddock, M.E. Parish, and D.P. Higgins, 1989**.** Bioconversion of (+)-limonene by *Pseudomonas gladioli. J. Food Sci.*, 54: 1241–1245.

Cadwallader, K.R. and R.J. Braddock, 1992. Enzymatic hydration of (4*R*)-(+)-limonene to (4*R*)-(+)-alpha-terpineol. *Dev. Food Sci.*, 29: 571–584.

Cantwell, S.G., E.P. Lau, D.S. Watt, and R.R. Fall, 1978. Biodegradation of acyclic isoprenoids by *Pseudomonas* species. *J. Bacteriol.*, 135: 324–333.

Chamberlain, E.M. and S. Dagley, 1968. The metabolism of thymol by a *Pseudomonas*. *Biochem. J.*, 110: 755–763.

Chapman, P.J., G. Meerman, and I.C. Gunsalus, 1965. The microbiological transformation of fenchone. *Biochem. Biophys. Res. Commun*., 20: 104–108.

Chapman, P.J., G. Meerman, I.C. Gunsalus, R. Srinivasan, and K.L. Rinehart Jr., 1966. A new acyclic acid metabolite in camphor oxidation. *J. Am. Chem. Soc*., 88: 618–619.

Christensen, M. and D.E. Tuthill, 1985. *Aspergillus*: An overview. In: *Advances in Penicillium and Aspergillus Systematics*, R.A. Samson and J.I. Pitt, eds, pp. 195–209. New York: Plenum Press.

Conrad, H.E., R. DuBus, and I.C. Gunsalus, 1961. An enzyme system for cyclic ketone lactonization, *Biochem. Biophys. Res. Commun.*, 6: 293–297.

Conrad, H.E., R. DuBus, M.J. Mamtredt, and I.C. Gunsalus, 1965a. Mixed function oxidation II. Separation and properties of the enxymes catalyzing camphor ketolactonization. *J. Biol. Chem.*, 240: 495–503.

Conrad, H.E., K. Lieb, and I.C. Gunsalus, 1965b. Mixed function oxidation III. An electron transport complex in camphor ketolactonization. *J. Biol. Chem.*, 240: 4029–4037.

David, L. and H. Veschambre, 1984. Preparation d'oxydes de linalol par bioconversion. *Tetrahadron Lett.*, 25: 543–546.

David, L. and H. Veschambre, 1985. Oxidative cyclization of linalol by various microorganisms. *Agric. Biol. Chem*., 49: 1487–1489.

DeFrank, J.J. and D.W. Ribbons, 1977a. *p*-Cymene pathway in *Pseudomonas putida*: Initial reactions. *J. Bacteriol.*, 129: 1356–1364.

DeFrank, J.J. and D.W. Ribbons, 1977b. *p*-Cymene pathway in *Pseudomonas putida*: Ring cleavage of 2,3-dihydroxy-*p*-cumate and subsequent reactions. *J. Bacteriol.*, 129: 1365–1374.

Demirci, F., 2000. *Microbial transformation of bioactive monoterpenes*. Ph.D. thesis, pp. 1–137. Anadolu University, Eskisehir, Turkey.

Demirci, F., H. Berber, and K.H.C. Baser, 2007. Biotransformation of *p*-cymene to thymoquinone. *Book of Abstracts of the 38th ISEO*, SL-1, p. 6.

Demirci, F., N. Kirimer, B. Demirci, Y. Noma, and K.H.C. Baser, 2001. The biotransformation of thymol methyl ether by different fungi. *XII Biotechnology Congr.*, Book of abstracts, p. 47.

Demirci, F., Y. Noma, N. Kirimer, and K.H.C. Baser, 2004. Microbial transformation of (–)-carvone. *Z. Naturforsch.*, 59c: 389–392.

Demyttenaere, J.C.R. and H.L. De Pooter, 1996. Biotransformation of geraniol and nerol by spores of *Penicillium italicum*. *Phytochemistry*, 41: 1079–1082.

Demyttenaere, J.C.R. and H.L. De Pooter, 1998. Biotransformation of citral and nerol by spores of *Penicillium digitatum. Flav. Fragr. J.*, 13: 173–176.

Demyttenaere, J.C.R., M. del Carmen Herrera, and N. De Kimpe, 2000. Biotransformation of geraniol, nerol and citral by sporulated surface cultures of *Aspergillus niger* and *Penicillium* sp. *Phytochemistry*, 55: 363–373.

Demyttenaere, J.C.R., I.E.I. Koninckx, and A. Meersman, 1996. Microbial production of bioflavours by fungal spores. In: *Flavour Science. Recent Developments*, A.J. Taylor and D.S. Mottram, eds, pp. 105–110. Cambridge, UK: The Royal Society of Chemistry.

Demyttenaere, J.C.R. and H.M. Willemen, 1998. Biotransformation of linalool to furanoid and pyranoid linalool oxides by *Aspergillus niger*. *Phytochemistry*, 47: 1029–1036.

Dhavalikar, R.S. and P.K. Bhattacharyya, 1966. Microbial transformation of terpenes. Part VIII. Fermentation of limonene by a soil Pseudomonad. *Indian J. Biochem*., 3: 144–157.

Dhavalikar, R.S., A. Ehbrecht, and G. Albroscheit, 1974. Microbial transformations of terpenoids: β-pinene. *Dragoco Rep.*, 3: 47–49.

Dhavalikar, R.S., P.N. Rangachari, and P.K. Bhattacharyya, 1966. Microbial transformation of terpenes. Part IX. Pathways of degradation of limonene in a soil Pseudomonad. *Indian J. Biochem*., 3: 158–164.

Farooq, A., M.I. Choudhary, S. Tahara, T.-U. Rahman, K.H.C. Baser, and F. Demirci, 2002. The microbial oxidation of (−)-β-pinene by *Botrytis cinerea*. *Z. Naturforsch.*, 57c: 686–690.

Fenaroli, G., 1975. Synthetic flavors. In: *Fenaroli's Handbook of Flavor Ingredients*, eds. T.E. Furia and N. Bellanca eds, Vol. 2, pp. 6–563. Cleveland, OH: CRC Press.

Flynn, T.M. and I.A. Southwell, 1979. 1,3-Dimethyl-2-oxabicyclo [2,2,2]-octane-3-methanol and 1,3-dimethyl-2-oxabicyclo[2,2,2]-octane- 3-carboxylic acid, urinary metabolites of 1,8-cineole. *Aus. J. Chem*., 32: 2093–2095.

Ganapathy, K. and P.K. Bhattacharyya, unpublished data.

Gibbon, G.H., N.F. Millis, and S.J. Pirt, 1972. Degradation of α-pinene by bacteria. *Proc. IV IFS, Ferment. Technol. Today*, pp. 609–612.

Gibbon, G.H. and S.J. Pirt, 1971. The degradation of α-pinene by *Pseudomonas* PX 1. *FEBS Lett.*, 18: 103–105.

Gondai, T., M. Shimoda, and T. Hirata, 1999. Asymmetric reduction of enone compounds by *Chlorella miniata*. *Proc. 43rd TEAC*, pp. 217–219.

Griffiths, E.T., S.M. Bociek, P.C. Harries, R. Jeffcoat, D.J. Sissons, and P.W. Trudgill, 1987b. Bacterial metabolism of alpha-pinene: Pathway from alpha-pinene oxide to acyclic metabolites in *Nocardia* sp. strain P18.3**.** *J. Bacteriol*., 169: 4972–4979.

Griffiths, E.T., P.C. Harries, R. Jeffcoat, and P.W. Trudgill, 1987a. Purification and properties of alpha-pinene oxide lyase from *Nocardia* sp. strain P18.3. *J. Bacteriol*., 169: 4980–4983.

Gunsalus, I.C., P.J. Chapman, and J.-F. Kuo, 1965. Control of catabolic specificity and metabolism. *Biochem. Biophys. Res. Commun.*, 18: 924–931.

Gyoubu, K. and M. Miyazawa, 2005. Biotransformation of (+)- and (−)-fenchone by liver microsomes. *Proc. 49th TEAC*, pp. 420–422.

Gyoubu, K. and M. Miyazawa, 2006. Biotransformation of (+)- and (−)-camphor by liver microsome. *Proc. 50th TEAC*, pp. 253–255.

Hagiwara, Y. and M. Miyazawa, 2007. Biotransformation of cineole by the larvae of common cutworm (*Spodoptera litura*) as a biocatalyst. *Proc. 51st TEAC*, pp. 304–305.

Hagiwara, Y., H. Takeuchi, and M. Miyazawa, 2006. Biotransformation of (+)-and (−)-menthone by the larvae of common cutworm (*Spodoptera litura*) as a biocatalyst. *Proc. 50th TEAC*, pp. 279–280.

Hamada, H. and T. Furuya, 2000. Hydroxylation of monoterpenes by plant suspension cells. *Proc. 44th TEAC*, pp. 167–168.

Hamada, H., T. Furuya, and N. Nakajima, 1996. The hydroxylation and glycosylation by plant catalysts. *Proc. 40th TEAC*, pp. 111–112.

Hamada, H., T. Harada, and T. Furuya, 2001. Hydroxylation of monoterpenes by algae and plant suspension cells. *Proc. 45th TEAC*, pp. 366–368.

Hamada, H., M. Kaji, T. Hirata, T. Furuya, 2003. Enantioselective biotransformation of monoterpenes by *Cyanobacterium*. *Proc. 47th TEAC*, pp. 162–163.

Hamada, H., Y. Kondo, M. Kaji, and T. Furuta, 2002. Glycosylation of monoterpenes by plant suspension cells. *Proc. 46th TEAC*, pp. 321–322.

Hamada, H., A. Matsumoto, and J. Takimura, 2004. Biotransformation of acyclic monoterpenes by biocatalysts of plant cultured cells and *Cyanobacterium*. *Proc. 48th TEAC*, pp. 393–395.

Hamada, H. and H. Yasumune, 1995. The hydroxylation of monoterpenoids by plant cell biotransformation. *Proc. 39th TEAC*, pp. 375–377.

Hartline, R.A. and I.C. Gunsalus, 1971. Induction specificity and catabolite repression of the early enzymes in camphor degradation by *Pseudomonas putida*. *J. Bacteriol.*, 106: 468–478.

Hashimoto Y. and M. Miyazawa, 2001. Microbial resolution of esters of racemic 2-*endo*-hydroxy-1,8-cineole by *Glomerella cingulata*. *Proc. 45th TEAC*, pp. 363–365.

Hayashi, T., T. Kakimoto, H. Ueda, and C. Tatsumi, 1969. Microbiological conversion of terpenes. Part VI. Conversion of borneol. *J. Agric. Chem. Soc. Jpn*., 43: 583–587.

Hayashi, T., H. Takashiba, S. Ogura, H. Ueda, and C. Tsutsumi, 1968. *Nippon Nogei-Kagaku Kaishi,* **42**: 190–196.

Hayashi, T., H. Takashiba, H. Ueda, and C. Tsutsumi, 1967. *Nippon Nogei Kagaku Kaishi*, 41.254. no. 79878g.

Hayashi, T., S. Uedono, and C. Tatsumi, 1972. Conversion of α-terpineol to 8,9-epoxy-*p*-menthan-1-ol. *Agric. Biol. Chem.*, 36: 690–691.

Hirata, T., K. Shimoda, and T. Gondai, 2000. Asymmetric hydrogenation of the C–C double bond of enones with the reductases from *Nicotiana tabacum*. *Chem. Lett*., 29: 850–851.

Hosler, P., 1969. U.S. Patent 3,458,399.

Hudlicky, T., D. Gonzales, and D.T. Gibson, 1999. Enzymatic dihydroxylation of aromatics in enantioselective synthesis: Expanding asymmetric methodology. *Aldrichim. Acta*, 32: 35–61.

Hungund, B.L., P.K. Bhattachayya, and P.N. Rangachari, 1970. Methylisopropyl ketone from a terpene fermentation by the soil Pseudomonad, PL-strain. *Indian J. Biochem*., 7: 80–81.

Ishida, T., Y. Asakawa, and T. Takemoto, T. Aratani, 1979. Terpenoid biotransformation in mammals. II. Biotransformation of *dl*-camphene. *J. Pharm. Sci.*, 68: 928–930.

Ishida, T., Y. Asakawa, and T. Takemoto, 1981a. Metabolism of myrtenal, pellillaldehyde and dehydroabietic acid in rabbits. *Res. Bull. Hiroshima Inst. Technol.*, 15: 79–91.

Ishida, T., Y. Asakawa, T. Takemoto, and T. Aratani, 1981b. Terpenoids biotransformation in mammals. III. Biotransformation of α-pinene, β-pinene, pinane, 3-carene, carane, myrcene, and *p*-cymene in rabbits. *J. Pharm. Sci.*, 70: 406–415.

Iscan, G., 2005. Unpublished data.

Ismaili-Alaoui, M., B. Benjulali, D. Buisson, and R. Azerad, 1992. Biotransformation of terpenic compounds by fungi I. Metabolism of *R*-(+)-pulegone. *Tetrahedron Lett.*, 33: 2349–2352.

Janssens, L., H.L. De Pooter, N.M. Schamp, and E.J. Vandamme, 1992. Production of flavours by microorganisms. *Process Biochem.*, 27: 195–215.

Joglekar, S.S. and R.S. Dhavlikar, 1969. Microbial transformation of terpenoids. I. Identification of metabolites produced by a Pseudomonad from citronellal and citral. *Appl. Microbiol.*, 18: 1084–1087.

Kaji, M., H. Hamada, and T. Furuya, 2002. Biotransformation of monoterpenes by *Cyanobacterium* and plant suspension cells. *Proc. 46th TEAC*, pp. 323–325.

Kamino, F. and M. Miyazawa, 2005. Biotransformation of (+)-and (−)-pinane-2,3-diol using plant pathogenic fungus, *Glomerella cingulata* as a biocatalyst. *Proc. 49th TEAC*, pp. 395–396.

Kamino, F., Y. Noma, Y. Asakawa, and M. Miyazawa, 2004. Biotransformation of (1*S*,2*S*,3*R*,5*S*)-(+)-pinane-2,3-diol using plant pathogenic fungus, *Glomerella cingulata* as a biocatalyst. *Proc. 48th TEAC*, pp. 383–384.

Kayahara, H., T. Hayashi, C. and Tatsumi, 1973. Microbiological conversion of (−)-perillaldehyde and *p*-mentha-1,3-dien-7-al. *J. Ferment. Technol.*, 51: 254–259.

Kieslich, K., W.-R. Abraham, and P. Washausen, 1985. Microbial transformations of terpenoids. In: *Topics in flavor research*, R.G. Berger, S. Nitz, and P. Schreier, eds, pp. 405–427. Marzling Hangenham: Eichborn.

Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, 1997. *Color Atlas and Textbook of Diagnostic Microbiology*, Philadelphia: Lippincott-Raven Publishers.

Kraidman, G., B.B. Mukherjee, and I.D. Hill, 1969. Conversion of limonene into an optically active isomer of α-terpineol by a *Cladosporium species*. *Bacteriological Proc.*, p. 63.

Krasnobajew, V., 1984. Terpenoids. In: *Biotechnology*, K. Kieslich, ed., Vol. 6a, pp. 97–125. Weinheim: Verlag Chemie.

Kumagae, S. and M. Miyazawa, 1999. Biotransformation of *p*-menthanes using common cutworm larvae, *Spodoptera litura* as a biocatalyst. *Proc. 43rd TEAC*, pp. 389–390.

Lassak, E.V., J.T. Pinkey, B.J. Ralph, T. Sheldon, and J.J.H. Simes, 1973. Extractives of fungi. V. Microbial transformation products of piperitone. *Aust. J. Chem*., 26: 845–854.

Liu, W., A. Goswami, R.P. Steffek, R.L. Chemman, F.S. Sariaslani, J.J. Steffens, and J.P.N. Rosazza, 1988. Stereochemistry of microbiological hydroxylations of 1,4-cineole. *J. Org. Chem*., 53: 5700–5704.

MacRae, I.C., V. Alberts, R.M. Carman, and I.M. Shaw, 1979. Products of 1,8-cineole oxidation by a Pseudomonad. *Aust. J. Chem*., 32: 917–922.

Madyastha, K.M. 1984. Microbial transformations of acyclic monoterpenes. *Proc. Indian Acad. Sci.* (*Chem. Sci.*), 93: 677–686.

Madyastha, K.M. and P.K. Bhattacharyya, 1968. Microbiological transformation of terpenes. Part XIII. Pathways for degradation of *p*-cymene in a soil pseudomonad (PL-strain). *Indian J. Biochem.*, 5: 161–167.

Madyastha, K.M. and V. Renganathan, 1983. Bio-degradation of acetates of geraniol, nerol and citronellol by *P. incognita*: Isolation and identification of metabolites. *Indian J. Biochem. Biophys*., 20: 136–140.

Madyastha, K.M. and N.S.R. Krishna Murthy, 1988a. Regiospecific hydroxylation of acyclic monoterpene alcohols by *Aspergillus niger*. *Tetrahedron Lett.* 29: 579–580.

Madyastha, K.M. and N.S.R. Krishna Murthy, 1988b. Transformations of acetates of citronellol, geraniol, and linalool by *Aspergillus niger*: Regiospecific hydroxylation of citronellol by a cell-free system. *Appl. Microbiol. Biotechnol.*, 28, 324–329.

Madyastha, K.M., P.K. Bhattacharyya, and C.S. Vaidyanathan, 1977. Metabolism of a monoterpene alcohol, linalool, by a soil pseudomonad. *Can. J. Microbiol.*, 23: 230–239.

Mattison, J.E., L.L. McDowell, and R.H. Baum, 1971. Cometabolism of selected monoterpenoids by fungi associated with monoterpenoid-containing plants. *Bacteriological Proc.*, p. 141.

Miyamoto, Y. and M. Miyazawa, 2001. Biotransformation of (+)- and (−)-borneol by the larvae of common cutworm (*Spodoptera litura*) as a biocatalyst. *Proc. 45th TEAC*, pp. 377–378.

Miyazato, Y. and M. Miyazawa, 1999. Biotransformation of (+)- and (−)-α-fenchyl acetated using plant parasitic fungus, *Glomerella cingulata* as a biocatalyst. *Proc. 43rd TEAC*, pp. 213–214.

Miyazawa, M., H. Furuno, K. Nankai, and H. Kameoka, 1991d. Biotransformation of verbenone by plant pathogenic microorganism, *Rhizoctonia solani*. *Proc. 35th TEAC*, pp. 274–275.

Miyazawa, M., H. Furuno, and H. Kameoka, 1992a. Biotransformation of thujone by plant pathogenic microorganism, *Botrytis allii* IFO 9430. *Proc. 36th TEAC*, pp. 197–198.

Miyazawa, M., H. Huruno, and H. Kameoka, 1991a. Biotransformation of (+)-pulegone to (−)-1*R*-8-hydroxy-4-*p*-menthen-3-one by *Botrytis allii*. *Chem. Express*, 6: 479–482.

Miyazawa, M., H. Huruno, and H. Kameoka, 1991b. *Chem. Express*, 6: 873.

Miyazawa, M., H. Kakita, M. Hyakumachi, K. Umemoto, and H. Kameoka, 1991e. Microbiological conversion of piperitone oxide by plant pathogenic fungi *Rhizoctonia solani*. *Proc. 35th TEAC*, pp. 276–277.

Miyazawa, M., H. Kakita, M. Hyakumachi, and H. Kameoka, 1992d. Biotransformation of monoterpenoids having *p*-menthan-3-one skeleton by *Rhizoctonis solani*. *Proc. 36th TEAC*, pp. 191–192.

Miyazawa, M., H. Kakita, M. Hyakumachi, K. Umemoto, and H. Kameoka, 1992e. Microbiological conversion of monoterpenoids containing *p*-menthan-3-one skeleton by plant pathogenic fungi *Rhizoctonia solani*. *Proc. 36th TEAC*, pp. 193–194.

Miyazawa, M., S. Kumagae, H. Kameoka, 1997a. Biotransformation of (−)-menthol and (+)-menthol by common cutworm Larvae, *Spodoptera litura* as a biocatalyst. *Proc. 41st TEAC*, pp. 391–392.

Miyazawa, M., S. Kumagae, H. Kameoka, 1997b. Biotransformation of (+)-*trans*-myrtanol and (−)-*trans*-myrtanol by common cutworm Larvae, *Spodoptera litura* as a biocatalyst. *Proc. 41st TEAC*, pp. 389–390.

Miyazawa, M. and Y. Miyamoto, 2004. Biotransformation of (+)-(1*R*, 2*S*)-fenchol by the larvae of common cutworm (*Spodoptera litura*). *Tetrahadron*, 60: 3091–3096.

Miyazawa, M., T. Murata, and H. Kameoka, 1998. Biotransformation of β-myrcene by common cutworm larvae, *Spodoptera litura* as a biocatalyst. *Proc. 42nd TEAC*, pp. 123–125.

Miyazawa, M., H. Nankai, and H. Kameoka, 1996a. Microbial oxidation of citronellol by *Glomerella cingulata*. *Nat. Prod. Lett.*, 8: 303–305.

Miyazawa, M., Y. Noma, K. Yamamoto, and H. Kameoka, 1983. Microbiological conversion of *d*- and *l*-limonene, *Proc. 27th TEAC*, pp. 147–149.

Miyazawa, M., Y. Noma, K. Yamamoto, and H. Kameoka, 1991c. Biotransformation of 1,4-cineole to 2-*endo*-hydroxy-1,4-cineole by *Aspergillus niger*. *Chem. Express*, 6: 771–774.

Miyazawa, M., Y. Noma, K. Yamamoto, and H. Kameoka, 1992b. Biohydroxylation of 1,4-cineole to 9-hydroxy-1,4-cineole by *Aspergillus niger*. *Chem. Express*, 7: 305–308.

Miyazawa, M., Y. Noma, K. Yamamoto, and H. Kameoka, 1992c. Biotransformation of 1,4-cineole to 3-*endo*-hydroxy-1,4-cineole by *Aspergillus niger. Chem. Express*, 7: 125–128.

Miyazawa, M., Y. Suzuki, and H. Kameoka, 1994b. Biotransformation of myrtanol by plant pathogenic microorganism, *Glomerella cingulata*, *Proc. 38th TEAC*, pp. 96–97.

Miyazawa, M., Y. Suzuki, and H. Kameoka, 1997c. Biotransformation of (−)- and (+)-isopinocamphenol by three fungi. *Phytochemistry*, 45: 945–950.

Miyazawa, M., T. Wada, and H. Kameoka, 1995a. Biotransformation of terpinene, limonene and α-phellandrene in common cutworm larvae, *Spodoptera litura* Fabricius, *Proc. 39th TEAC*, pp. 362–363.

Miyazawa, M., T. Wada, and H. Kameoka, 1996b. Biotransformation of *p*-menthanes using common cutworm larvae, *Spodoptera litura* as a biocatalyst. *Proc. 40th TEAC*, pp. 80–81.

Miyazawa, M., K. Yamamoto, Y. Noma, and H. Kameoka, 1990a. Bioconversion of (+)-fenchone to (+)-6-*endo*-hydroxyfenchone by *Aspergillus niger*. *Chem. Express*, 5: 237–240.

Miyazawa, M., K. Yamamoto, Y. Noma, and H. Kameoka, 1990b. Bioconversion of (+)-fenchone to 5-*endo*-hydroxyfenchone by *Aspergillus niger*. *Chem. Express*, 5: 407–410.

Miyazawa, M., H. Yanahara, and H. Kameoka, 1995c. Biotransformation of *trans*-pinocarveol by plant pathogenic microorganism, *Glomerella cingulata*, and by the larvae of common cutworm, *Spodoptera litura* Fabricius. *Proc. 39th TEAC*, pp. 360–361.

Miyazawa, M., H. Yanagihara, and H. Kameoka, 1996c. Biotransformation of pinanes by common cutworm larvae, *Spodoptera litura* as a biocatalyst. *Proc. 40th TEAC*, pp. 84–85.

Miyazawa, M., K. Yokote, and H. Kameoka, 1994a. Biotransformation of linalool oxide by plant pathogenic microorganisms, *Glomerella cingulata*. *Proc. 38th TEAC*, pp. 101–102.

Miyazawa, M., K. Yokote, and H. Kameoka, 1995b. Biotransformation of 2-*endo*-hydroxy-1,4-cineole by plant pathogenic microorganism, *Glomerella cingulata*. *Proc. 39th TEAC*, pp. 352–353.

Mizutani, S., T. Hayashi, H. Ueda, and C. Tstsumom, 1971. Microbiological conversion of terpenes. Part IX. Conversion of linalool. *Nippon Nogei Kagaku Kaishi*, 45: 368–373.

Moroe, T., S. Hattori, A. Komatsu, and Y. Yamaguchi, 1971. Japanese Patent, 2.036. 875. no. 98195t.

Mukherjee, B.B., G. Kraidman, and I.D. Hill, 1973. Synthesis of glycols by microbial transformation of some monocyclic terpenes. *Appl. Microbiol.*, 25: 447–453.

Mukherjee, B.B., G. Kraidman, I.D. Hill, 1974. Transformation of 1-menthene by a *Cladosporium*: Accumulation of β-isopropyl glutaric acid in the growth medium. *Appl. Microbiol.*, 27: 1070–1074.

Murakami, T., I. Ichimoto, and C. Tstsumom, 1973. Microbiological conversion of linalool. *Nippon Nogei Kagaku Kaishi*, 47: 699–703.

Murata, T. and M. Miyazawa, 1999. Biotransformation of dihydromyrcenol by common cutworm larvae, *Spodoptera litura* as a biocatalyst. *Proc. 43rd TEAC*, pp. 393–394.

Nakanishi, K. and M. Miyazawa, 2004. Biotransformation of (−)-menthone by human liver microsomes. *Proc. 48th TEAC*, pp. 401–402.

Nakanishi, K. and M. Miyazawa, 2005. Biotransformation of (+)- and (−)- menthol by liver microsomal humans and rats. *Proc. 49th TEAC*, pp. 423–425.

Nishimura, H., S. Hiramoto, and J. Mizutani, 1983a. Biological activity of bottrospicatol and related compounds produced by microbial transformation of (−)-*cis*-carveol towards plants. *Proc. 27th TEAC*, pp. 107–109.

Nishimura, H., S. Hiramoto, J. Mizutani, Y. Noma, A. Furusaki, and T. Matsumoto, 1983b. Structure and biological activity of bottrospicatol, a novel monoterpene produced by microbial transformation of (−)-*cis*-carveol. *Agric. Biol. Chem*., 47: 2697–2699.

Nishimura, H. and Y. Noma, 1996. Microbial transformation of monoterpenes: flavor and biological activity. In: *Biotechnology for Improved Foods and Flavors*, G.R. Takeoka, R. Teranishi, P.J. Williams, and A. Kobayashi, A., ACS Symp. Ser. 637, pp.173–187. American Chemical Society, Washington, DC.

Nishimura, H., Y. Noma, and J. Mizutani, 1982. *Eucalyptus* as biomass. Novel compounds from microbial conversion of 1,8-cineole. *Agric. Biol. Chem.*, 46: 2601–2604.

Nishimura, H., D.M. Paton, and M. Calvin, 1980. *Eucalyptus radiata* oil as a renewable biomass. *Agric. Biol. Chem*., 44: 2495–2496.

Noma, Y., 1976. Microbiological conversion of carvone. Biochemical reduction of terpenes, part VI. *Ann. Res. Stud. Osaka Joshigakuen Junior College*, 20: 33–47.

Noma, Y., 1977. Conversion of the analogues of carvone and dihydrocarvone by *Pseudomonas ovalis*, strain 6-1, Biochemical reduction of terpenes, part VII. *Nippon Nogeikagaku Kaishi*, 51: 463–470.

Noma, Y., 1979a. Conversion of (−)-carvone by *Nocardia lurida* A-0141 and *Streptosporangium roseum* IFO3776. Biochemical reduction of terpenes, part VIII. *Nippon Nogeikagaku Kaishi*, 53: 35–39.

Noma, Y., 1979b. On the pattern of reaction mechanism of (+)-carvone conversion by actinomycetes. Biochemical reduction of terpenes, part X, *Ann. Res. Stud. Osaka Joshigakuen Junior College*, 23: 27–31.

Noma, Y., 1980. Conversion of (−)-carvone by strains of *Streptomyces*, A-5–1 and *Nocaradia*, 1–3–11. *Agric. Biol. Chem*., 44: 807–812.

Noma, Y., 1984. Microbiological conversion of carvone, *Kagaku to Seibutsu*, 22: 742–746.

Noma, Y., 1988. Formation of *p*-menthane-2,8-diols from (−)-dihydrocarveol and (+)-dihydrocarveol by *Aspergillus* spp., *The Meeting of Kansai Division of The Agricultural and Chemical Society of Japan*, Kagawa, p. 28.

Noma, Y., 2000. unpublished data.
Noma, Y., 2007. Microbial production of mosquitocidal (1*R*,2*S*,4*R*)-(+)-menthane- 2,8-diol. In: *Aromatic Plants from Asia their Chemistry and Application in Food and Therapy*, L. Jiarovetz, N.X. Dung, and V.K. Varshney, pp. 169–186. Dehradun: Har Krishan Bhalla & Sons.
Noma, Y., and Y. Asakawa, 1992. Enantio- and diastereoselectivity in the biotransformation of carveols by *Euglena gracilis* Z. *Phytochem.*, 31: 2009–2011.
Noma, Y. and Y. Asakawa, 1995. *Aspergillus* spp.: Biotransformation of Terpenoids and Related Compounds. In: *Biotechnology in Agriculture and Forestry*, *Vol. 33. Medicinal and Aromatic Plants VIII*, Y.P.S. Bajaj, ed., pp. 62–96. Berlin: Springer.
Noma, Y. and Y. Asakawa, 1998. *Euglena gracilis* Z: Biotransformation of terpenoids and related compounds. In: *Biotechnology in Agriculture and Forestry, Vol. 41. Medicinal and Aromatic Plants X*, Y.P.S. Bajaj, ed., pp. 194–237. Berlin Heidelberg: Springer.
Noma, Y. and Y. Asakawa, 2005a. New metabolic pathways of β-pinene and related compounds by *Aspergillus niger*. *Book of Abstracts of the 36th ISEO*, p. 32.
Noma, Y. and Y. Asakawa, 2005b. Microbial transformation of (−)-myrtenol and (−)-nopol. *Proc. 49th TEAC*, pp. 78–80.
Noma, Y. and Y. Asakawa, 2006a. Biotransformation of (+)-limonene and related compounds by *Citrus* pathogenic fungi. *Proc. 50th TEAC*, pp. 431–433.
Noma, Y. and Y. Asakawa, 2006b. Biotransformation of β-pinene, myrtenol, nopol and nopol benzyl ether by *Aspergillus niger* TBUYN-2. *Book of Abstracts of the 37th ISEO*, p. 144.
Noma, Y. and Y. Asakawa, 2006c. Microbial transformation of (−)-nopol benzyl ether. *Proc. 50th TEAC*, pp. 434–436.
Noma, Y. and Y. Asakawa, 2007a. Biotransformation of limonene and related compounds by newly isolated low temperature grown *citrus* pathogenic fungi and red yeast. *Book of Abstracts of the 38th ISEO*, p. 7.
Noma, Y. and Y. Asakawa, 2007b. Microbial transformation of limonene and related compounds. *Proc. 51st TEAC*, pp. 299–301.
Noma, Y. and Y. Asakawa, 2008. New metabolic pathways of (+)-carvone by Citrus pathogenic *Aspergillus niger* Tiegh CBAYN and *A. niger* TBUYN-2, *Proc. 52nd TEAC*, pp. 206–208.
Noma, Y. and M. Iwami, 1994. Separation and identification of terpene convertible actinomycetes: *S. bottropensis* SY-2-1, *S. ikutamanensis* Ya-2-1 and *S. humidus* Tu-1. *Bull. Tokushima Bunri Univ.*, 47: 99–110.
Noma, Y., M. Miyazawa, K. Yamamoto, H. Kameoka, T. Inagaki, and H. Sakai, 1984. Microbiological conversion of perillaldehyde. Biotransformation of *l*- and *dl*-perillaldehyde by *Streptomyces ikutamanensis*, Ya-2-1, *Proc. 28th TEAC*, pp. 174–176.
Noma, Y., H. Nishimura, and C. Tatsumi, 1980. Biotransformation of carveol by Actinomycetes. 1. Biotransformation of (−)-*cis*-carveol and (−)-*trans*-carveol by *Streptomyces bottropensis,* SY-2-1, *Proc. 24th TEAC*, pp. 67–70.
Noma, Y. and H. Nishimura, 1980. Microbiological transformation of 1,8-cineole. Oxidative products from 1,8-cineole by *S. bottropensis*, SY-2-1. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 28.
Noma, Y. and H. Nishimura, 1981. Microbiological transformation of 1,8-cineole. Production of 3β-hydroxy-1,8-cineole from 1,8-cineole by *S. ikutamanensis*, Ya-2-1. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 196.
Noma, Y. and H. Nishimura, 1982. Biotransformation of carvone. 4. Biotransformation of (+)-carvone by *Streptomyces bottropensis*, SY-2-1. *Proc. 26th TEAC*, pp. 156–159.
Noma, Y. and H. Nishimura, 1983a. Biotransformation of (−)-carvone and (+)-carvone by *S. ikutamanensis* Ya-2-1. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 390.
Noma, Y. and H. Nishimura, 1983b. Biotransformation of carvone. 5. Microbiological transformation of dihydrocarvones and dihydrocarveols, *Proc. 27th TEAC*, pp. 302–305.
Noma, Y. and H. Nishimura, 1984. Microbiological conversion of carveol. Biotransformation of (−)-*cis*-carveol and (+)-*cis*-carveol by *S. bottropensis*, Sy-2-1. *Proc. 28th TEAC*, pp. 171–173.
Noma, Y. and H. Nishimura, 1987. Bottrospicatols, novel monoterpenes produced on conversion of (−)- and (+)-*cis*-carveol by *Streptomyces*. *Agric. Biol. Chem*., 51: 1845–1849.
Noma, Y., H. Nishimura, S. Hiramoto, M. Iwami, and C. Tstsumi, 1982. A new comound, (4*R*, 6*R*)-(+)-6,8-oxidomenth-1-en-9-ol produced by microbial conversion of (−)-*cis*-carveol. *Agric. Biol. Chem*., 46: 2871–2872.
Noma, Y., S. Nonomura, and H. Sakai, 1974a. Conversion of (−)-carvotanacetone and (+)-carvotanacetone by *Pseudomonas ovalis*, strain 6-1, *Agric. Biol. Chem*., 38: 1637–1642.

Noma, Y., S. Nonomura, H. Ueda, and C. Tatsumi, 1974b. Conversion of (+)-carvone by *Pseudomonas ovalis*, strain 6-1(1). *Agric. Biol. Chem*., 38: 735–740.

Noma, Y., S. Nonomura, H. Ueda, H. Sakai, and C. Tstusmi, 1974c. Microbial transformation of carvone. *Proc. 18th TEAC*, pp. 20–23.

Noma, Y., S. Nonomura, and H. Sakai, 1975. Epimerization of (−)-isodihydrocarvone to (−)-dihydrocarvone by *Pseudomonas fragi* IFO 3458. *Agric. Biol. Chem*., 39: 437–441.

Noma, Y. and S. Nonomura, 1974. Conversion of (−)-carvone and (+)-carvone by a strain of *Aspergillus niger*. *Agric. Biol. Chem*., 38: 741–744.

Noma, Y. and H. Sakai, 1984. Investigation of the conversion of (−)-perillyl alcohol, 1,8-cineole, (+)-carvone and (−)-carvone by rare actinomycetes. *Ann. Res. Stud. Osaka Joshigakuen Junior College*, 28: 7–18.

Noma, Y. and C. Tatsumi, 1973. Conversion of (−)-carvone by *Pseudomonas ovalis*, strain 6-1(1), Microbial conversion of terpenes part XIII. *Nippon Nogeikagaku Kaishi*, 47: 705–711.

Noma, Y., M. Toyota, and Y. Asakawa, 1985a. Biotransformation of (−)-carvone and (+)-carvone by *Aspergillus* spp. *Annual Meeting of Agricultural and Biological Chemistry*, Sapporo, p. 68.

Noma, Y., M. Toyota, and Y. Asakawa, 1985b. Biotransformation of carvone. 6. Biotransformation of (−)-carvone and (+)-carvone by a strain of *Aspergillus niger*. *Proc. 29th TEAC*, pp. 235–237.

Noma, Y., M. Toyota, Y. and Asakawa, 1985c. Microbiological conversion of (−)-carvotanacetone and (+)-carvotanacetone by *S. bottropensis* SY-2-1. *Proc. 29th TEAC*, pp. 238–240.

Noma, Y., M. Toyota, and Y. Asakawa, 1986. Reduction of terpene aldehydes and epoxidation of terpene alcohols by *S. ikutamanensis*, Ya-2-1. *Proc. 30th TEAC*, pp. 204–206.

Noma, Y., M. Toyota, and Y. Asakawa, 1988a. Microbial transformation of thymol formation of 2-hydroxy-3-*p*-menthen-5-one by *Streptomyces humidus*, Tu-1. *Proc. 28th TEAC*, pp. 177–179.

Noma, Y., H. Takahashi, M. Toyota, and Y. Asakawa, 1988b. Microbiological conversion of (−)-carvotanacetone and (+)-carvotanacetone by a strain of *Aspergillus niger*. *Proc. 32nd TEAC*, pp. 146–148.

Noma, Y., H. Takahashi, and Y. Asakawa, 1989. Microbiological conversion of menthol. Biotransformation of (+)-menthol by a strain of *Aspergillus niger*. *Proc. 33rd TEAC*, pp. 124–126.

Noma, Y., H. Takahashi, and Y. Asakawa, 1990. Microbiological conversion of *p*-menthane 1. Formation of *p*-menthane-1,9-diol from *p*-menthane by a strain of *Aspergillus niger*. *Proc. 34th TEAC*, pp. 253–255.

Noma, Y., H. Takahashi, and Y. Asakawa, 1991a. Biotransformation of terpene aldehyde by *Euglena gracilis* Z. *Phytochem.*, 30: 1147–1151.

Noma, Y., N. Miki, E. Akehi, E. Manabe, and Y. Asakawa, 1991b. Biotransformation of monoterpenes by photosynthetic marine algae, *Dunaliella tertiolecta*, *Proc. 35th TEAC*, pp. 112–114.

Noma, Y., E. Akehi, N. Miki, and Y. Asakawa, 1992a. Biotransformation of terpene aldehyde, aromatic aldehydes and related compounds by *Dunaliella tertiolecta. Phytochemistry*, 31: 515–517.

Noma, Y., S. Yamasaki, and Asakawa Y. 1992b. Biotransformation of limonene and related compounds by *Aspergillus cellulosae*. *Phytochemistry*, 31: 2725–2727.

Noma, Y., H. Takahashi, T. Hashimoto, and Y. Asakawa, 1992c. Biotransforamation of isopiperitenone, 6-gingerol, 6-shogaol and neomenthol by a strain of *Aspergillus niger*. *Proc. 37th TEAC*, pp. 26–28.

Noma, Y., A. Sogo, S. Miki, N. Fujii, T. Hashimoto, and Y. Asakwawa, 1992d. Biotransformation of terpenoids and related compounds. *Proc. 36th TEAC*, pp. 199–201.

Noma, Y., H. Takahashi, and Y. Asakawa, 1993. Formation of 8 kinds of *p*-menthane-2,8-diols from carvone and related compounds by *Euglena gracilis* Z. Biotransformation of monoterpenes by photosynthetic microorganisms. Part VIII. *Proc. 37th TEAC*, pp. 23–25.

Noma, Y., T. Higata, T. Hirata, Y. Tanaka, T. Hashimoto, and Y. Asakawa, 1995. Biotransformation of [6-^{2}H]-(−)-carvone by *Aspergillus niger*, *Euglena gracilis* Z and *Dunaliella tertiolecta*, *Proc. 39th TEAC*, pp. 367–368.

Noma, Y., K. Hirata, and Y. Asakawa, 1996. Biotransformation of 1,8-cineole. Why do the biotransformed 2α- and 3α-hydroxy-1,8-cineole by *Aspergillus niger* have no optical activity? *Proc. 40th TEAC*, pp. 89–91.

Noma, Y., K. Matsueda, I. Maruyama, and Y. Asakawa, 1997. Biotransformation of terpenoids and related compounds by *Chlorella* species. *Proc. 41st TEAC*, pp. 227–229.

Noma, Y., J. Watanabe, T. Hashimoto, and Y. Asakawa, 2001. Microbiological transformation of β-pinene. *Proc. 45th TEAC*, pp. 88–90.

Noma, Y., M. Furusawa, T. Hashimoto, and Y. Asakawa, 2002. Stereoselective formation of (1*R*, 2*S*, 4*R*)-(+)-*p*-menthane-2,8-diol from α-pinene. *Book of Abstracts of the 33rd ISEO*, p. 142.

Noma, Y., F. Kamino, T. Hashimoto, and Y. Asakawa, 2003. Biotransformation of (+)- and (−)-pinane-2,3-diol and related compounds by *Aspergillus niger*. *Proc. 47th TEAC*, pp. 91–93.

Noma, Y., M. Furusawa, T. Hashimoto, and Y. Asakawa, 2004. Biotransformation of (+)- and (−)-3-pinanone by *Aspergillus niger*. *Proc. 48th TEAC*, pp. 390–392.

Noma, Y., T. Hashimoto, S. Uehara, and Y. Asakawa, 2009. unpublished data.

Nonoyama, H., H. Matsui, M. Hyakumachi, and M. Miyazawa, 1999. Biotransformation of (−)-menthone using plant parasitic fungi, *Rhizoctonia solani* as a biocatalyst. *Proc. 43rd TEAC*, pp. 387–388.

Ohsawa, M. and Miyazawa, M. 2001. Biotransformation of (+)- and (−)-isopulegol by the larvae of common cutworm (*Spodoptera litura*) as a biocatalyst. *Proc. 45th TEAC*, pp. 375–376.

Omata, T. Iwam N. oto, T. Kimura, A. Tanaka, S. Fukui, 1981. Stereoselective hydrolysis of *dl*-menthyl succinate by gel-entrapped *Rhodotorula minuta* var. *texensis* cells in organic solvent. *Appl. Microbiol. Biotechnol.*, 11: 119–204.

Oosterhaven, K., K.J. Hartmans, and J.J.C. Scheffer, 1995a. Inhibition of potato sprouts growth by carvone enantiomers and their bioconversion in sprouts. *Potato Res.*, 38: 219–230.

Oosterhaven, K., B. Poolman, and E.J. Smid, 1995b. *S*-Carvone as a natural potato sprouts inhibiting, fungistatic and bacteriostatic compound. *Ind. Crops Prod.*, 4: 23–31.

Oritani, T. and K. Yamashita, 1973a. Microbial *dl*-acyclic alcohols, *Agric. Biol. Chem*., 37: 1923–1928.

Oritani, T. and Yamashita, K. 1973b. Microbial resolution of racemic 2- and 3-alkylcyclohexanols. *Agric. Biol. Chem*., 37: 1695–1700.

Oritani, T. and K. Yamashita, 1973c. Microbial resolution of *dl*-isopulegol. *Agric. Biol. Chem*., 37: 1687–1689.

Oritani, T. and K. Yamashita, 1973d. Microbial resolution of racemic carvomenthols. *Agric. Biol. Chem*., 37: 1691–1694.

Oritani, T. and K. Yamashita, 1974. Microbial resolution of (±)-borneols. *Agric. Biol. Chem*., 38: 1961–1964.

Oritani, T. and Yamashita, K. 1980. Optical resolution of *dl*-β, γ-unsaturated terpene alcohols by biocatalyst of microorganism. *Proc. 24th TEAC*, pp. 166–169.

Pfrunder, B. and Ch. Tamm, 1969a. Mikrobiologische Umwandlung von bicyclischen monoterpenen durch *Absidia orchidis* (Vuill.) Hagem. 2. Teil: Hydroxylierung von Fenchon und Isofenchon. *Helv. Chim. Acta.*, 52: 1643–1654.

Pfrunder, B. and Ch. Tamm, 1969b. Mikrobiologische Umwandlung von bicyclischen monoterpenen durch *Absidia orchidis* (Vuill.) Hagem. 1. Teil: Reduktion von Campherchinon und Isofenchonchinon. *Helv. Chim. Acta.*, 52: 1630–1642.

Prema, B.R. and P.K. Bhattachayya, 1962. Microbiological transformation of terpenes. II. Transformation of α-pinene. *Appl. Microbiol.*, 10: 524–528.

Rama Devi, J. and P.K. Bhattacharyya, 1977a. Microbiological transformations of terpenes. Part XXIV. Pathways of degradation of linalool, geraniol, nerol and limonene by *Pseudomonas incognita*, linalool strain. *Indian J. Biochem. Biophys.*, 14: 359–363.

Rama Devi, J. and P.K. Bhattacharyya, 1977b. Microbiological transformation of terpenes. Part XXIII. Fermentation of geraniol, nerol and limonene by soil Pseudomonad, *Pseudomonas incognita* (linalool strain). *Indian J. Biochem. Biophys.*, 14: 288–291.

Rama Devi, J., S.G. Bhat, and P.K. Bhattacharyya, 1977. Microbiological transformations of terpenes. Part XXV. Enzymes involved in the degradation of linalool in the *Pseudomonas incognita*, linalool strain. *Indian J. Biochem. Biophys.*, 15: 323–327.

Rama Devi, J. and P.K. Bhattacharyya, 1978. Molecular rearrangements in the microbiological transformations of terpenes and the chemical logic of microbial processes. *J. Indian Chem. Soc*., 55: 1131–1137.

Rapp. A. and H. Mandery, 1988. Influence of *Botrytis cinerea* on the monoterpene fraction wine aroma. In: *Bioflavour'87. Analysis – Biochemistry – Biotechnology*, ed. Schreier P., Walter de Gruyter and Co., Berlin, pp. 445–452.

Saeki M. and N. Hashimoto, 1968. Microbial transformation of terpene hydrocarbons. Part I. Oxidation products of *d*-limonene and *d*-pentene. *Proc. 12th TEAC*, pp. 102–104.

Saeki, M. and N. Hashimoto, 1971. Microorganism biotransformation of terpenoids. Part II. Production of *cis*-terpin hydrate and terpineol from *d*-limonene. *Proc. 15th TEAC*, pp. 54–56.

Saito, H. and M. Miyazawa, 2006. Biotransformation of 1,8-cineole by *Salmonella typhimurium* OY1001/3A4. *Proc. 50th TEAC*, pp. 275–276.

Savithiry, N., T.K. Cheong, and P. Oriel, 1997. Production of alpha-terpineol from *Escherichia coli* cells expressing thermostable limonene hydratase. *Appl. Biochem. Biotechnol.*, 63–65: 213–220.

Sawamura, Y., S. Shima, H. Sakai, and C. Tatsumi, 1974. Microbiological conversion of menthone. *Proc. 18th TEAC*, pp. 27–29.

Schwammle, B., E. Winkelhausen, S. Kuzmanova, and W. Steiner, 2001. Isolation of carvacrol assimilating microorganisms. *Food Technol. Biotechnol.*, 39: 341–345.

Seubert, W. and E. Fass, 1964a. Studies on the bacterial degradation of isoprenoids. V. The mechanism of isoprenoid degradation. *Biochem. Z.*, 341: 35–44.

Seubert, W. and E. Fass, 1964b. Studies on the bacterial degradation of isoprenoids. IV. The purification and properties of beta-isohexenylglutaconyl-COA-hydratase and beta-hydroxy-beta-isohexenylglutaryl-COA-lyase. *Biochem. Z.*, 341: 23–34.

Seubert, W. and U. Remberger, 1963. Studies on the bacterial degradation of isoprenoid compounds. II. The role of carbon dioxide. *Biochem. Z.*, 338: 245–246.

Seubert, W., E. Fass, and U. Remberger, 1963. Studies on the bacterial degradation of isoprenoid compounds. III. Purification and properties of geranyl carboxylase. *Biochem. Z.*, 338: 265–275.

Shima, S., Y. Yoshida, Y. Sawamura, and C. Tstsumi, 1972. Microbiological conversion of perillyl alcohol. *Proc. 16th TEAC*, pp. 82–84.

Shimoda, K., D.I. Ito, S. Izumi, and T. Hirata, 1996. Novel reductase participation in the syn-addition of hydrogen to the C=C bond of enones in the cultured cells of *Nicotiana tabacum***.** *J. Chem. Soc., Perkin Trans*. 1, 355–358.

Shimoda, K., T. Hirata, and Y. Noma, 1998. Stereochemistry in the reduction of enones by the reductase from *Euglena gracilis. Z. Phytochem.*, 49: 49–53.

Shimoda, K., S. Izumi, and T. Hirata, 2002. A novel reductase participating in the hydrogenation of an exocyclic C–C double bond of enones from *Nicotiana tabacum***.** *Bull. Chem. Soc. Jpn*., 75: 813–816.

Shimoda, K., N. Kubota, H. Hamada, and M. Kaji, 2003. *Cyanobacterium* catalyzed asymmetric reduction of enones. *Proc. 47th TEAC*, pp. 164–166.

Shindo, M., T. Shimada, and M. Miyazawa, 2000. Metabolism of 1,8-cineole by cytochrome P450 enzymes in human and rat liver microsomes. *Proc. 44th TEAC*, pp. 141–143.

Shukla, O.P., and P.K. Bhattacharyya, 1968. Microbiological transformations of terpenes: Part XI—Pathways of degradation of α- & β-pinenes in a soil Pseudomonad (PL-strain). *Indian J. Biochem*., 5: 92–101.

Shukla, O.P., M.N. Moholay, and P.K. Bhattacharyya, 1968. Microbiological transformation of terpenes: Part X—Fermantation of α- & β-pinenes by a soil Pseudomonad (PL-strain). *Indian J. Biochem*., 5: 79–91.

Shukla, O.P., R.C. Bartholomeus, and I.C. Gunsalus, 1987. Microbial transformation of menthol and menthane-3,4-diol. *Can. J. Microbiol.*, 33: 489–497.

Southwell, I.A. and T.M. Flynn, 1980. Metabolism of α- and β-pinene, *p*-cymemene and 1,8-cineole in the brush tail possum. *Xenobiotica*, 10: 17–23.

Suga, T. and T. Hirata, 1990. Biotransformation of exogenous substrates by plant cell cultures. *Phytochemistry*, 29: 2393–2406.

Suga, T., T. Hirata, and H. Hamada, 1986. The stereochemistry of the reduction of carbon–carbon double bond with the cultured cells of *Nicotiana tabacum. Bull. Chem. Soc. Jpn*., 59: 2865–2867.

Sugie, A. and M. Miyazawa, 2003. Biotransformation of (−)-α-pinene by human liver microsomes. *Proc. 47th TEAC*, pp. 159–161.

Swamy, G.K., K.L. Khanchandani, and P.K. Bhattacharyya, 1965. Symposium on recent advances in the chemistry of terpenoids, Natural Institute of Sciences of India, New Dehli, p. 10.

Takagi, K., Y. Mikami, Y. Minato, I. Yajima, and K. Hayashi, 1972. Manufacturing metho of carvone by microorganisms, *Japanese Patent* 72-38998.

Takahashi, H., Y. Noma, M. Toyota, and Y. Asakawa, 1994. The biotransformation of (−)- and (+)-neomenthols and isomenthols by *Aspergillus niger*. *Phytochemistry*, 35: 1465–1467.

Takeuchi, H. and M. Miyazawa, 2004. Biotransformation of nerol by the larvae of common cutworm (*Spodoptera litura*) as a biocatalyst. *Proc. 48th TEAC*, pp. 399–400.

Takeuchi, H. and M. Miyazawa, 2005. Biotransformation of (−)- and (+)-citronellene by the larvae of common cutworm (*Spodoptera litura*) as biocatalyst. *Proc. 49th TEAC*, pp. 426–427.

Trudgill, P.W., 1990. Microbial metabolism of terpenes—recent developments. *Biodegradation* 1: 93–105.

Tsukamoto, Y., S. Nonomura, H. Sakai, and C. Tatsumi, 1974. Microbiological oxidation of *p*-menthane 1. Formation of formation of *p-cis*-menthan-1-ol. *Proc. 18th TEAC*, pp. 24–26.

Tsukamoto, Y., S. Nonomura, and H. Sakai, 1975. Formation of *p-cis*-menthan-1-ol from *p*-menthane by *Pseudomonas mendociana* SF. *Agric. Biol. Chem.*, 39: 617–620.

Van der Werf, M.J., J.A.M. de Bont, and D.J. Leak, 1997. Opportunities in microbial biotransformation of monoterpenes. *Adv. Biochem. Eng./Biotechnol.*, 55: 147–177.

Van der Werf, M.J. and J.A.M. de Bont, 1998a. Screening for microorganisms converting limonene into carvone. In: *New frontiers in screening for microbial biocatalysts*, *Proc. Int. Symp.*, Ede, The Netherlands, K. Kieslich, C.P. Beek, J.A.M. van der Bont, and W.J.J. van den Tweel, eds, Vol. 53, pp. 231–234. Studies in Organic Chemistry.

Van der Werf, M.J., K.M. Overkamp, and J.A.M. de Bont, 1998b. Limonene-1,2-epoxide hydrolase from *Rhodococcus erythropolis* DCL14 belongs to a novel class of epoxide hydrolases. *J. Bacteriol.*, 180: 5052–5057.

van Dyk, M.S., E. van Rensburg, I.P.B. Rensburg, and N. Moleleki, 1998. Biotransformation of monoterpenoid ketones by yeasts and yeast-like fungi, *J. Mol. Catal. B: Enzym.*, 5: 149–154.

Verstegen-Haaksma, A.A., H.J. Swarts, B.J.M. Jansen, A. de Groot, N. Bottema-MacGillavry, and B. Witholt, 1995. Application of *S*-(+)-carvone in the synthesis of biologically active natural products using chemical transformations and bioconversions. *Ind. Crops Prod.*, 4: 15–21.

Watanabe, T., H. Nomura, T. Iwasaki, A. Matsushima, and T. Hirata, 2007. Cloning of pulegone reductase and reduction of enones with the recombinant reductase. *Proc. 51st TEAC*, pp. 323–325.

Watanabe, Y. and T. Inagaki, 1977a. Japanese Patent 77.12.989. No. 187696x.

Watanabe, Y. and T. Inagaki, 1977b. Japanese Patent 77.122.690. No. 87656g.

Wolf-Rainer, A., 1994. Phylogeny and biotransformation. Part 5. Biotransformation of isopinocampheol. *Z. Naturforsch.*, 49c: 553–560.

Yamada, K., S. Horiguchi, and J. Tatahashi, 1965. Studies on the utilization of hydrocarbons by microorganisms. Part VI. Screening of aromatic hydrocarbon-assimilating microorganisms and cumic acid formation from *p*-cymene. *Agric. Biol. Chem.*, 29: 943–948.

Yamaguchi, Y., A. Komatsu, and T. Moroe, 1977. Asymmetric hydrolysis of *dl*-menthyl acetate by *Rhodotorula mucilaginosa*. *J. Agric. Chem. Soc. Jpn.*, 51: 411–416.

Yamamoto, K., M. Miyazawa, H. Kameoka, and Y. Noma, 1984. Biotransformation of *d*- and *l*-fenchone by a strain of *Aspergillus niger*. *Proc. 28th TEAC*, pp. 168–170.

Yamanaka, T. and M. Miyazawa, 1999. Biotransformation of (−)-*trans*-verbenol by common cutworm larvae, *Spodoptera litura* as a biocatalyst. *Proc. 43rd TEAC*, pp. 391–392.

Yawata, T., M, Ogura, K. Shimoda, S. Izumi, and T. Hirata, 1998. Epoxidation of monoterpenes by the peroxidase from the cultured cells of *Nicotiana tabacum*, *Proc. 42nd TEAC*, pp. 142–144.

Yonemoto, N., S. Sakamoto, T. Furuya, and H. Hamada, 2005. Preparation of (−)-perillyl alcohol oligosaccharides. *Proc. 49th TEAC*, pp. 108–110.

15 Biotransformation of Sesquiterpenoids, Ionones, Damascones, Adamantanes, and Aromatic Compounds by Green Algae, Fungi, and Mammals

Yoshinori Asakawa and Yoshiaki Noma

CONTENTS

15.1 INTRODUCTION

Recently, environment-friendly green or clean chemistry is emphasized in the field of organic and natural product chemistry. Noyori's high-efficient production of (−)-menthol using (*S*)-BINAP-Rh

catalyst is one of the most important green chemistries (Tani et al., 1982; Otsuka and Tani, 1991) and 1000 ton of (−)-menthol has been produced by this method in 1 year. On the other hand, enzymes of microorganisms and mammals are able to transform a huge variety of organic compounds, such as mono- sesqui-, and diterpenoids, alkaloids, steroids, antibiotics, and amino acids from crude drugs and spore-forming green plants to produce pharmacologically and medicinally valuable substances.

Since Meyer and Neuberg (1915) studied the microbial transformation of citronellal, there are a great number of reports concerning biotrasformation of essential oils, terpenoids, steroids, alkaloids, and acetogenins using bacteria, fungi, and mammals. In 1988 Mikami (Mikami, 1988) reported the review article of biotransformation of terpenoids entitled "Microbial Conversion of Terpenoids." Lamare and Furstoss (1990) reviewed biotransformation of more than 25 sesquiterpenoids by microorganisms. In this chapter, the recent advances in the biotransformation of natural and synthetic compounds; sesquiterpenoids, ionones, α-damascone, and adamantanes, and aromatic compounds, using microorganisms including algae and mammals are described.

15.2 BIOTRANSFORMATION OF SESQUITERPENOIDS BY MICROORGANISMS

15.2.1 Highly Efficient Production of Nootkatone (2) from Valencene (1)

The most important and expensive grapefruit aroma, nootkatone (**2**), decreases the somatic fat ratio (Haze et al., 2002), and therefore its highly efficient production has been requested by the cosmetic and fiber industrial sectors. Previously, valencene (**1**) from the essential oil of Valencia orange was converted into nootkatone (**2**) by biotransformation using *Enterobacter* species only in 12% yield (Dhavlikar and Albroscheit, 1973), *Rodococcus* KSM-5706 in 0.5% yield with a complex mixture (Okuda et al., 1994), and using Cytochrome P450 (CYP450) in 20% yield with other complex products (Sowden et al., 2005). Nootkatone (**2**) was chemically synthesized from valencene (**1**) with $AcOOCMe_3$ in three steps and chromic acid in low yield (Wilson and Saw, 1978) and using surface-functionalized silica supported by metal catalysts such as Co^{2+}, Mn^{2+}, and so on with *tert*-butyl hydroperoxide in 75% yield (Salvador and Clark, 2002). However, these synthetic methods are not safe because they involve toxic heavy metals. An environment-friendly method for the synthesis of nootkatone that does not use any heavy metals such as chromium and manganese must be designed. The commercially available and cheap sesquiterpene hydrocarbon (+)-valencene (**1**) ($[\alpha]_D$ + 84.6°, c = 1.0) obtained from Valencia orange oil was very efficiently converted into nootkatone (**2**) by biotransformations using *Chlorella* (Hashimoto et al., 2003a), *Mucor* species (Hashimoto et al., 2003), *Botryosphaeria dothidea*, and *Botryodiplodia theobromae* (Furusawa et al., 2005, 2005a; Noma et al., 2001a).

Chlorella fusca var. *vacuolata* IAMC-28 (Figure 15.1) was inoculated and cultivated while stationary under illumination in Noro medium $MgCl_2{\cdot}6H_2O$ (1.5 g), $MgSO_4{\cdot}7H_2O$ (0.5 g), KCl (0.2 g), $CaCl_2{\cdot}2H_2O$ (0.2 g), KNO_3 (1.0 g), $NaHCO_3$ (0.43 g), TRIS (2.45 g), K_2HPO_4 (0.045 g), Fe-EDTA (3.64 mg), EDTA-2Na (1.89 mg), $ZnSO_4{\cdot}7H_2O$ (1.5 g), H_3BO_2 (0.61 mg), $CoCl_2{\cdot}6H_2O$ (0.015 mg), $CuSO_4{\cdot}5H_2O$ (0.06 mg), $MnCl_2{\cdot}4H_2O$ (0.23 mg), and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.38 mg), in distilled H_2O 1 L (pH 8.0). Czapek-peptone medium [1.5% sucrose, 1.5% glucose, 0.5% polypeptone, 0.1% K_2HPO_4, 0.05% $MgSO_4{\cdot}7H_2O$, 0.05% KCl, and 0.001% $FeSO_4{\cdot}7H_2O$, in distilled water (pH 7.0)] was used for the biotransformation of substrate by microorganism. *Aspergillus niger* was isolated in our laboratories from soil in Osaka prefecture, and was identified according to its physiological and morphological characters.

(+)-Valencene (**1**) (20 mg/50 mL) isolated from the essential oil of Valencia orange was added to the medium and biotransformed by *Chlorella fusca* for a further 18 days to afford nootkatone (**2**) [gas chromatography-mass spectrometry (GC-MS) peak area: 89%; isolated yield: 63%] (Figure 15.2) (Furusawa et al., 2005, 2005a; Noma et al., 2001a). The reduction of **2** with $NaBH_4$ and $CeCl_3$ gave

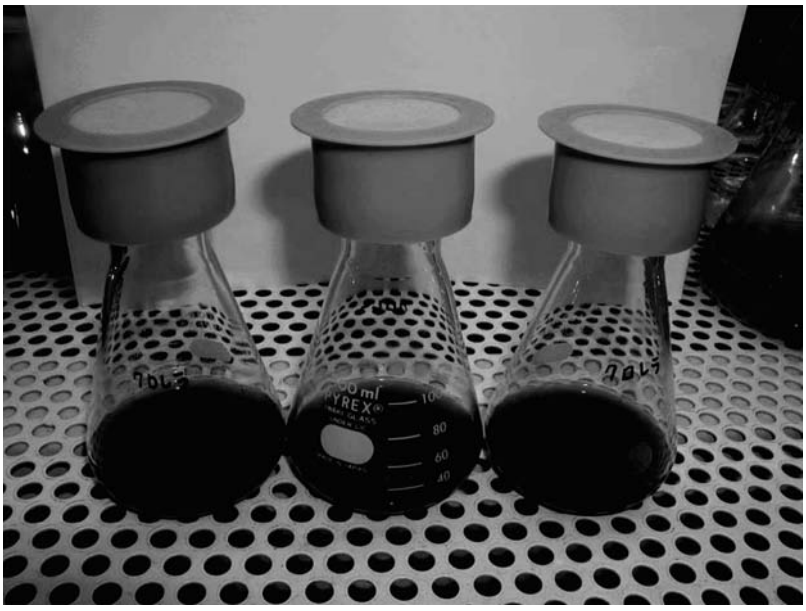


FIGURE 15.1 *Chlorella fusca* var. *vacuolata*.

2α-hydroxyvalencene (**3**) in 87% yield, followed by Mitsunobu reaction with *p*-nitrobenzoic acid, triphenylphosphine, and diethyl azodicarboxylate to give nootkatol (2β-hydroxyvalencene) (**4**), possessing calcium-antagonistic activity isolated from *Alpinia oxyphylla* (Shoji et al., 1984) in 42% yield. Compounds **3** and **4** thus obtained were easily biotransformed by *Chlorella fusca* and *Chlorella pyrenoidosa* for only 1 day to give nootkatone (**2**) in good yield (80–90%), respectively.

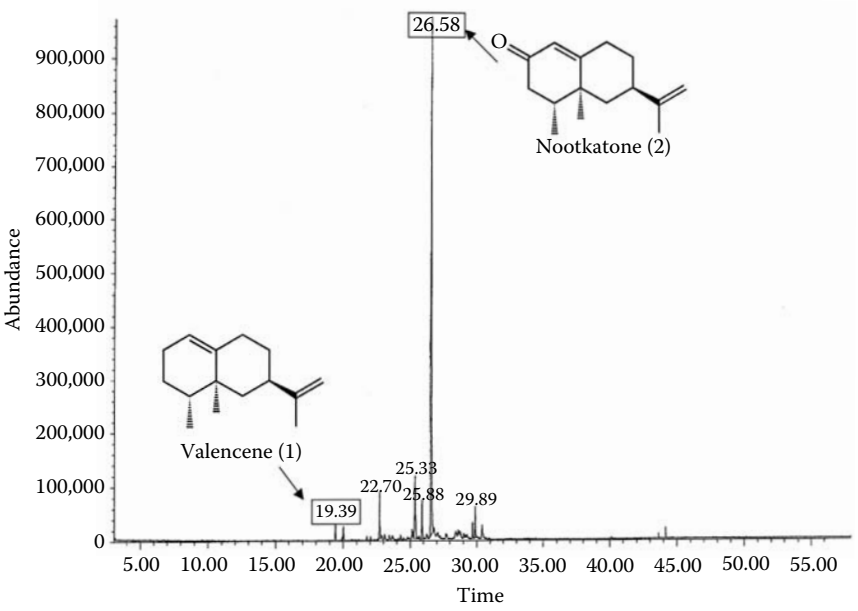


FIGURE 15.2 Total ion chromatogram of metabolites of valencene (**1**) by *Chlorella fusca* var. *vacuolata*.

TABLE 15.1
Conversion of Valencene (1) to Nootkatone (2) by *Chlorella* sp. for 14 Days

	Metabolites (% of the Total in GC-MS)				
***Chlorella* sp.**	**Valencene (1)**	**2α-Nootkatol (3)**	**2β-Nootkatol (4)**	**Nootkatone (2)**	**Conversion Ratio (%)**
C. fusca	11	0	0	89	89
C. pyrenoidosa	7	0	0	93	93
C. vulgaris	0	0	0	100	100

The biotransformation of compound **1** was further carried out by *Chlorella pyrenoidosa* and *Chlorella vulgaris* (Furusawa et al., 2005, 2005a) and soil bacteria (Noma et al., 2001) to give nootkatone in good yield (Table 15.1).

In the time course of the biotransformation of **1** by *Chlorella pyrenoidosa*, the yield of nootkatone (**2**) and nootkatol (**4**) without 2α-hydroxyvalencene (**3**) increased with the decrease in that of **1**, and subsequently the yield of **2** increased with decrease in that of **3**. In the metabolic pathway of valencene (**1**), **1** was slowly converted into nootkatol (**4**), and subsequently **4** was rapidly converted into **2**, as shown in Figure 15.3.

A fungus strain from the soil adhering to the thalloid liverwort *Pallavicinia subciliata*, *Mucor* species, which was inoculated and cultivated statically in Czapek-peptone medium (pH 7.0) at 30°C for 7 days. Compound **1** (20 mg/50 mL) was added to the medium and incubated for a further 7 days.

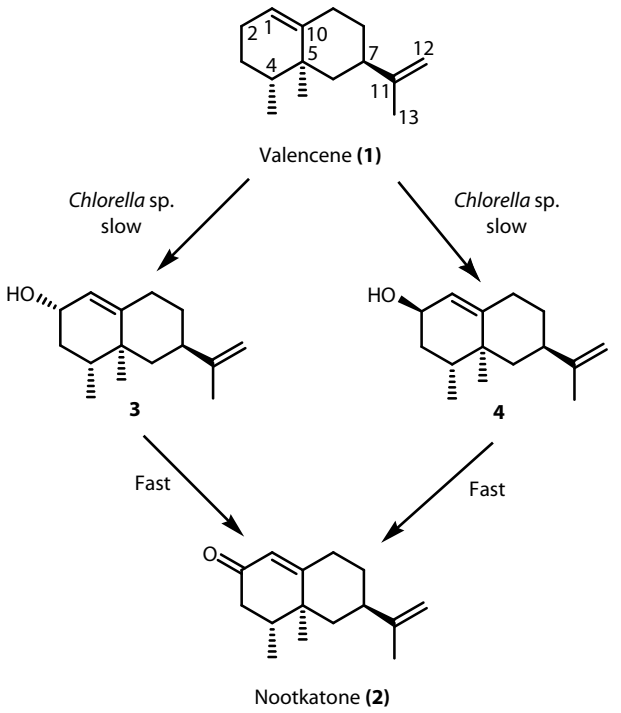


FIGURE 15.3 Biotransformation of valencene (**1**) by *Chlorella* species.

Nootkatone (**2**) was then obtained in very high yield (82%) (Furusawa et al., 2005; Noma et al., 2001a).

The biotransformation from **1** to **2** was also examined using the plant pathogenic fungi *Botryosphaeria dothidea* and *Botryodiplodia theobromae* (a total of 31 strains) separated from fungi infecting various types of fruit, and so on. *Botryosphaeria dothidea* and *Botryodiplodia theobromae* were both inoculated and cultivated while stationary in Czapek-peptone medium (pH 7.0) at 30°C for 7 days. The same size of the substrate **1** was added to each medium and incubated for a further 7 days to obtain nootkatone (42–84%) (Furusawa et al., 2005).

The expensive grapefruit aromatic, nootkatone (**2**) used by cosmetic and fiber manufacturers was obtained in high yield by biotransformation of (+)-valencene (**1**), which can be cheaply obtained from Valencia orange, by *Chlorella* species, fungi such as *Mucor* species, *Botryosphaeria dothidea*, and *Botryodiplodia theobromae*. This is a very inexpensive and clean oxidation reaction, which does not use any heavy metals, and thus this method is expected to find applications in the industrial production of nootkatone.

15.2.2 Biotransformation of Valencene (1) by *Aspergillus niger* and *Aspergillus wentii*

Valencene (**1**) from Valencia orange oil was cultivated by *Aspergillus niger* in Czapek-peptone medium, for 5 days to afford six metabolites **5** (1.0%), **6** and **7** (13.5%), **8** (1.1%), **9** (1.5%), **10** (2.0%), and **11** (0.7%), respectively. Ratio of compounds **6** (11*S*) and **7** (11*R*) was determined as 1:3 by HPLC analysis of their thiocarbonates (**12** and **13**) (Noma et al., 2001a) (Figure 15.4).

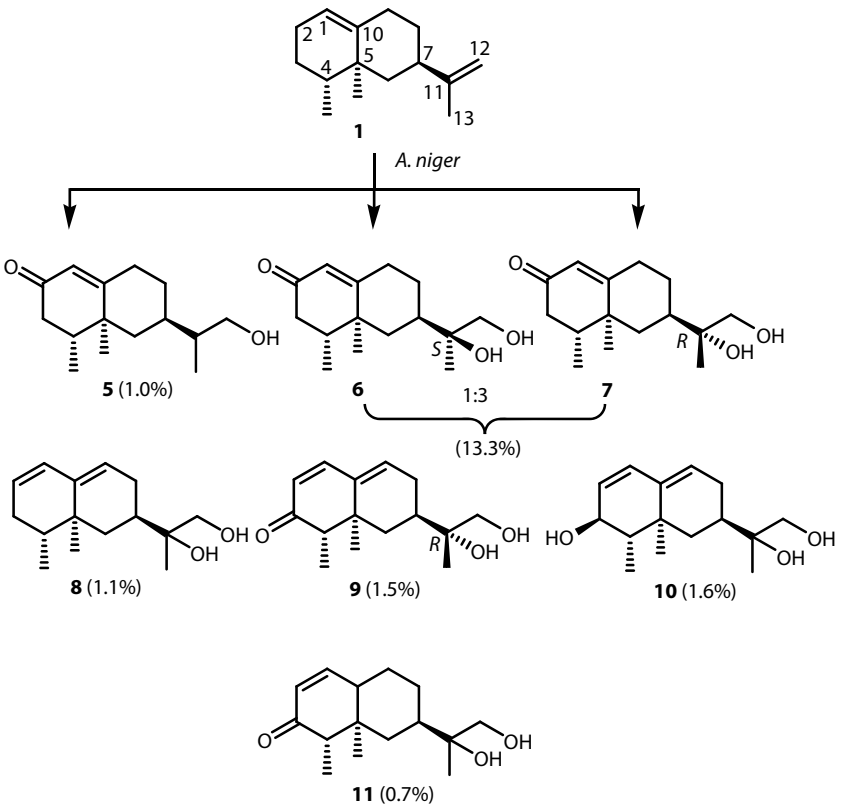


FIGURE 15.4 Biotransformation of valencene (**1**) by *Aspergillus niger*.

Compounds **8**–**11** could be biosynthesized by elimination of a hydroxy group of 2-hydroxyvalencenes (**3**, **4**). Compound **3** was biotransformed for 5 days by *Aspergillus niger* to give three metabolites **6** and **7** (6.4%), **8** (34.6%), and **9** (5.5%), respectively. Compound **4** was biotransformed for 5 days by *Aspergillus niger* to give three metabolites: **6** and **7** (21.8%), **9** (5.5%), and **10** (10.4%), respectively (Figure 15.5).

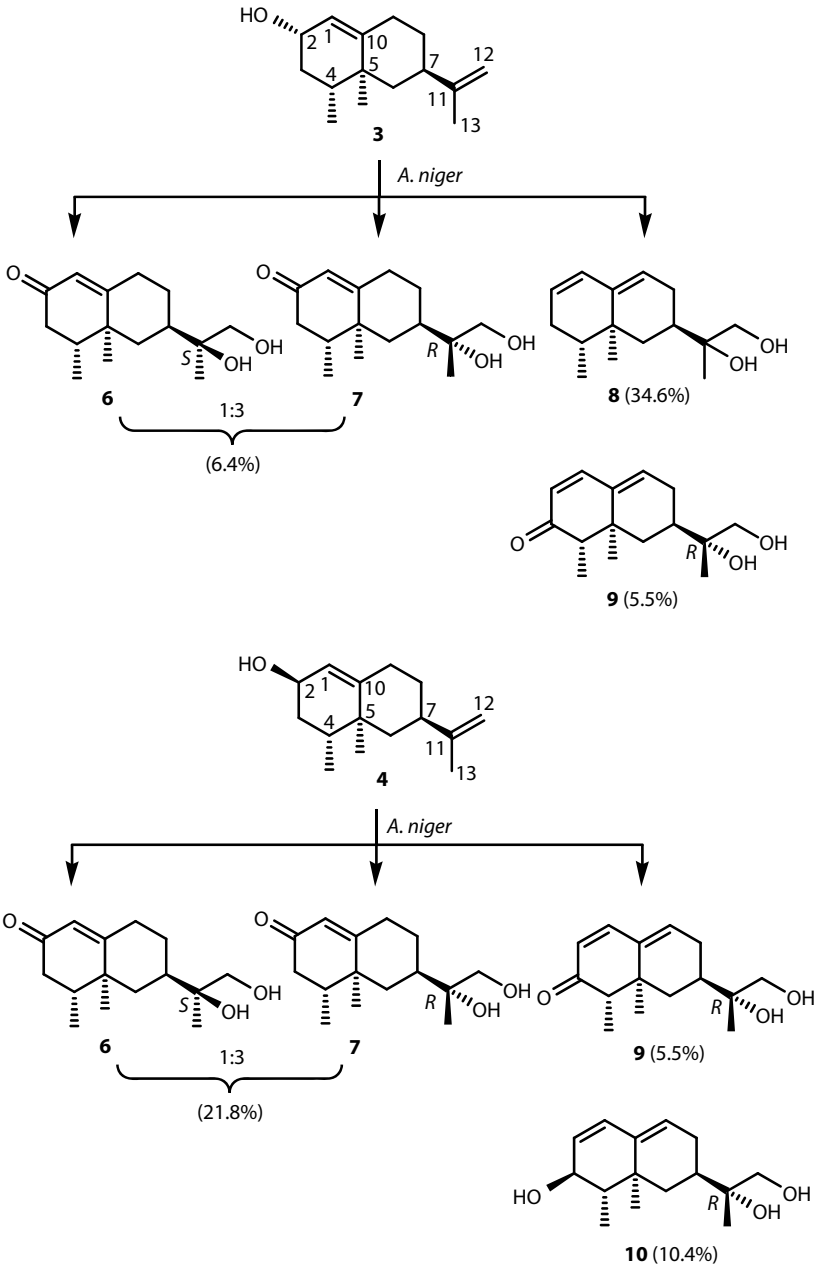


FIGURE 15.5 Biotransformation of 2α-hydroxyvalencene (**3**) and 2β-hydroxyvalencene (**4**) by *Aspergillus niger*.

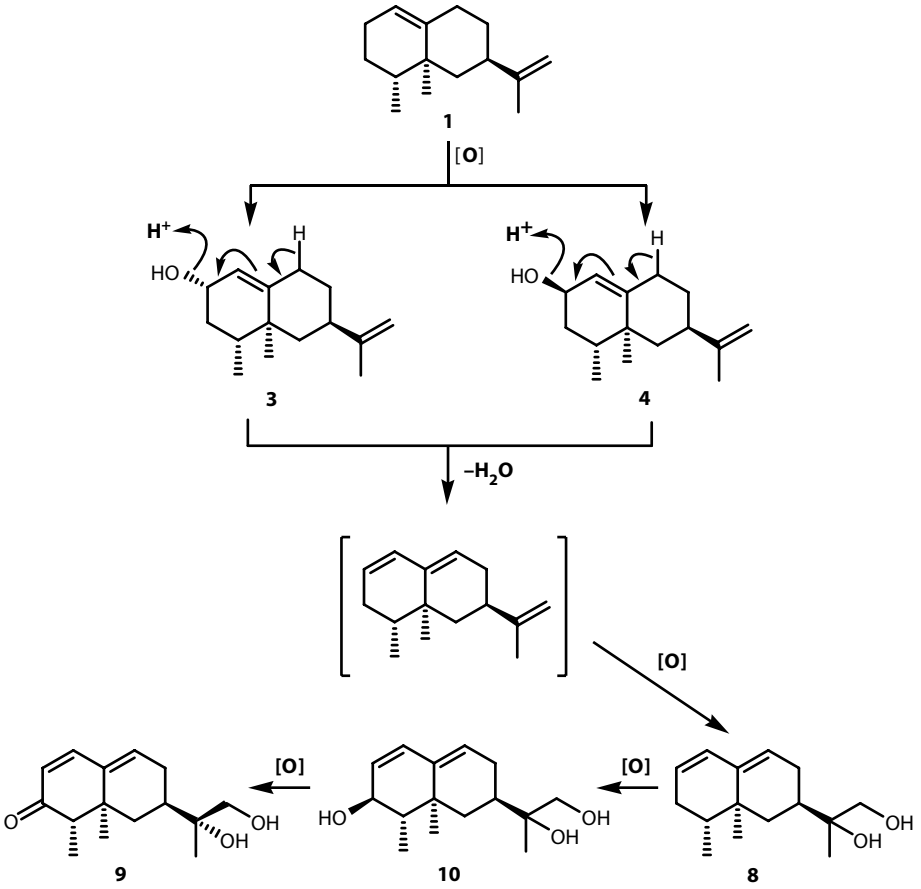


FIGURE 15.6 Possible pathway of biotransformation of valencene (**1**) by *Aspergillus niger.*

Both ratios of **6** (11*S*) and **7** (11*R*) obtained from **3** and **4** were 1:3, respectively. From the above results, plausible metabolic pathways of valencene (**1**) and 2-hydroxyvalencene (**3**, **4**) by *Aspergillus niger* are shown in Figure 15.6 (Noma et al., 2001a).

Aspergillus wentii and *Eurotrium purpurasens* converted valencene (**1**) to 11,12-epoxide (**14a**) and the same diol (**6**, **7**) (Takahashi and Miyazawa, 2005) as well as nootkatone (**2**) and 2α-hydroxyvalencene (**3**) (Takahashi and Miyazawa, 2006).

Kaspera et al. (2005) reported that valencene (**1**) was incubated in submerged cultures of the ascomycete *Chaetomium globosum*, to give nootkatone (**2**), 2α-hydroxyvalencene (**3**), and valencene 11,12-epoxide (**14a**), together with a valencene ketodiol, valencenediols, a valencene ketodiol, a valencene triol, or valencene epoxydiol that were detected by liquid chromatography-mass spectrocopy (LC-MS) spectra and GC-MS of trimethyl silyl derivatives. These metabolites are accumulated preferably inside the fungal cells (Figure 15.7).

The metabolites of valencene, nootkatone (**2**), (**3**), and (**14a**), indicated grapefruit with sour and citrus with bitter odor, respectively. Nootkatone 11,12-epoxide (**14**) showed no volatile fragrant properties.

15.2.3 Biotransformation of Nootkatone (2) by *Aspergillus niger*

Aspergillus niger was inoculated and cultivated rotatory (100 rpm) in Czapek-peptone medium at 30°C for 7 days. (+)-Nootkatone (**2**), ($[\alpha]_D$ + 193.5°, *c* = 1.0), (80 mg/200 mL), which was isolated

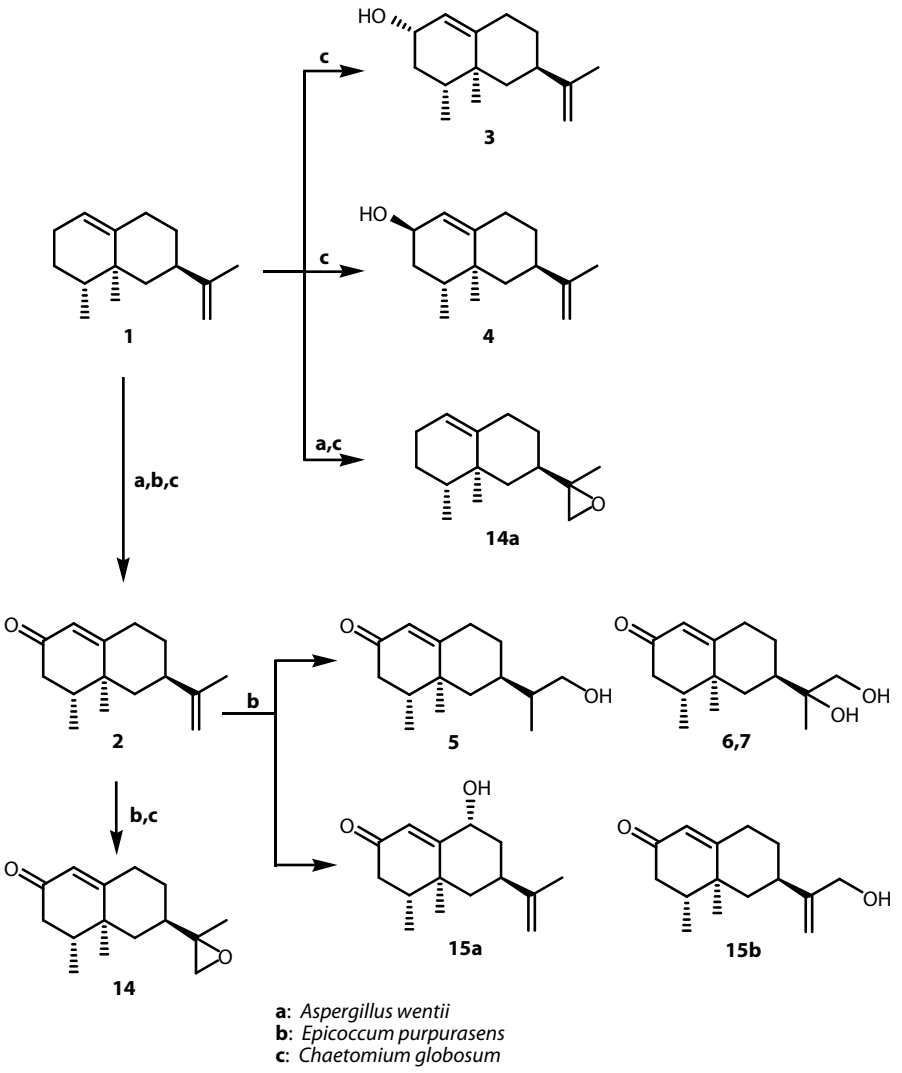


FIGURE 15.7 Biotransformation of valencene (**1**) and nootkatone (**2**) by *Aspergillus wentii, Epicoccum purpurascens,* and *Chaetomium globosum*.

from the essential oil of grapefruit, was added to the medium and further cultivated for 7 days to obtain two metabolites, 12-hydroxy-11,12-dihydronootkatone (**5**) (10.6%) and C11 stereo-mixtures (51.5%) of nootkatone-11*S*,12-diol (**6**) and its 11*R* isomer (**7**) (11*R*:11*S* = 1:1) (Hashimoto et al., 2000a; Noma et al., 2001a; Furusawa et al., 2003) (Figure 15.8).

11,12-epoxide (**14**) obtained by epoxidation of nootkatone (**2**) with *m*CPBA was biotransformed by *Aspergillus niger* for 1 day to afford **6** and **7** (11*R*:11*S* = 1:1) in good yield (81.4%). 1-aminobenzotriazole, an inhibitor of CYP450, inhibited the oxidation process of **1** into compounds **5**–**7** (Noma et al., 2001a). From the above results, possible metabolic pathways of nootkatone (**2**) by *Aspergillus niger* might be considered as shown in Figure 15.9.

The same substrate was incubated with *Aspergillus wentii* to produce diol (**6**, **7**) and 11,12--epoxide (**14**) (Takahashi and Miyazawa, 2005).

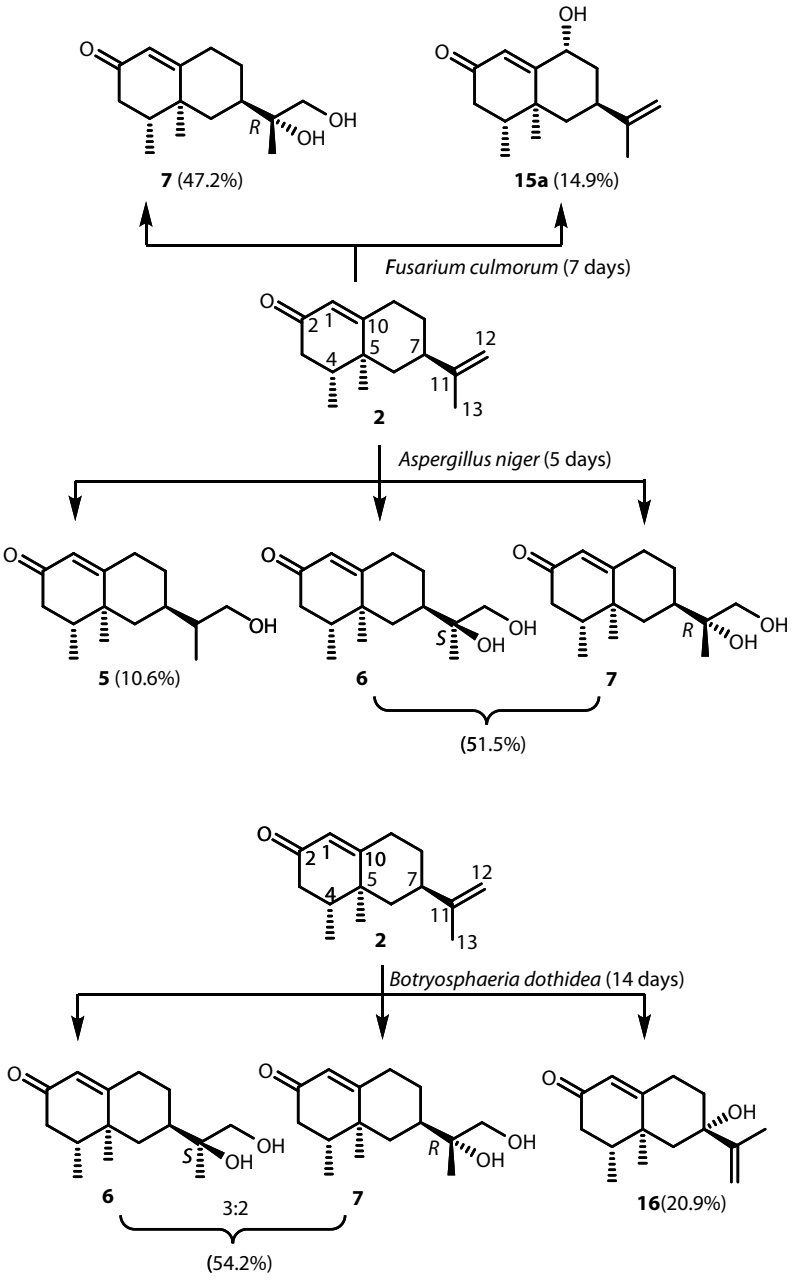


FIGURE 15.8 Biotransformation of nootkatone (**2**) by *Fusarium culmorum*, *Aspergillus niger*, and *Botryosphaeria dothidea*.

15.2.4 Biotransformation of Nootkatone (2) by *Fusarium culmorum* and *Botryosphaeria dothidea*

(+)-Nootkatone (**2**) was added to the same medium as mentioned above including *Fusarium culmorum* to afford nootkatone-11*R*,12-diol (**7**) (47.2%) and 9β-hydroxynootkatone (**15**) (14.9%) (Noma et al., 2001a).

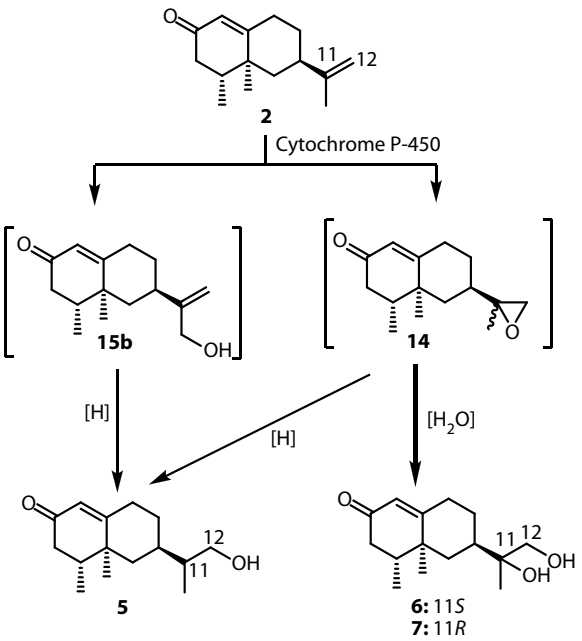


FIGURE 15.9 Possible pathway of biotransformation of valencene (**1**) by Cytochrome P-450.

Compound **7** was stereospecifically obtained at C11 by biotransformation of **1**. Purity of compound **7** was determined as ca. 95% by high performance liquid chromatography (HPLC) analysis of the thiocarbonate (**13**).

The biotransformation of nootkatone (**2**) was examined by the plant pathogenic fungus, *Botryosphaeria dothidea* separated from the fungus that infected the peach. (+)-Nootkatone (**2**) was cultivated with *Botryosphaeria dothidea* (Peach PP8402) for 14 days to afford nootkatone diols (**6** and **7**) (54.2%) and 7α-hydroxynootkatone (**16**) (20.9%). Ratio of compounds **6** and **7** was determined as 3:2 by HPLC analysis of the thiocarbonates (**12**, **13**) (Noma et al., 2001a). Nootkatone (**2**) was administered into rabbits to give the same diols (**6**, **7**) (Asakawa et al., 1986; Ishida, 2005).

Epicoccum purpurascens also biotransformed nootkatone (**2**) to **5**–**7**, **14**, and **15a** (Takahashi and Miyazawa, 2006).

The biotransformation of **2** by *Aspergillus niger* and *Botryosphaeria dothidea* resembled to that of the oral administration to rabbit since the ratio of the major metabolites 11*S*- (**6**) and 11*R*-nootkatone-11,12-diol (**7**) was similar. It is noteworthy that the biotransformation of **2** by *Fusarium culmorum* affords stereospecifically nootkatone-11*R*, 12-diol (**7**) (Noma et al., 2001a) (Figure 15.10).

Metabolites **3**–**5**, **12**, and **13** from (+)-nootkatone (**2**) and **14**–**17** from (+)-valencene (**1**) did not show an effective odor.

Dihydronootkatone (**17**), which shows that citrus odor possesses antitermite activity, was also treated in *Aspergillus niger* to obtain 11*S*-mono- (**18**) and 11*R*-dihydroxylated products (**19**) (the ratio 11*S* and 11*R* = 3:2). On the other hand, *Aspergillus cellulosae* reduced ketone group at C2 of **17** to give 2α- (**20**) (75.7%) and 2β-hydroxynootkatone (**21**) (0.7%) (Furusawa et al., 2003) (Figure 15.11).

Tetrahydronootkatone (**22**) also shows antitermite and mosquito-repellant activity. It was incubated with *Aspergillus niger* to give two similar hydroxylated compounds (**23**, 13.6% and **24**, 9.9%) to those obtained from **17** (Furusawa, 2006) (Figure 15.12).

8,9-Dehydronootkatone (**25**) was incubated with *Aspergillus niger* to give four metabolites, a unique acetonide (**26**, 15.6%), monohydroxylated (**27**, 0.2%), dihydroxylated (**28**, 69%), and a carboxyl derivative (**29**, 0.8%) (Figure 15.13).

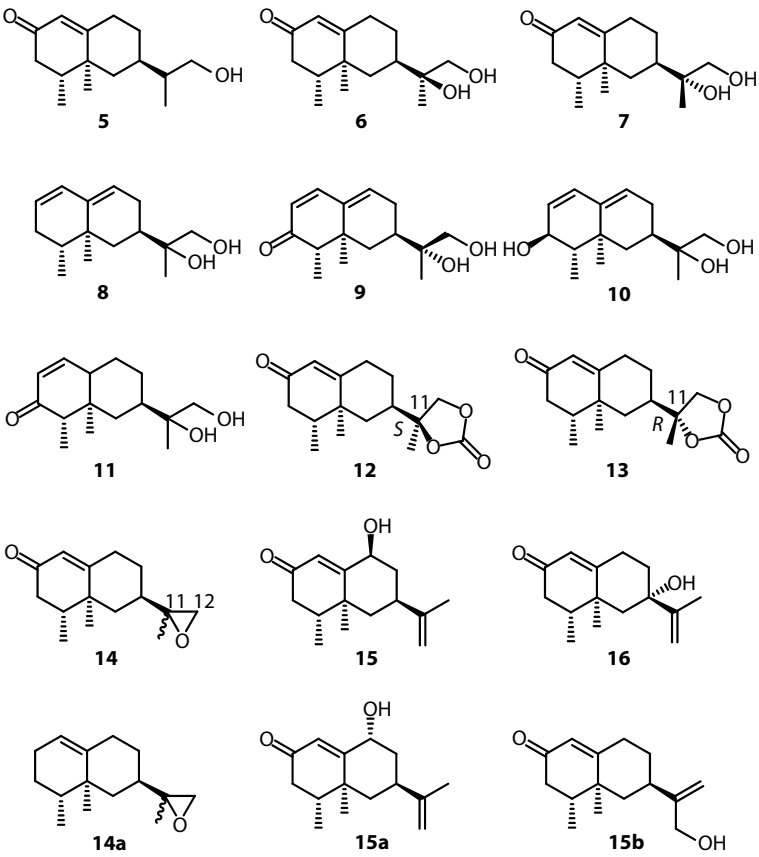


FIGURE 15.10 Metabolites (**5**–**11**, **14**–**15b**) from valencene (**1**) and nootkatone (**2**) by various microorganisms.

When the same substrate was treated in *Aspergillus sojae* IFO 4389, compound **25** was converted to the different monohydroxylated product (**30**, 15.8%) from that mentioned above. *Aspergillus cellulosae* is an interesting fungus since it did not give any same products as mentioned above; in place, it produced trinorsesquitepene ketone (**31**, 6%) and nitrogen-containing aromatic compound (**32**) (Furusawa et al., 2003) (Figure 15.14).

Mucor species also oxidized compound **25** to give three metabolites, 13-hydroxy-8,9-dehydronootkatone (**33**, 13.2%), an epoxide (**34**, 5.1%), and a diol (**35**, 19.9%) (Furusawa et al., 2003). The same substrate was investigated with cultured suspension cells of the liverwort, *Marchantia polymorpha* to afford **33** (Hegazy et al., 2005) (Figure 15.15).

Although *Mucor* species could give nootkatone (**21**) from valencene (**1**), this fungus biotransformed the same substrate (**25**) to the same alcohol (**30**, 13.2%) obtained from the same starting compound (**25**) in *Aspergillus sojae*, a new epoxide (**34**, 5.1%) and a diol (**35**, 9.9%).

The metabolites (**3**, **4**, **20**, **21**) inhibited the growth of lettuce stem, and **3** and **4** inhibited germination of the same plant (Hashimoto and Asakawa, 2007).

Valerianol (**35a**), from *Valeriana officinalis* whose dried rhizome is traditionally used for its carminative and sedative properties, was biotransformed by *Mucor plumbeus*, to produce three metabolites, a bridged ether (**35b**), and a triol (**35c**), which might be formed via C1–C10 epoxide, and **35d** arises from double dehydration (Arantes et al., 1999). In this case, allylic oxidative compounds have not been found (Figure 15.16).

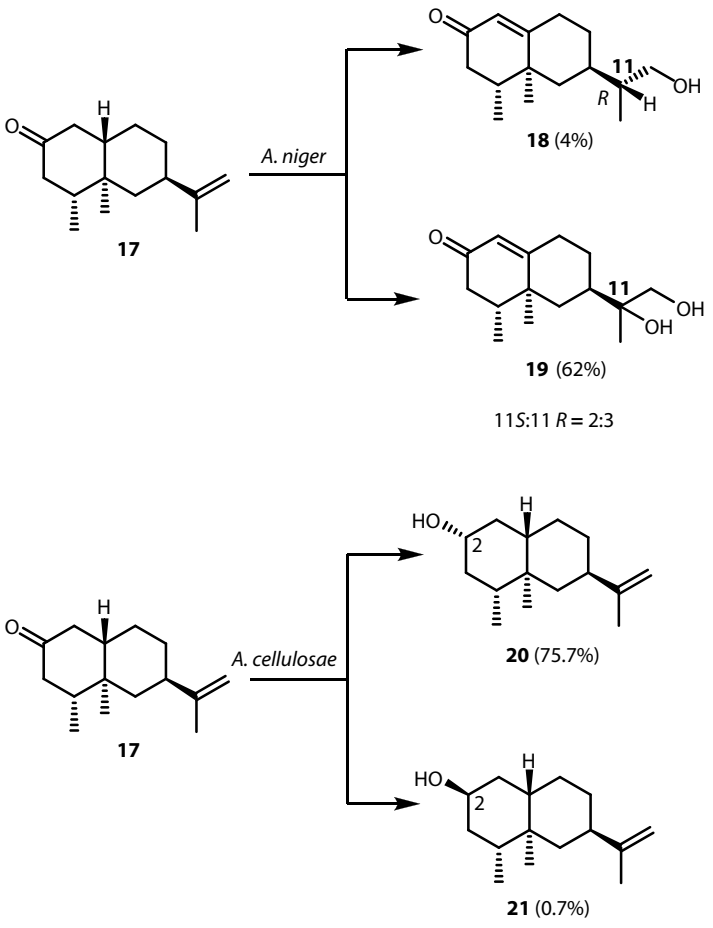


FIGURE 15.11 Biotransformation of dihydronootkatone (**17**) by *Aspergillus niger* and *Aspergillus cellulosae*.

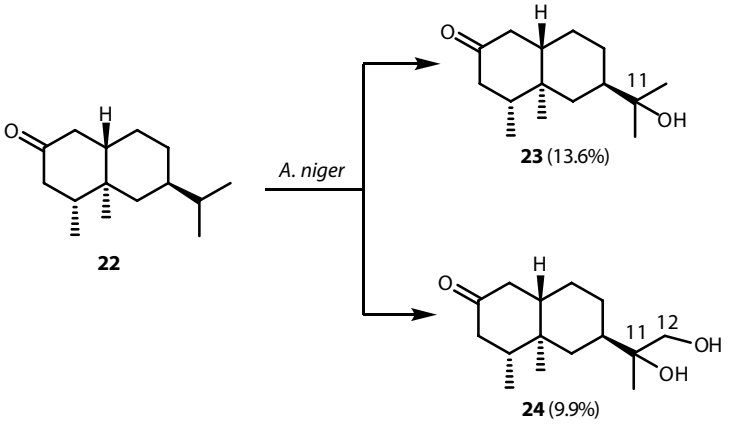


FIGURE 15.12 Biotransformation of tetrahydronootkatone (**22**) by *Aspergillus niger*.

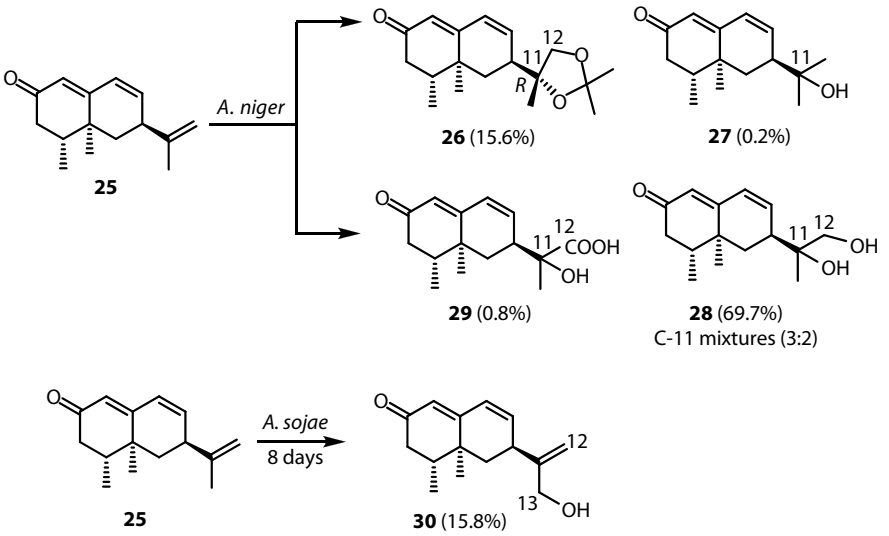


FIGURE 15.13 Biotransformation of 8,9-dehydronootkatone (**25**) by *Aspergillus sojae*.

15.2.5 Biotransformation of (+)-1(10)-Aristolene (36) from the Crude Drug *Nardostachys chinensis* by *Chlorella fusca*, *Mucor* species, and *Aspergillus niger*

The structure of sesquiterpenoid, (+)-1(10)-aristolene (= calarene) (**36**) from the crude drug *Nardostachys chinensis* was similar to that of nootkatone. 2-Oxo-1(10)-aristolene (**38**) shows antimelanin inducing activity and excellent citrus fragrance. On the other hand, the enantiomer (**37**) of **36** and (+)-aristolone (**41**) were also found in the liverworts as the natural products. In order

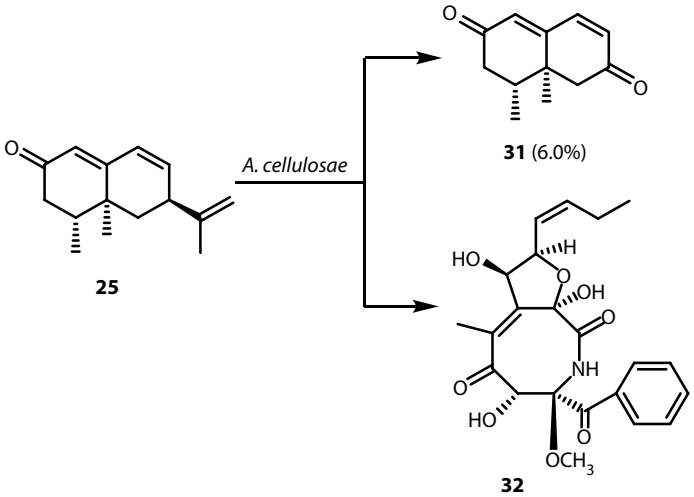


FIGURE 15.14 Biotransformation of 8,9-dehydronootkatone (**25**) by *Aspergillus cellulosae*.

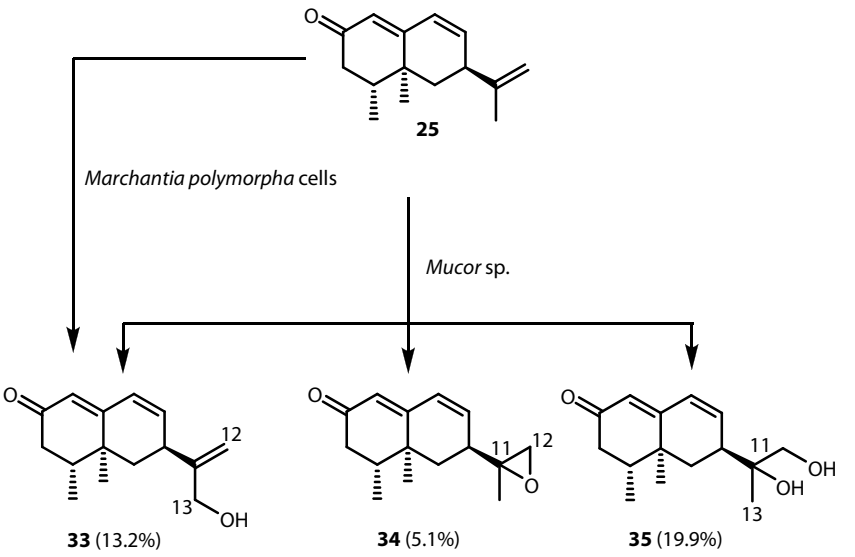


FIGURE 15.15 Biotransformation of 8,9-dehydronootkatone (**25**) by *Marchantia polymorpha* and *Mucor* species.

to obtain compound **38** and its analogues, compound **36** was incubated with *Chlorella fusca* var. *vacuolata* IAMC-28, *Mucor* species, and *Aspergillus niger* (Furusawa et al., 2006a) (Figure 15.17).

Chlorella fusca was inoculated and cultivated stationary in Noro medium (pH 8.0) at 25°C for 7 days and (+)-1(10)-aristolene (**36**) (20 mg/50 mL) was added to the medium and further incubated for 10–14 days and cultivated stationary under illumination (pH 8.0) at 25°C for 7 days to afford 1(10)-aristolen-2-one (**38**, 18.7%), (−)-aristolone (**39**, 7.1%), and 9-hydroxy-1(10)-aristolen-2-one (**40**). Compounds **38** and **40** were found in *Aristolochia* species (Figure 15.18).

Mucor species was inoculated and cultivated rotatory (100 rpm) in Czapek-peptone medium (pH 7.0) at 30°C for 7 days. (+)-1(10)-Aristolene (**36**) (100 mg/200 mL) was added to the medium and further for 7 days. The crude metabolites contained **38** (0.9%) and **39** (0.7%) as very minor products (Figure 15.19).

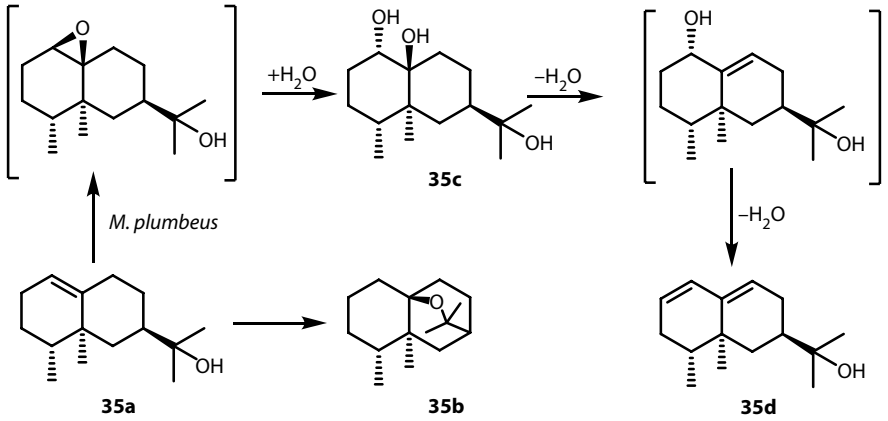


FIGURE 15.16 Biotransformation of valerianol (**35a**) by *Mucor plumbeus.*

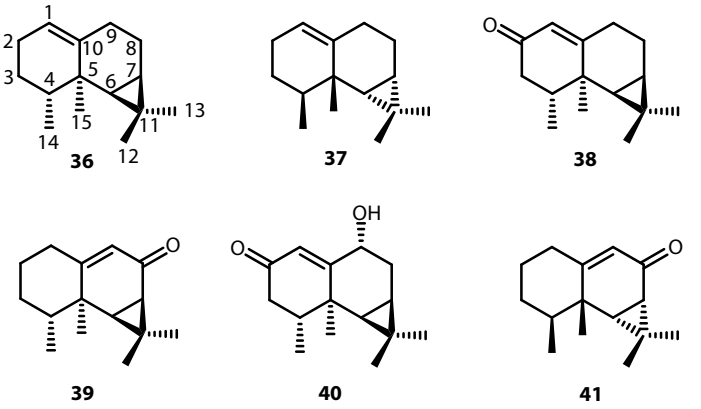


FIGURE 15.17 Naturally occurring aristolane sesquiterpenoids.

Although *Mucor* species produced a large amount of nootkatone (**2**) from valencene (**1**), however, only poor yield of similar products as those from valencene (**1**) was seen in the biotransformation of tricyclic substrate (**36**). Possible biogenetic pathway of (+)-1(10)-aristolene (**36**) is shown in Figure 15.20.

Aspergillus niger was inoculated and cultivated rotatory (100 rpm) in Czapek-peptone medium (pH 7.0) at 30°C for 3 days. (+)-1(10)-Aristolene (**36**) (100 mg/200 mL) was added to the medium and further for 7 days. From the crude metabolites four new metabolic products (**42**, 1.3%), (**43**, 3.2%), (**44**, 0.98%), and (**45**, 2.8%) were obtained in very poor yields (Figure 15.21). Possible metabolic pathways of **36** by *Aspergillus niger* are shown in Figure 15.22.

Commercially available (+)-1(10)-aristolene (**36**) was treated with *Diplodia gossypina* and *Bacillus megaterium*. Both microorganisms converted **36** to four (**46**–**49**; 0.8%, 1.1, 0.16%, 0.38%) and six metabolites, (**40**, **50–55**; 0.75%, 1.0%, 1.0%, 2.0%, 1.1%, 0.5%, 0.87%), together with **40** (0.75%) respectively (Abraham et al., 1992) (Figure 15.23).

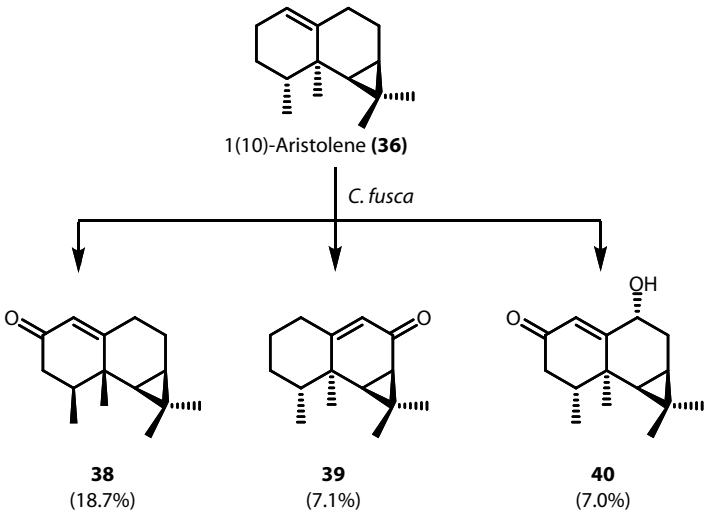


FIGURE 15.18 Biotransformation of 1(10)-aristolene (**36**) by *Chlorella fusca*.

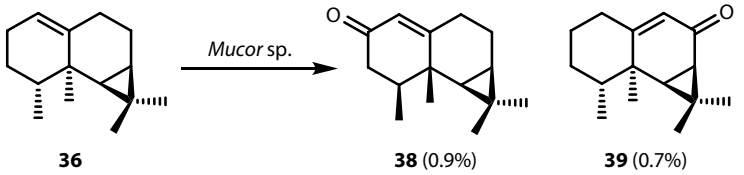


FIGURE 15.19 Biotransformation of 1(10)-aristolene (**36**) by *Mucor* species.

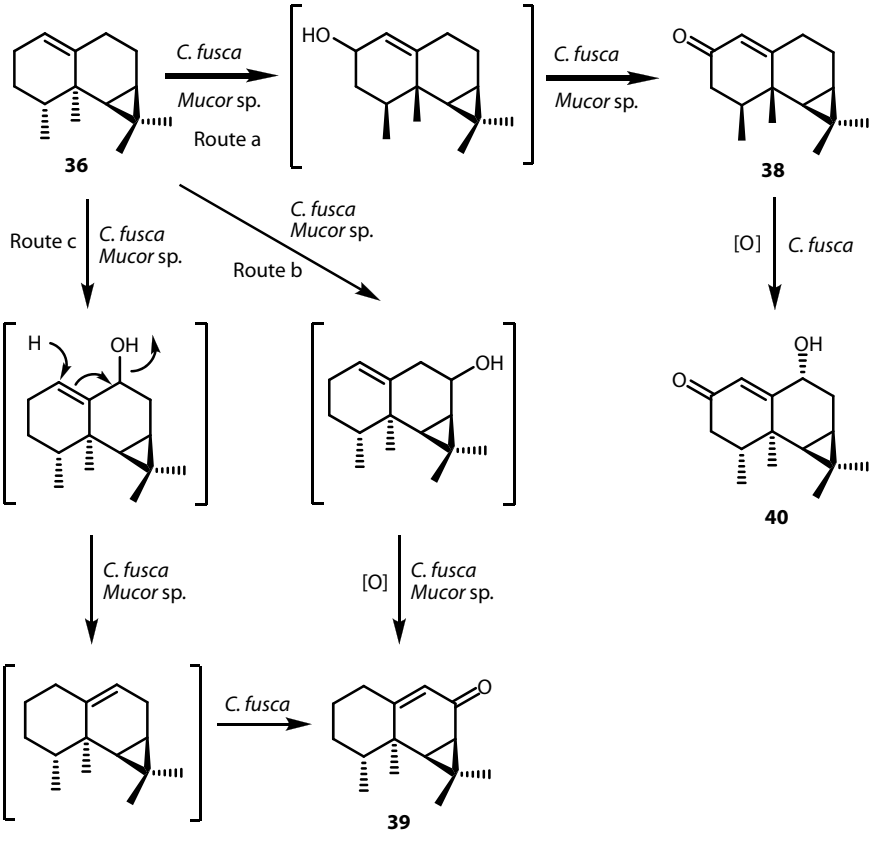


FIGURE 15.20 Possible pathway of biotransformation of 1(10)-aristolene (**36**) by *Chlorella fusca* and *Mucor* species.

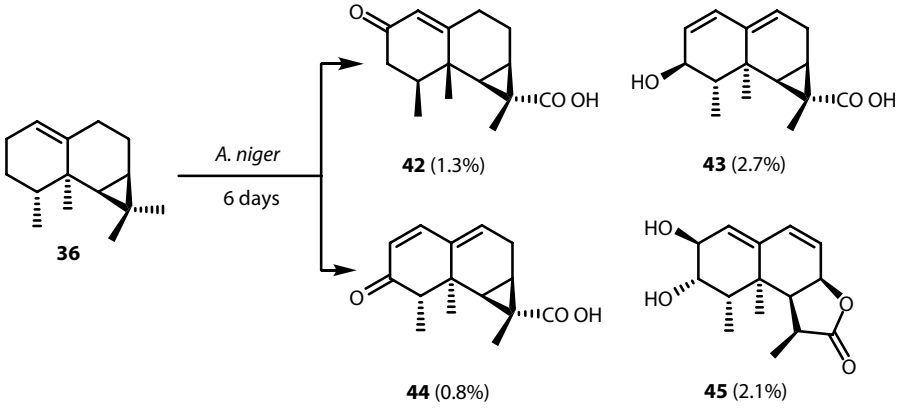


FIGURE 15.21 Biotransformation of 1(10)-aristolene (**36**) by *Aspergillus niger.*

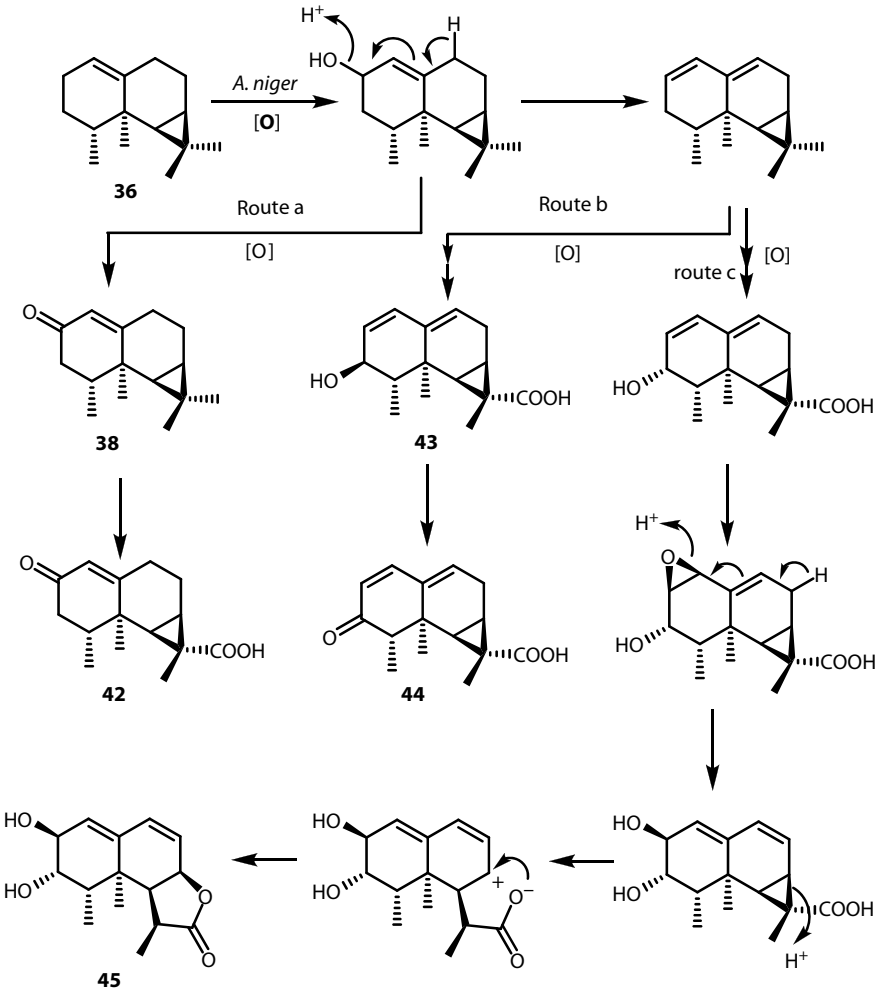


FIGURE 15.22 Possible pathway of biotransformation of 1(10)-aristolene (**36**) by *Aspergillus niger.*

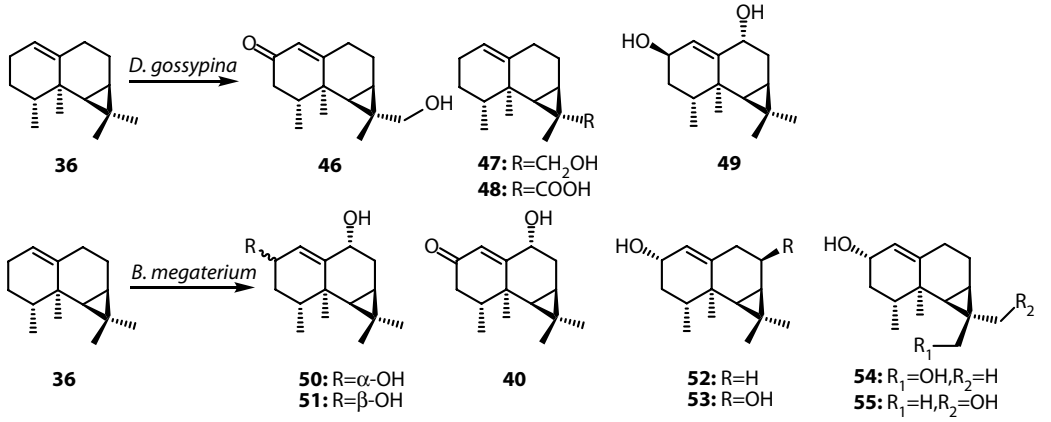


FIGURE 15.23 Biotransformation of 1(10)-aristolene (**36**) by *Diplodia gossypina* and *Bacillus megaterium.*

It is noteworthy that *Chlorella* and *Mucor* species introduce hydroxyl group at C2 of the substrate (**36**) as seen in the biotransformation of valencene (**1**) while *Diplodia gossypina* and *Bacillus megaterium* oxidizes C2, C8, C9, and/or 1,1-dimethyl group on a cyclopropane ring. *Aspergillus niger* oxidizes not only C2 but also stereoselectively oxidized one of the gem-dimethyl groups on cyclopropane ring. Stereoselective oxidation of one of gem-dimethyl of cyclopropane and cyclobutane derivatives is observed in biotransformation using mammals (see later).

15.2.6 Biotransformation of Various Sesquiterpenoids by Microorganisms

Aromadendrane-type sesquiterpenoids have been found not only in higher plants but also in liverworts and marine sources. Three aromadendrenes (**56**, **57**, **58**) were biotransformed by *Diplodia gossypina*, *Bacillus megaterium*, and *Mycobacterium smegmatis* (Abraham et al., 1992). Aromadendrene (**56**) (800 mg) was converted by *Bacillus megaterium* to afford a diol (**59**) and a triol (**60**) of which **59** (7 mg) was the major product. The triol (**60**) was also obtained from the metabolite of (+)-(1*R*)-aromadendrene (**56**) by the plant pathogen *Glomerella cingulata* (Miyazawa et al., 1995a). *allo*-Aromadendrene (**57**) (1.2 g) was also treated in *Mycobacterium smegmatis* to afford **61** (10 mg) (Abraham et al., 1992) (Figure 15.24).

The same substrate was also incubated with *Glomerella cingulata* to afford C10 epimeric triol (**62**) (Miyazawa et al., 1995a). Globulol (**58**) (400 mg) was treated in *Mycobacterium smegmatis* to give only a carboxylic acid (**63**) (210 mg). The same substrate (**58**) (1 g) was treated in *Diplodia gossypina* and *Bacillus megaterium* to give two diols, **64** (182 mg), **65** and a triol (**66**) from the former and **67**–**69** from the latter organism among which **64** (60 mg) was predominant (Abraham et al., 1992). *Glomerella cingulata* and *Botrytis cinerea* also bioconverted globulol (**58**) to diol (**64**) regio- and stereoselectively (Miyazawa et al., 1994) (Figures 15.25 and 15.26).

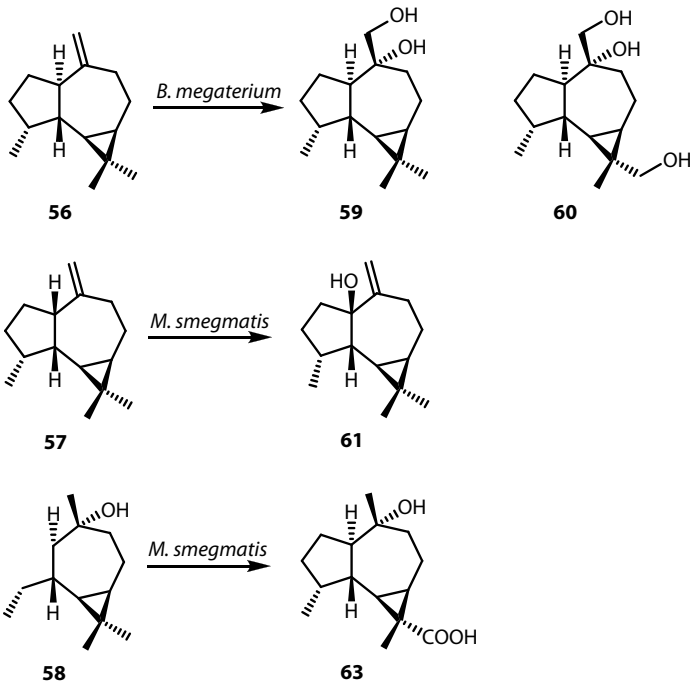


FIGURE 15.24 Biotransformation of aromadendrene (**56**), alloaromadendrene (**57**), and globulol (**58**) by *Bacillus megaterium* and *Mycobacterium smegmatis*.

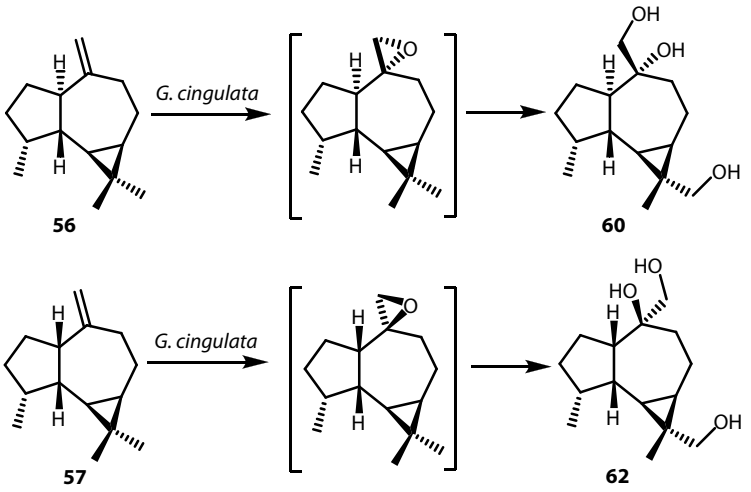


FIGURE 15.25 Biotransformation of aromadendrene (**56**) and alloaromadendrene (**57**) by *Glomerella cingulata*.

Globulol (**58**) (1.5 g) and 10-epiglobulol (**70**) (1.2 mL) were separately incubated with *Cephalosporium aphidicola* in shake culture for 6 days to give the same diol **64** (780 mg) as obtained from the same substrate by *Bacillus megaterium* mentioned above and **71** (720 mg), (Hanson et al., 1994). *Aspergillus niger* also converted globulol (**58**) and epiglobulol (**70**) to a diol (**64**) and

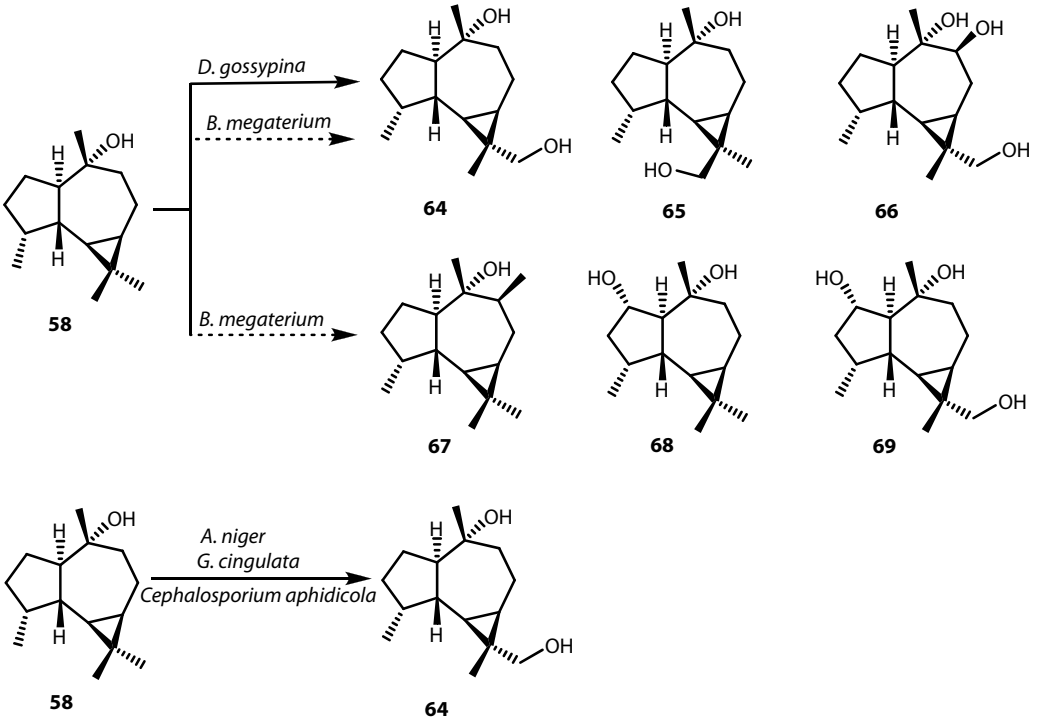


FIGURE 15.26 Biotransformation of globulol (**58**) by various microorganisms.

three 13-hydroxylated globulol (**71**, **72**, **74**) and 4α-hydroxylated product (**73**). The epimerization at C4 is very rare example (Hayashi et al., 1998).

Ledol (**75**), an epimer at C1 of globulol was incubated with *Glomerella cingulata* to afford C13 carboxylic acid (**76**) (Miyazawa et al., 1994) (Figure 15.27).

Squamulosone (**77**), aromadendr-1(10)-en-9-one isolated from *Hyptis verticillata* (Labiatae), was reduced chemically to give **78**–**82**, which were incubated with the fungus *Curvularia lunata* in two different growth media (Figure 15.28).

From **78**, two metabolites **80** and **83** were obtained. Compound **79** and **80** were metabolized to give ketone **81** as the sole product and **78** and **83**, respectively. From compound **81**, two metabolites, **79** and **84** were obtained (Figure 15.29). From the metabolite of the substrate (**82**), five products (**84**–**88**) were isolated (Collins, Reynold, and Reese, 2002) (Figure 15.30).

Squamulosone (**77**) was treated in the fungus *Mucor plumbeus* ATCC 4740 to give not only cyclopentanol derivatives (**89**, **90**) but also C12 hydroxylated products (**91**–**93**) (Collins, Ruddock, et al., 2002) (Figure 15.31).

Spathulenol (**94**), which is found in many essential oils, was fed by *Aspergillus niger* to give a diol (**95**) (Higuchi et al., 2001). *Ent*-10β-hydroxycyclocolorenone (**96**) and myli-4(15)-en-9-one (**96a**) isolated from the liverwort *Mylia taylorii* were incubated with *Aspergillus niger* IFO 4407 to give C10 epimeric product (**97**) (Hayashi et al., 1999) and 12-hydroxylated product (**96b**), respectively (Nozaki et al., 1996) (Figures 15.32 and 15.33).

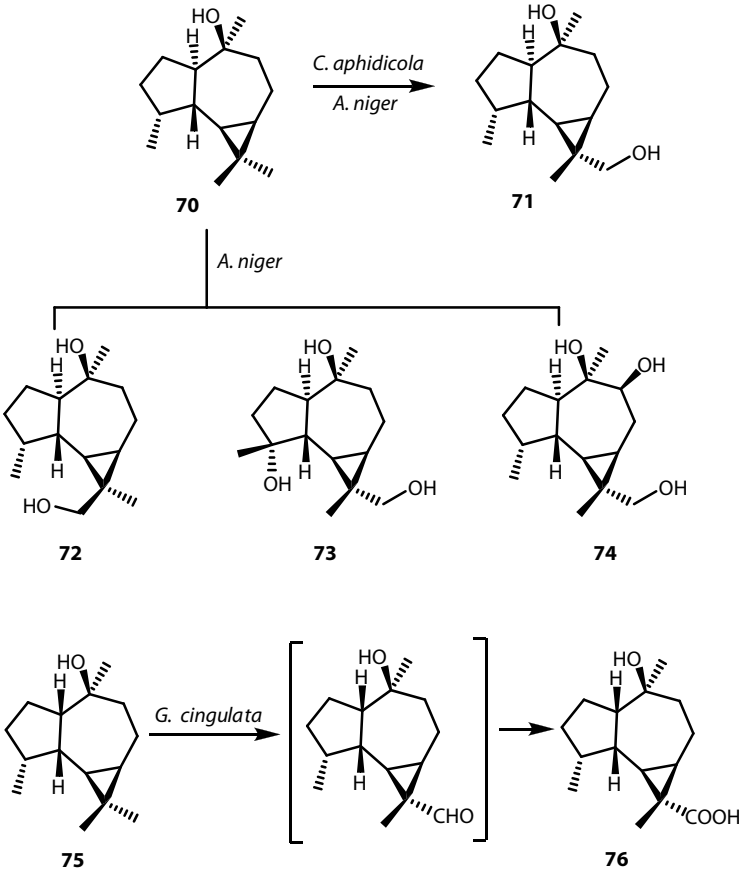


FIGURE 15.27 Biotransformation of 10-epi-glubulol (**70**) and ledol (**75**) by *Cephalosporium aphidicola*, *Aspergillus niger*, and *Glomerella cingulata*.

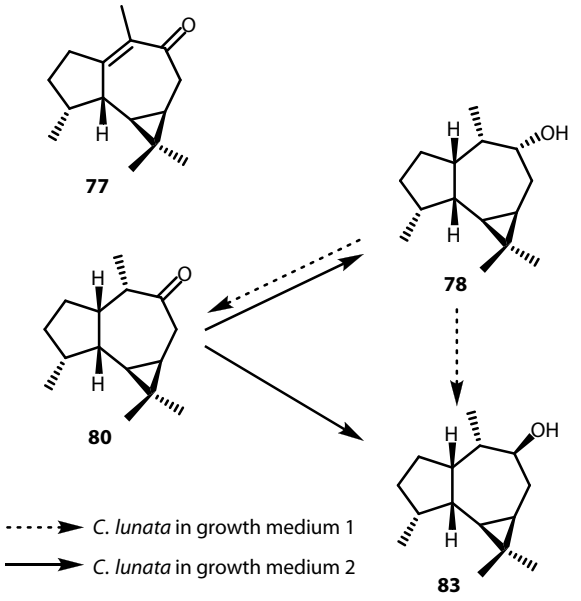


FIGURE 15.28 Biotransformation of aromadendra-9-one (**80**) by *Curvularia lunata*.

(+)-*ent*-Cyclocolorenone (**98**) $[\alpha]_D - 405°$ (c = 8.8, EtOH), one of the major compounds isolated from the liverwort *Plagiochila sciophila* (Asakawa, 1982, 1995), was treated by *Aspergillus niger* to afford three metabolites, 9-hydroxycyclocolorenone (**99**, 15.9%) 12-hydroxy-(+)-cyclocolorenone (**100**, 8.9%) and a unique cyclopropane-cleaved metabolite, 6β-hydroxy-4,11-guaiadien-3-one (**101**, 35.9%), and 6β,7β-dihydroxy-4,11-guaiadien-3-one (**102**, trace), of which **101** was the major component. The enantiomer (**103**) $[\alpha]_D + 402°$ (c = 8.8, EtOH) of **98** isolated from *Solidago altissima* was biotransformed by the same organism to give 13-hydroxycycolorenone (**103a**, 65.5%), the enantiomer of **100**, 1β,13-dihydroxycyclocolorenone (**103b**, 5.0%), and its C11-epimer (**103c**)

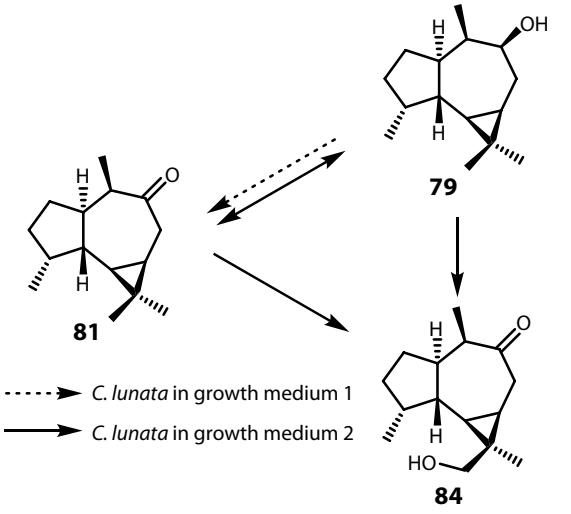


FIGURE 15.29 Biotransformation of 10-epi-aromadendra-9-one (**81**) by *Curvularia lunata*.

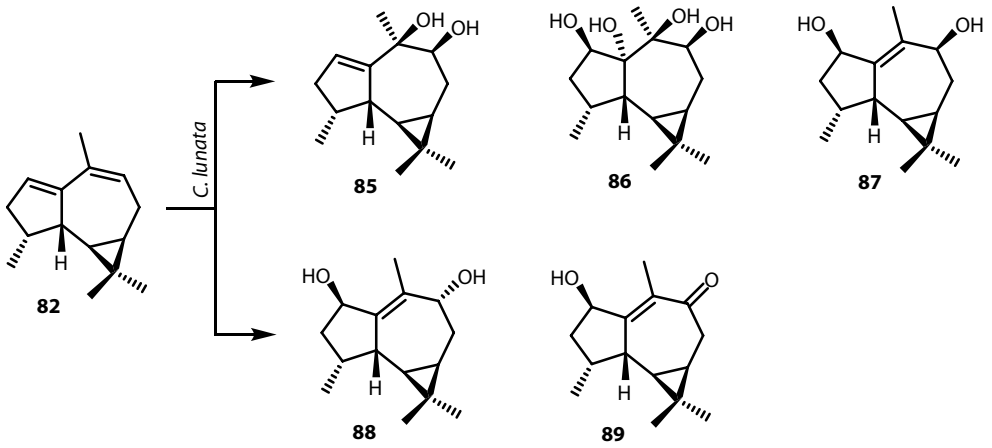


FIGURE 15.30 Biotransformation of aromadendr-1(10),9-diene (**82**) by *Curvularia lunata*.

(Furusawa et al., 2005b, 2006a). It is noteworthy that no cyclopropane-cleaved compounds from **103** have been detected in the crude metabolites even in GC-MS analysis (Figure 15.34).

Plagiochiline A (**104)** that shows potent insect antifeedant, cytotoxicity, and piscidal activity are very pungent 2,3-secoaromadendrane sesquiterpenoids having 1,1-dimethyl cyclopropane ring, isolated from the liverwort *Plagiochila fruticosa*. Plagiochilide (**105**) is the major component of this liverwort. In order to get more pungent component, the lactone (**105**, 101 mg) was incubated with

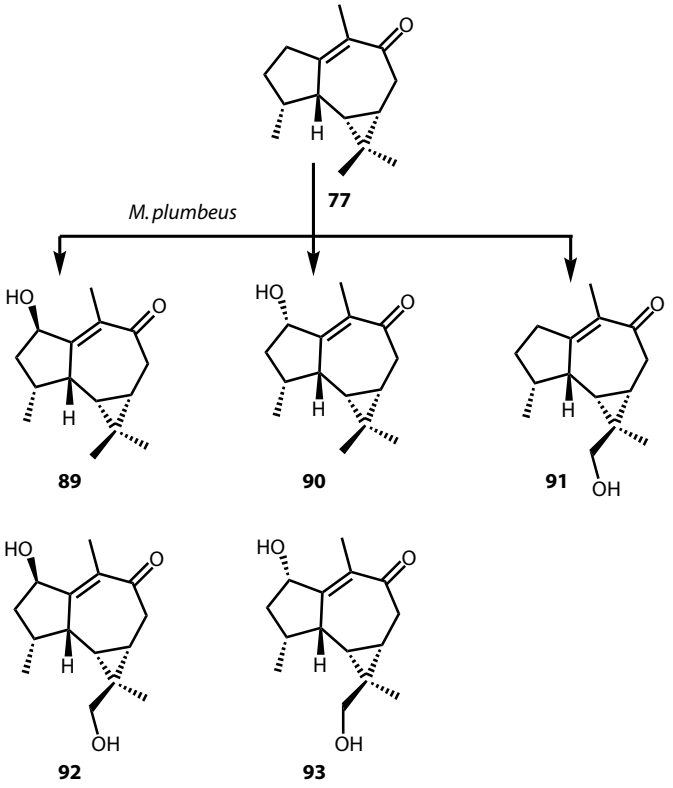
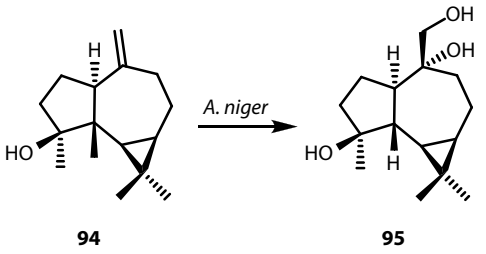


FIGURE 15.31 Biotransformation of squamulosone (**77**) by *Mucor plumbeus*.



94 **95**

FIGURE 15.32 Biotransformation of spathulenol (**94**) by *Aspergillus niger.*

Aspergillus niger to give two metabolites **106** (32.5%) and **107** (9.7%). Compound **105** was incubated in *Aspergillus niger* including 1-aminobenzotriazole, the inhibitor of CYP450, to produce only **106**, since this enzyme plays an important role in the formation of carboxylic acid (**107**) from primary alcohol (**106**). Unfortunately, two metabolites show nothing hot taste (Hashimoto et al., 2003c; Furusawa et al., 2006) (Figure 15.35).

Partheniol, 8α-hydroxybicyclogermacrene (**108**) isolated from *Parthenium argentatum* × *Parthenium Tometosa*, was cultured in the media of *Mucor circinelloides* ATCC 15242 to afford six metabolites, a humulane (**109**), three maaliane- (**110**, **112**, **113**), an aromadendrane- (**111**), and a tricylohumulane triol (**114**), the isomer of compound (**111**). Compounds **110**, **111**, and **114** were isolated as their acetates (Figure 15.36).

Compounds **110** might originate from the substrate by acidic transannular cyclization since the broth was pH 6.4 just before extraction (Maatooq, 2002).

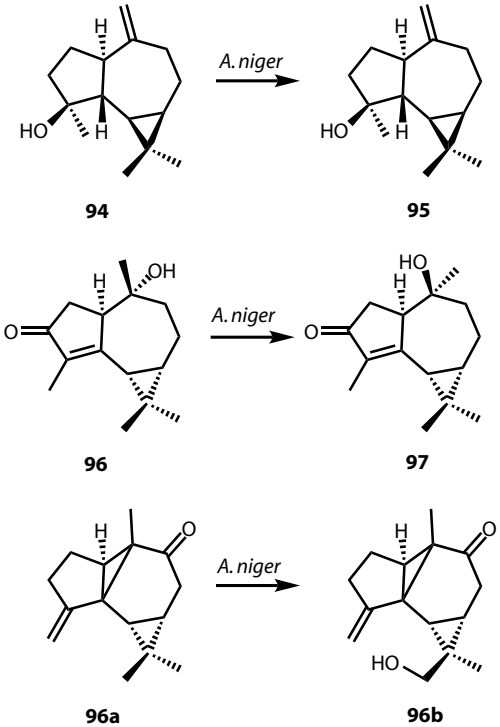


FIGURE 15.33 Biotransformation of spathulenol (**94**), *ent*-10β-hydroxycyclocolorenone (**96**) and myli-4-(15)-en-9-one (**96a**) by *Aspergillus niger.*

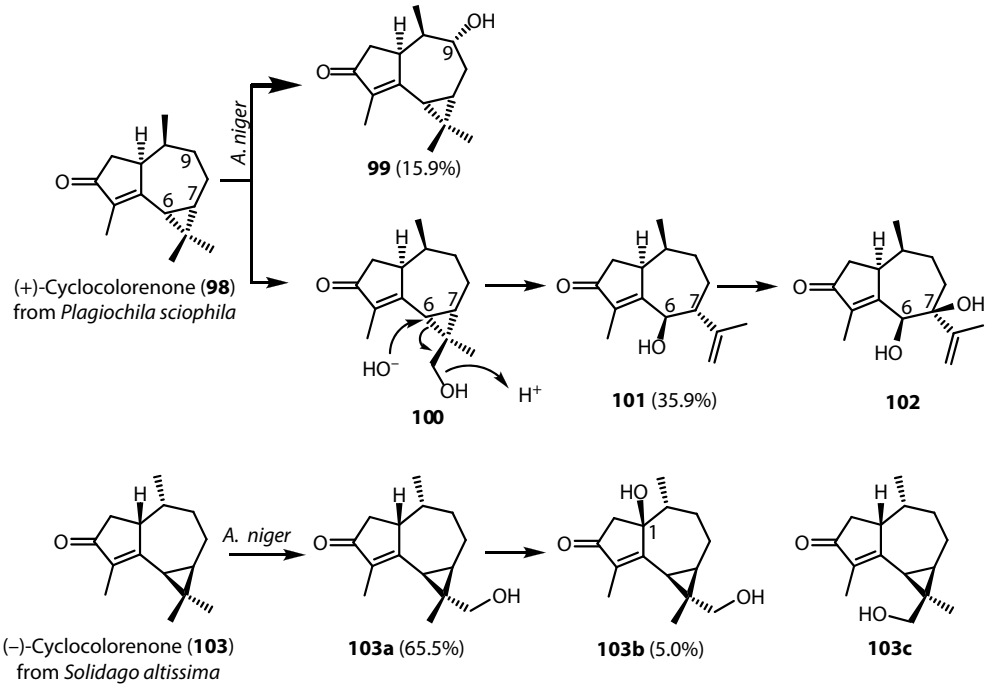


FIGURE 15.34 Biotransformation of (+)-cyclocolorenone (**98**) and (–)-cyclocolorenone (**103**) by *Aspergillus niger*.

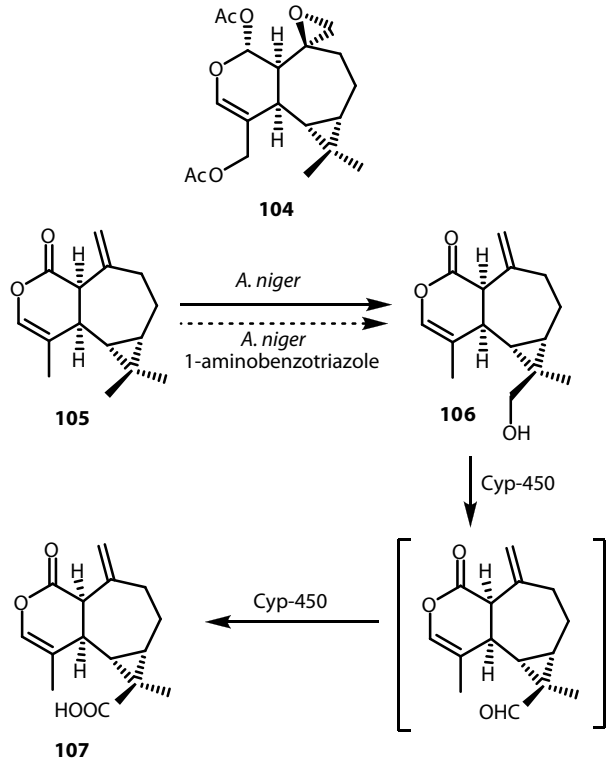


FIGURE 15.35 Biotransformation of plagiochiline C (**104**) by *Aspergillus niger*.

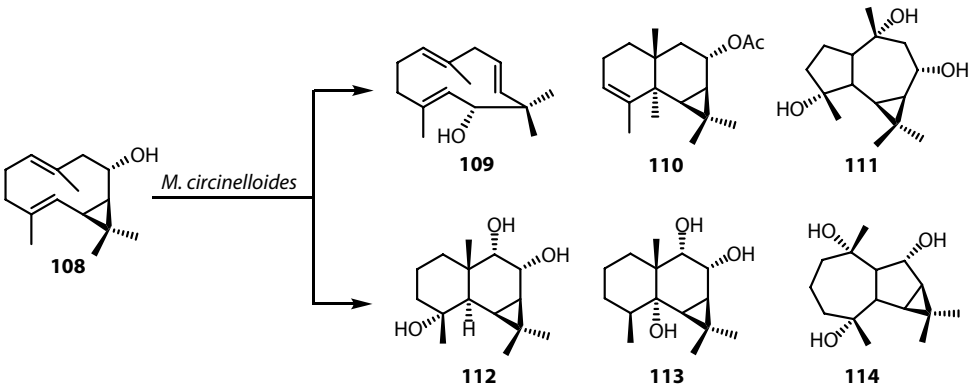


FIGURE 15.36 Biotransformation of 8α-hydroxybicyclogermacrene (**108**) by *Mucor circinelloides*.

The same substrate (**108**) was incubated with the fungus *Calonectria decora* to afford six new metabolites (**108a**–**108f**). In these reactions, hydroxylation, epoxidation, and *trans*-annular cyclization were evidenced (Maatooq, 2002b) (Figure 15.37).

ent-Maaliane-type sesquiterpene alcohol, 1α-hydroxymaaliene (**115**), isolated from the liverwort *Mylia taylorii*, was treated in *Aspergillus niger* to afford two primary alcohols (**116**, **117**) (Morikawa et al., 2000). Such an oxidation pattern of 1,1-dimethyl group on the cyclopropane ring has been found in aromadendrane series as described above, and mammalian biotransformation of a monoterpene hydrocarbon, Δ^3-carene (Ishida et al., 1981) (Figure 15.38).

9(15)-Africanene (**117a**), a tricyclic sesquiterpene hydrocarbon isolated from marine soft corals of *Simularia* species, was biotransformed by *Aspergillus niger* and *Rhizopus oryzae* for 8 days to give 10α-hydroxy- (**117b**) and 9α,15-epoxy derivative (**117c**) (Venkateswarlu et al., 1999) (Figure 15.39).

Germacrone (**118**), (+)-germacrone-4,5-epoxide (**119**), and curdione (**120**) isolated from *Curcuma aromatica*, which has been used as crude drug, was incubated with *Aspergillus niger.* From compound **119** (700 mg), two naturally occurring metabolites, zedoarondiol (**121**) and isozedoarondiol (**122**), were obtained (Takahashi, 1994). Compound (**119**) was cultured in callus of *Curcuma zedoaria* and *Curcuma aromatica* to give the same secondary metabolites **121**, **122**, and **124** (Sakui et al., 1988) (Figures 15.40 and 15.41).

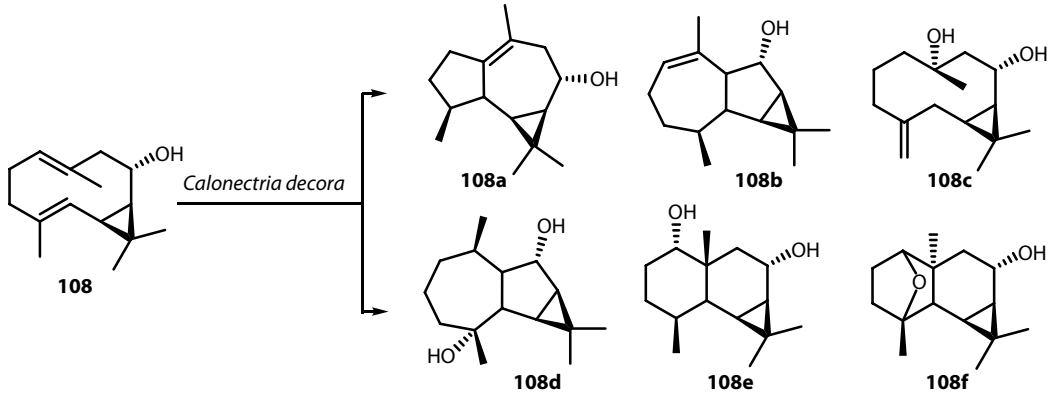


FIGURE 15.37 Biotransformation of 8α-hydroxybicyclogermacrene (**108**) by *Calonectria decora*.

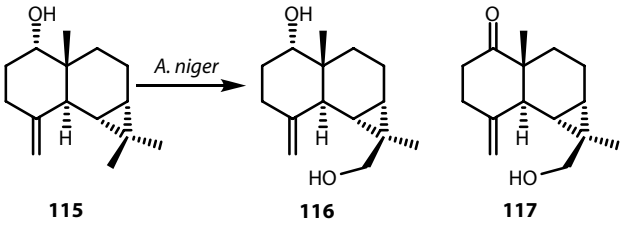


FIGURE 15.38 Biotransformation of 1α-hydroxymaaliene (**115**) *Aspergillus niger.*

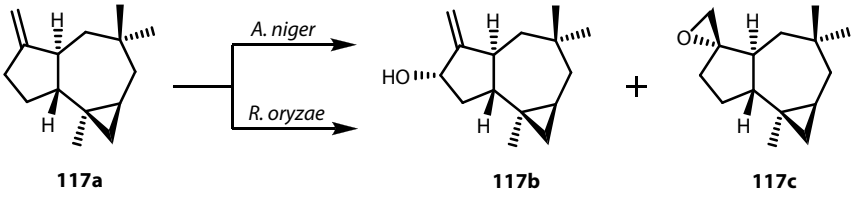


FIGURE 15.39 Biotransformation of 9(15)-africanene (**117a**) by *Aspergillus niger* and *Rhizopus oryzae.*

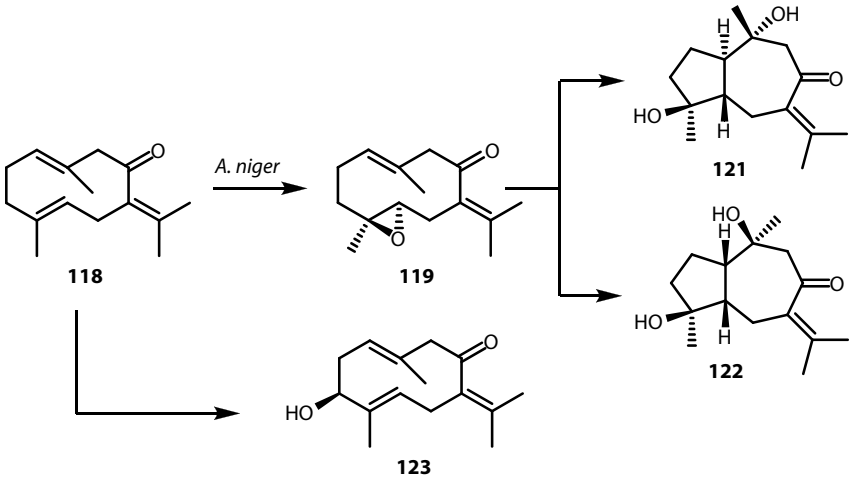


FIGURE 15.40 Biotransformation of germacrone (**118**) by *Aspergillus niger.*

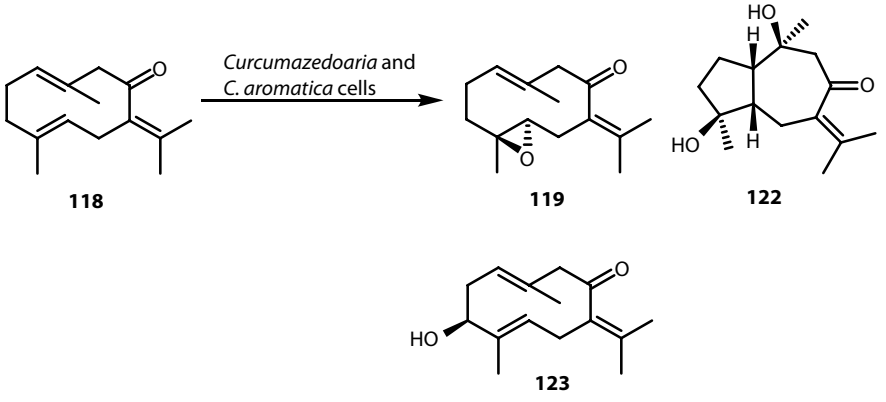


FIGURE 15.41 Biotransformation of germacrone (**118**) by *Curcuma zedoaria* and *Curcuma aromatica* cells.

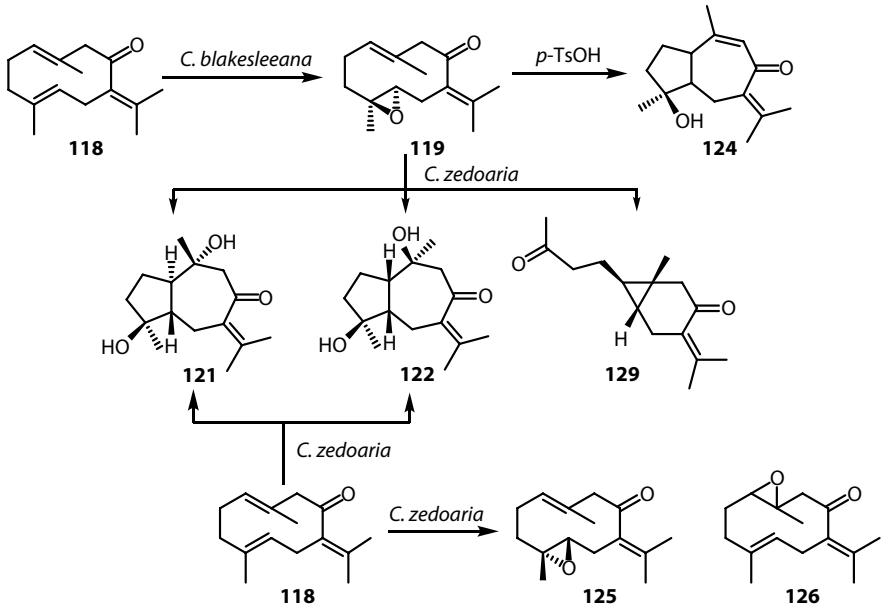


FIGURE 15.42 Biotransformation of germacrone (**118**) by *Cunninghamella blakeseeana* and *Curcuma zedoaria* cells.

Aspergillus niger biotransformed germacrone (**118**, 3g) to very unstable 3β-hydroxygermacrone (**123**), and 4,5-epoxygermacrone (**119**) which was further converted to two guaiane sesquiterpenoids (**121**) and (**122**) through *trans*-annular-type reaction (Takahashi, 1994). The same substrate was incubated in the microorganism, *Cunninghamella blakeseeana* to afford germacrone-4,5-epoxide (**119**) (Hikino et al., 1971) while the treatment of **118** in the callus of *Curcuma zedoaria* gave four metabolites **121**, **122**, **125**, and **126** (Sakamoto et al., 1994) (Figure 15.42).

The same substrate (**118**) was treated in plant cell cultures of *Solidago altissima* (Asteraceae) for 10 days to give various hydroxylated products (**121**, **127**, **125**, **128**–**132**) (Sakamoto et al., 1994). Guaiane (**121**) underwent further rearrangement C4–C5, cleavage and C5–C10 *trans*-annular cyclization to the bicyclic hydroxyketone (**128**) and diketone (**129**) (Sakamoto et al., 1994) (Figure 15.43).

Curdione (**120**) was also treated in *Aspergillus niger* to afford two allylic alcohols (**133**, **134**) and a spirolactone (**135**). *Curcuma aromatica* and *Curcuma wenyujin* produced spirolactone (**135**) which might be formed from curdione via *trans*-annular reaction *in vivo* was biotransformed to spirolactone diol (**135**) (Asakawa et al., 1991; Sakui et al., 1992) (Figure 15.44).

Aspergillus niger also converted shiromodiol diacetate (**136**) isolated from *Neolitsea sericea* to 2β-hydroxy derivative (**137**) (Nozaki et al., 1996) (Figure 15.45).

Twenty strains of filamentous fungi and four species of bacteria were screened initially by thin layer chromatography (TLC) for their biotransformation capacity of curdione (**120**). *Mucor spinosus*, *Mucor polymorphosporus*, *Cunninghamella elegans*, and *Penicillium janthinellum* were found to be able to biotransform curdione (**120**) to more polar metabolites. Incubation of curdione with *Mucor spinosus*, which was most potent strain to produce metabolites, for 4 days using potato medium gave five metabolites (**134**, **134a**–**134d**) among which compounds **134c** and **134d** are new products (Ma et al., 2006) (Figure 15.46).

Many eudesmane-type sesquiterpenoids have been biotransformed by several fungi and various oxygenated metabolites obtained.

β-Selinene (**138**) is ubiquitous sesquiterpene hydrocarbon of seed oil from many species of Apiaceae family; for example, *Cryptotenia canadensis* var. *japonica*, which is widely used as vegetable for Japanese soup. β-Selinene was biotransformed by plant pathogenic fungus *Glomerella*

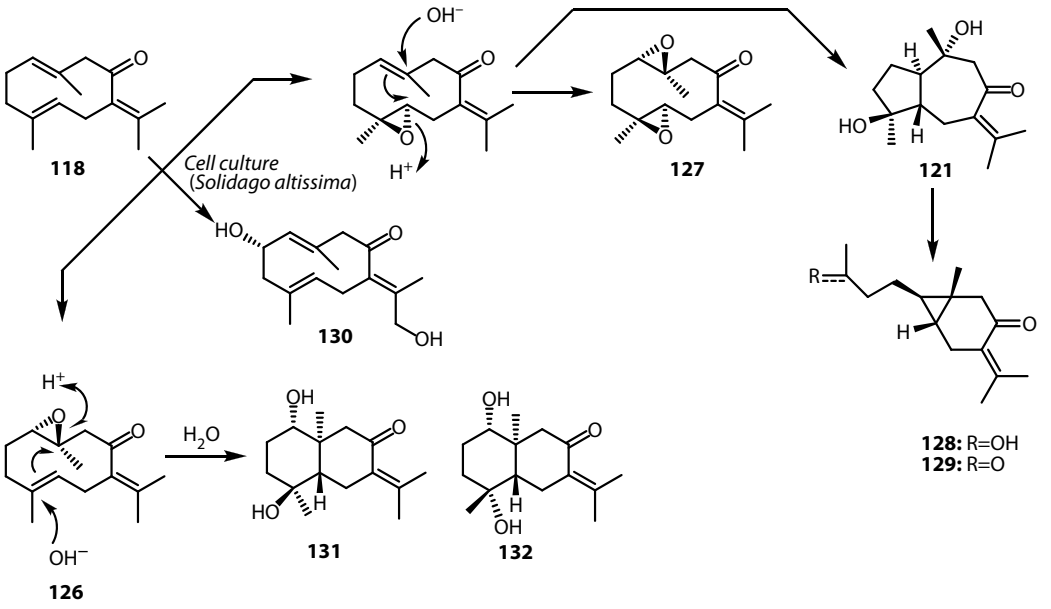


FIGURE 15.43 Biotransformation of germacrone (**118**) by *Solidago altissima* cells.

cingulata to give an epimeric mixtures (1:1) of 1β,11,12-trihydroxy product (**139**) (Miyazawa et al., 1997a). The same substrate was treated in *Aspergillus wentii* to give 2α,11,12-trihydroxy derivative (**140**) (Takahashi et al., 2007).

Eudesm-11(13)-en-4,12-diol (**141**) was biotransformed by *Aspergillus niger* to give 3β-hydroxy derivative (**142**) (Hayashi et al., 1999).

α-Cyperone (**143**) was fed by *Collectotrichum phomoides* (Lamare and Furstoss, 1990) to afford 11,12-diol (**144**) and 12-manool (**145**) (Higuchi et al., 2001) (Figure 15.47).

The filamentous fungi *Gliocladium roseum* and *Exserohilum halodes* were used as the bioreactors for 4β-hydroxyeudesmane-1,6-dione (**146**) isolated from *Sideritis varoi* subsp. *cuatrecasasii*. The former fungus transformed **146** to 7α-hydroxyl- (**147**), 11-hydroxy- (**148**), 7α,11-dihydroxy- (**149**), 1α,11-dihydroxy- (**150**), and 1α,8α-dihydroxy derivatives (**151**) while *Exserohilum halodes* gave only 1α-hydoxy product (**152**) (Garcia-Granados et al., 2001) (Figure 15.48).

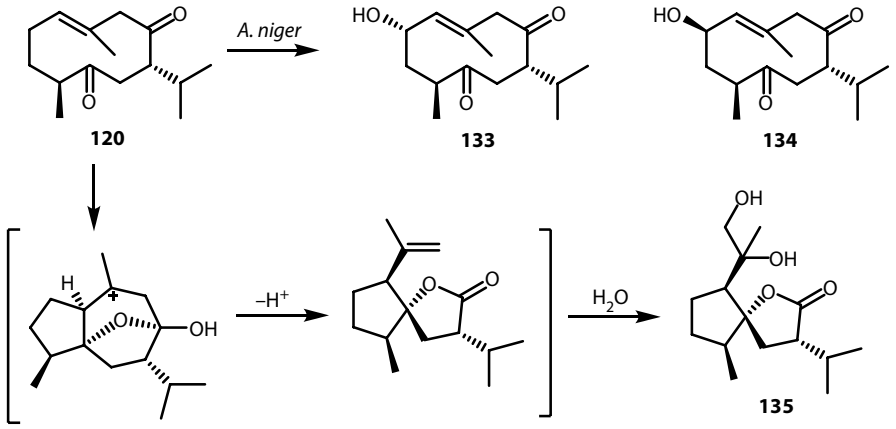
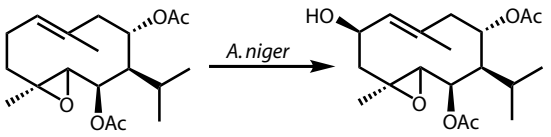


FIGURE 15.44 Biotransformation of curdione (**120**) by *Aspergillus niger*.



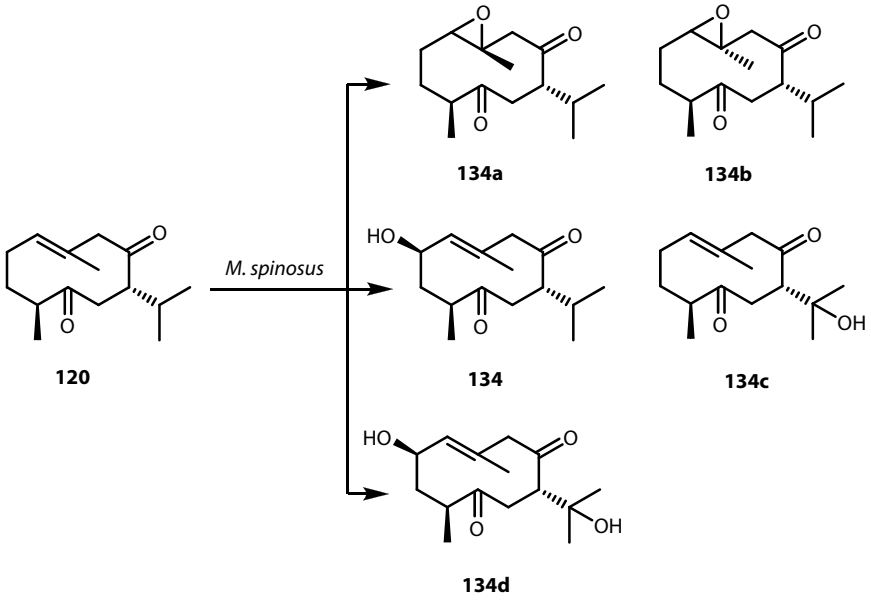
136 137

FIGURE 15.45 Biotransformation of shiromodiol diacetate (**136**) by *Aspergillus niger.*

Orabi (2000) reported that *Beauvaria bassiana* is the most efficient microorganism to metabolize plectanthone (**152a**) among 20 microorganisms, such as *Absidia glauca, Aspergillus flavipes, Beauvaria bassiana, Cladosporium resinae, Penicillium frequentans*, and so on. The substrate (**152a**) was incubated with *Beauvaria bassiana* to give metabolites **152b** (2.1%), **152c** (21.2%), **152d** (2.5%), **152e** (no data), and **152f** (1%) (Figure 15.49).

(–)-α-Eudesmol (**153**) isolated from the liverwort *Porella stephaniana* was treated by *Aspergillus cellulosae* and *Aspergillus niger* to give 2-hydroxy (**154**) and 2-oxo derivatives (**155**), among which the latter product was predominantly obtained. This bioconversion was completely blocked by 1-aminobenzotriazole, CYP450 inhibitor. Compound **155** has been known as natural product, isolated from *Pterocarpus santalinus* (Noma et al., 1996). Biotransformation of α-eudesmol (**153**) isolated from the dried *Atractylodes lancea* was reinvestigated by *Aspergillus niger* to give 2-oxo-11,12-dihydro-α-eudesmol (**156**) together with 2-hydroxy- (**154**), and 2-oxo-α-eudesmol (**155**). β-Eudesmol (**157**) was treated in *Aspergillus niger*, with the same culture medium to afford 2α- (**158**) and 2β-hydroxy-α-eudesmol (**159**) and 2α,11,12-trihydroxy-β-eudesmol (**160**) and 2-oxo derivative (**161**), which was further isomerized to compound **162** (Noma et al., 1996, 1997) (Figure 15.50).

Three new hydroxylated metabolites (**157b**–**157d**) along with a known **158** and (**157e**–**157g**) were isolated from the biotransformation reaction of a mixture of β- (**157**) and γ-eudesmols (**157a**) by *Gibberella suabinetii*. The metabolites proved a super activity of the hydroxylase, dehydrogenase, and isomerase enzymes. The hydroxylation is a common feature; on the contrary, cyclopropyl ring formation like compound (**158d**) is very rare (Maatooq, 2002a) (Figure 15.51).



120 134a 134b 134 134c 134d

FIGURE 15.46 Biotransformation of curdione (**120**) by *Mucor spinosus.*

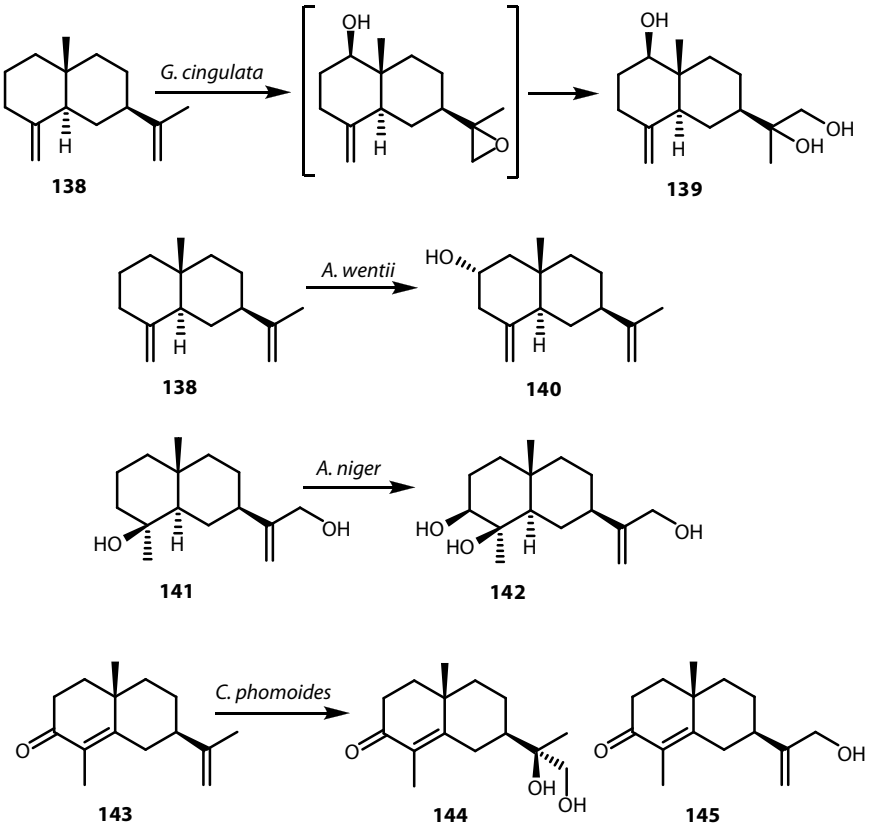


FIGURE 15.47 Biotransformation of eudesmenes (**138**, **141**, **143**) by *Aspergillus wentii*, *Glomerella cingulata*, and *Collectotrium phomoides*.

A furanosesquiterpene, atractylon (**163**) obtained from *Atractylodis* rhizoma was treated with the same fungus to yield atractylenolide III (**164**) possessing inhibition of increased vascular permeability in mice induced by acetic acid (Hashimoto et al., 2001).

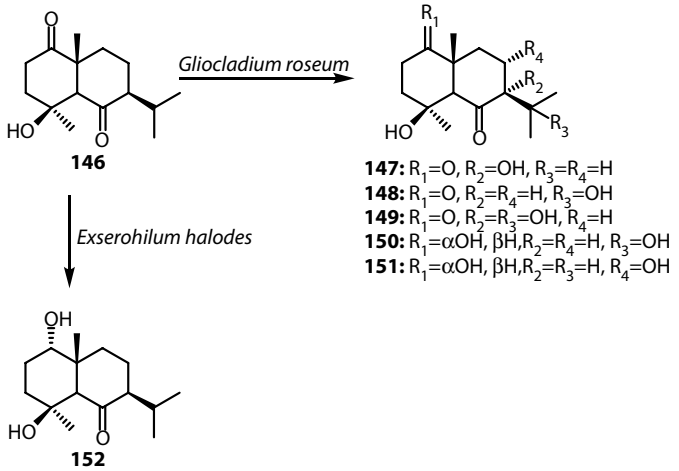


FIGURE 15.48 Biotransformation of 4β-hydroxy-eudesmane-1,6-dione (**146**) by *Gliocladium roseum* and *Exserohilum halodes*.

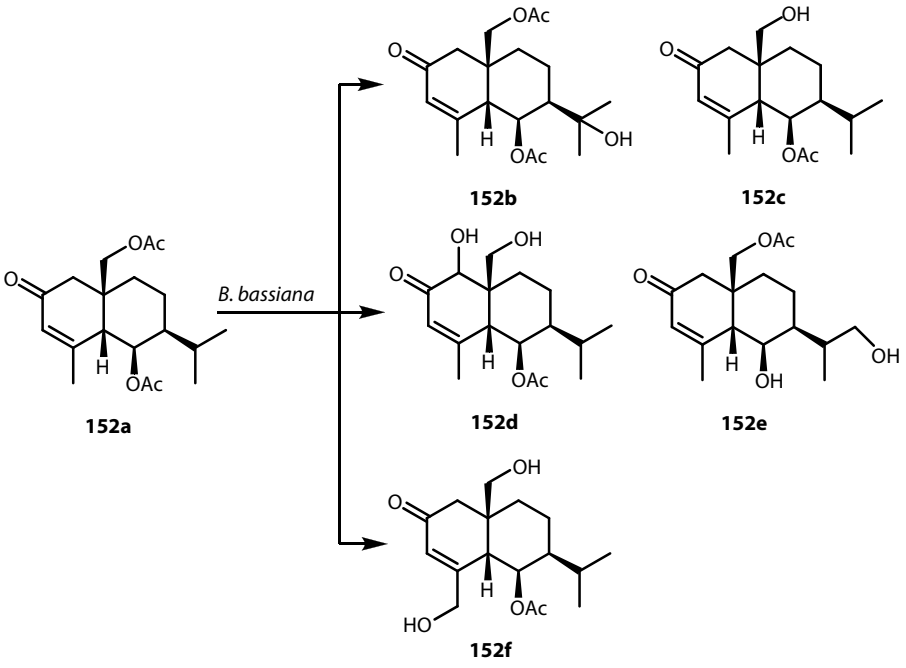


FIGURE 15.49 Biotransformation of eudesmenone (**152a**) by *Beauvaria bassiana*.

The biotransformation of sesquiterpene lactones have been carried out by using different microorganisms.

Costunolide (**165**), a very unstable sesquiterpene γ-lactone, from *Saussurea radix*, was treated in *Aspergillus niger* to produce three dihydrocostunolides (**166**–**168**) (Clark and Hufford, 1979). Costunolide is easily converted into eudesmanolides (**169**–**172**) in diluted acid, thus **166**–**168** might be biotransformed after being cyclized in the medium including the microorganisms. If the crude

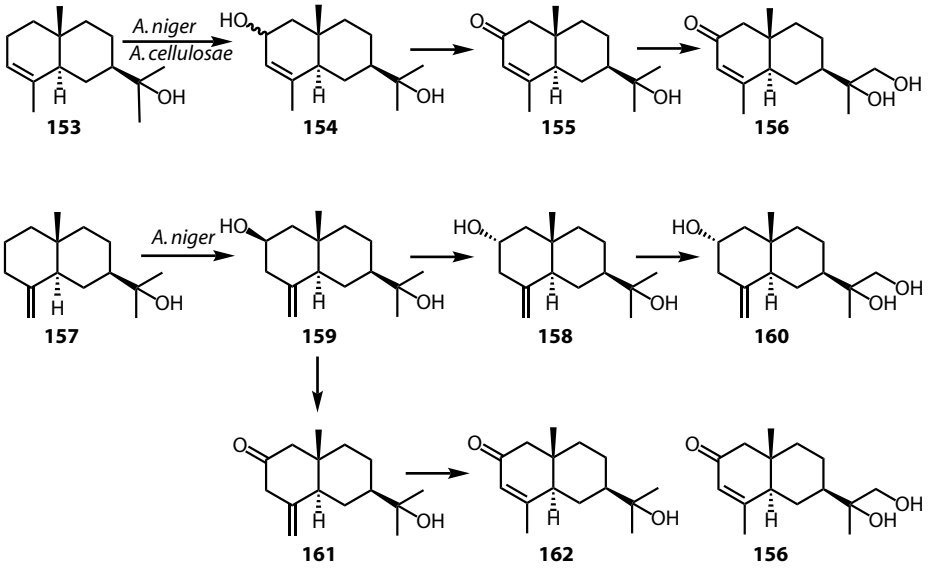


FIGURE 15.50 Biotransformation of α-eudesmol (**153**) and β-eudesmol (**157**) by *Aspergillus niger* and *Aspergillus cellulosae*.

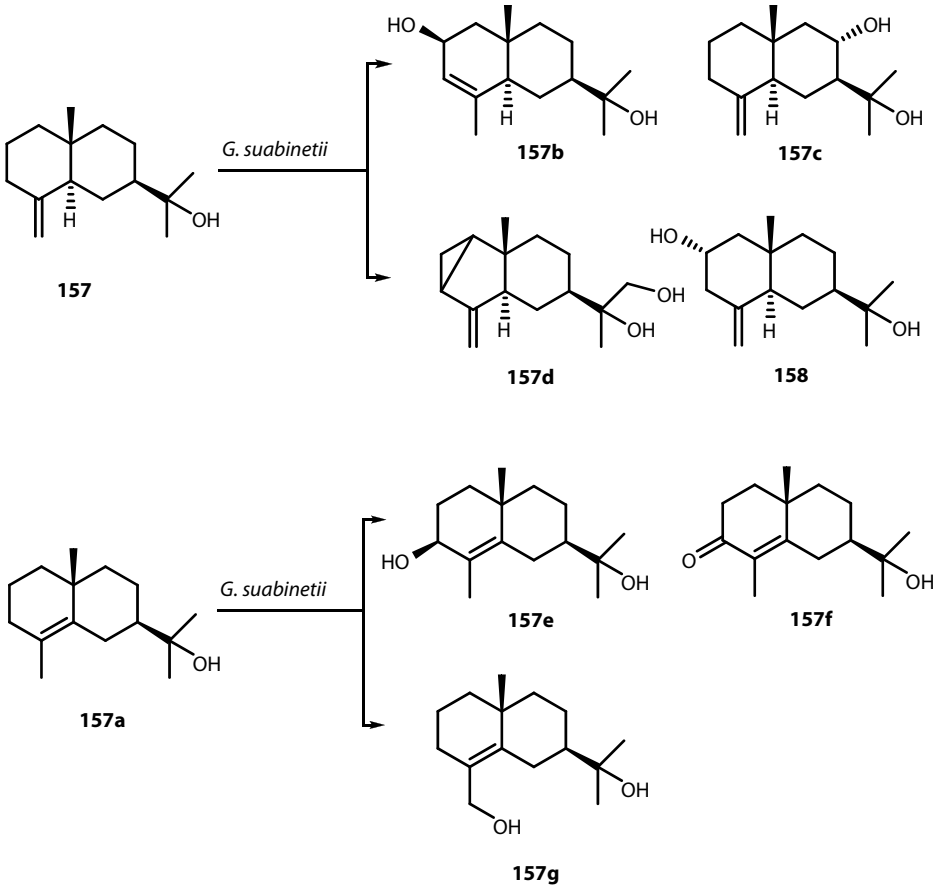


FIGURE 15.51 Biotransformation of β-eudesmol (**157**) and γ-eudesmol (**157a**) by *Gibberella suabinetii*.

drug including costunolide (**165**) is orally administered, **165** will be easily converted into **169**–**172** by stomach juice (Figure 15.52).

(+)-Costunolide (**165**), (+)-cnicin (**172a**), and (+)-salonitgenolide (**172b**) were incubated with *Cunninghamella echinulata* and *Rhizopus oryzae*.

The former fungus converted compound **165**, to four metabolites, (+)-11β,13-dihydrocostunolide (**165a**), 1β-hydroxyeudesmanolide, (+)-santamarine (**166a**), (+)-reynosin (**166b**), and (+)-1β-hydroxyarbusculin A (**168a**), which might be formed from 1β,10α-epoxide (**166c**). Treatment of **172a** with *Cunninghamella echinulata* gave (+)-salonitenolide (**172b**) (Barrero et al., 1999) (Figure 15.53).

α-Cyclocostunolide (**169**), β-cyclocostunolide (**170**), and γ-cyclocostunolide (**171**) prepared from costunolide were cultivated in *Aspergillus niger*, respectively. From the metabolite of **169**, four dihydro lactones (**173**–**176**) were obtained, among which sulfur-containing compound (**176**) was predominant (Figure 15.54).

The same substrate (**169**) was cultivated for 3 days by *Aspergillus cellulosae* to afford a sole metabolite, 11β,13-dihydro-α-cyclocostunolide (**177**). Possible metabolic pathways of **169** by both microorganisms were shown in Figure 15.55.

A double bond at C11–C13 of **169** was firstly reduced stereoselectively to afford **177**, followed by oxidation at C2 to give **173**, and then further oxidation occurred to furnish two hydroxyl derivatives (**174**, **175**) in *Aspergillus niger*. The sulfide compound (**176**) might be formed from **175** or by Michel condensation of ethyl 2-hydroxy-3-mercaptopropanate, which might originate from Czapek-peptone medium into exomethylene group of α-cyclocostunolide (Hashimoto et al., 1999a, 2001).

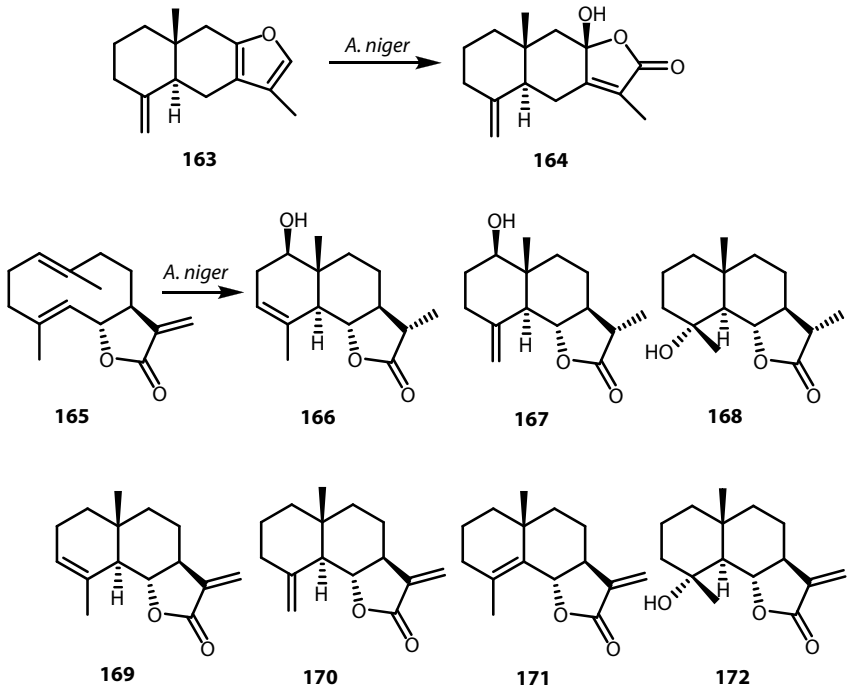


FIGURE 15.52 Biotransformation of atractylon (**163**) and costunolide (**165**) by *Aspergillus niger.*

Aspergillus niger converted β-cyclocostunolide (**170**) to 2-oxygenated metabolites (**173**, **174**, **178**–**181**) of which **173** was predominant. It is suggested that compound **173** and **174** might be formed during biotransformation period since metabolite media after 7 days was acidic (pH 2.7). Surprisingly, *Aspergillus cellulose* gave a sole 11β,13-dihydro-β-cyclocostunolide (**182**), which was abnormally folded in the mycelium of *Aspergillus cellulosae* as a crystal form after biotransformation of **170**. On the other hand, the metabolites were normally liberated in medium outside of the mycelium of *Aspergillus niger* and *Botryosphaeria dothidea* (Hashimoto et al., 1999a, 2001) (Figure 15.56).

Botryosphaeria dothidea has no stereoselectivity to reduce C11–C13 double bond of β-cyclocostunolide (**170**) since this organism gave two dihydro derivatives **182** (16.7%) and **183** (37.8%), respectively, as shown in Figure 15.57.

It is noteworthy that both α- and β-cyclocostunolides were biotransformed by *Aspergillus niger* to give the sulfur-containing metabolites (**176**, **181**). Possible biogenetic pathway of **170** is shown in Figure 15.58.

When γ-cyclocostunolide (**171**) was cultivated in *Aspergillus niger* to give dihydro-α-santonin (**187**, 25%) and its related C11,C13 dihydro derivatives (**184**–**186**, **188**, **189**) were obtained as a small amount. Compound **186** was recultivated for 2 days by the same organism as mentioned above to afford **187** (25%) and 5β-hydroxy-α-cyclocostunolide (**189**, 54%). Recultivation of **185** for 2 days by *Aspergillus niger* afforded compound **187** as a sole metabolite. During the biotransformation of **171**, no sulfur-containing product was obtained. Both *Aspergillus cellulosae* and *Botryosphaeria dothidea* produced only dihydro-γ-cyclocostunolide (**184**) from the substrate (**171**) (Hashimoto et al., 1999a, 2001) (Figure 15.59).

Santonin (**190**) has been used as vermicide against round warm. *Cunninghamella blakesleeana* and *Aspergillus niger* converted **190**–**187** (Atta-ur Rahman et al., 1998). When **187** was fed by *Aspergillus niger* for one week to give 2β-hydroxy-1,2-dihydro-α-santonin (**188**, 39%) as well as 1β-hydroxy-1,2-dihydro-α-santonin (**195**, 6.5%), 9β-hydroxy-1,2-dihydro-α-santonin (**196**, 6.9%), and α-santonin (**190**, 5.4%), which might be obtained from dehyroxylation of **188**, as a minor

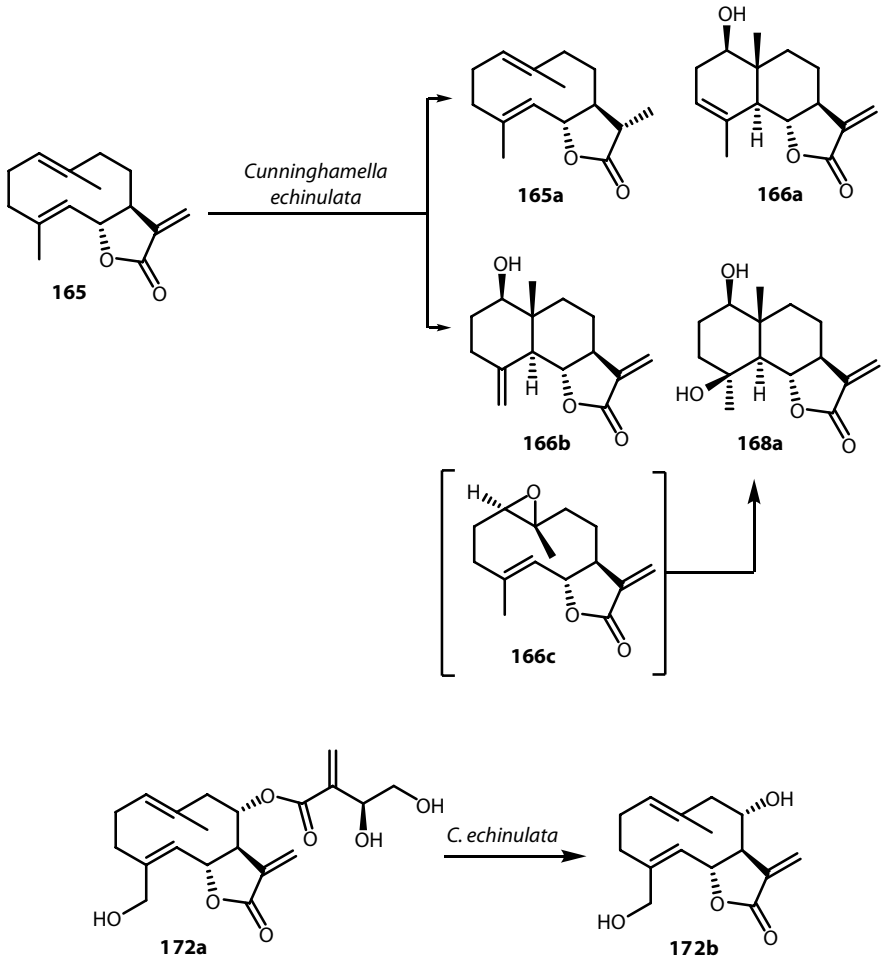


FIGURE 15.53 Biotransformation of costunolide (**165**) and its derivative (**172a**) by *Cunninghamella vechinulata* and *Rhizopus oryzae*.

component (Hashimoto et al., 2001). Compound **188** was isolated from the crude metabolite of γ-cyclocostunolide (**171**) by *Aspergillus niger* as mentioned above (Figure 15.60).

It was treated with *Aspergillus niger* for 7 days to give **191** (18.3%), **192** (2.3%), **193** (19.3%), and **194** (3.5%) of which **193** was the major metabolite. Compound **191** was isolated from dog's urine after the oral administration of **190**. The structure of compound **194** was established as lumisantonin obtained by the photoreaction of **190**. α-Santonin **190** was not converted into 1,2-dihydro derivative by *Aspergillus niger*, whereas the other strain of *Aspergillus niger* gave a single product, 1,2-dihydro-α-santonin (**187**) (Hashimoto et al., 2001) (Figure 15.61).

Ata and Nachtigall (2004) reported that α-santonin (**190**) was incubated with *Rhizopus stolonifer* to give (**187a**), and (**183b**), while with *Cunninghamella bainieri*, *Cunninghamella echinulata*, and *Mucor plumbeus* to afford the known 1,2-dihydro-α-santonin (**187**) (Figure 15.62).

α-Santonin (**190**) and 6-*epi*-α-santonin (**198**) were cultivated in *Absidia coerulea* for 2 days to give 11β-hydroxy- (**191**, 71.4%) and 8α-hydroxysantonin (**197**, 2.0%), while 6-*epi*-santonin (**198**) afforded four major products (**199**–**201**, **206**) and four minor analogues (**202**, **203**–**205**). *Asparagus officinalis* also biotransformed α-santonin (**190**) into three eudesmanolides (**187**, **207**, **208**) and a guaianolide (**209**) in a small amount. 6-*Epi*-santonin (**198**) was also treated in the same bioreactor

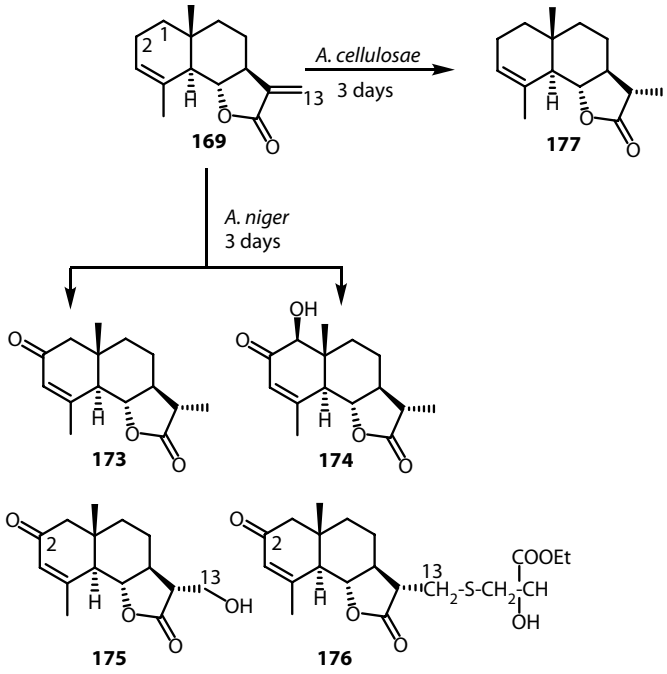


FIGURE 15.54 Biotransformation of α-cyclocostunolide (**169**) by *Aspergillus niger* and *Aspergillus cellulosae*.

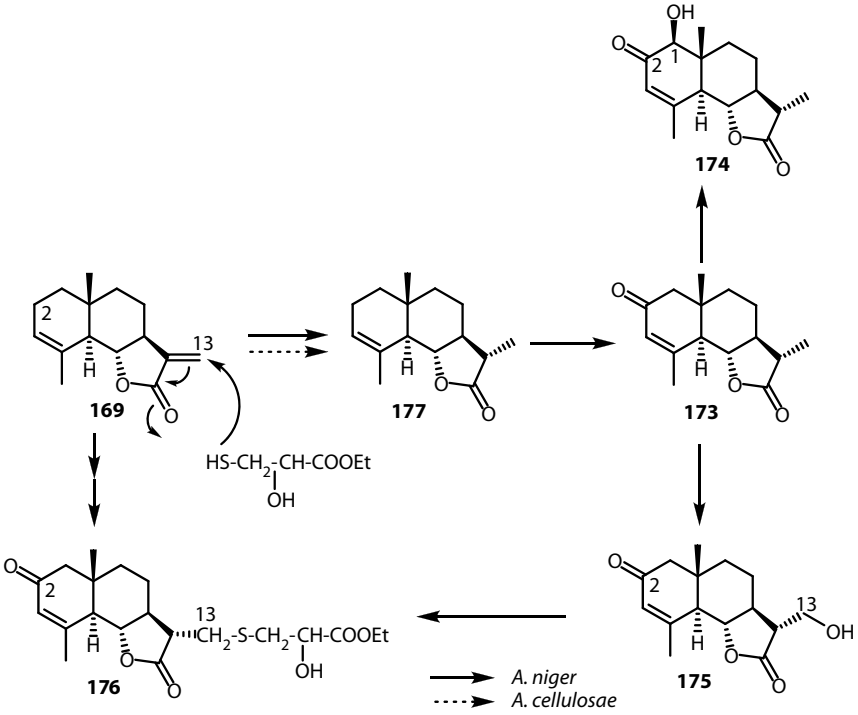


FIGURE 15.55 Possible pathway of biotransformation of α-cyclocostunolide (**169**) by *Aspergillus niger* and *Aspergillus cellulosae*.

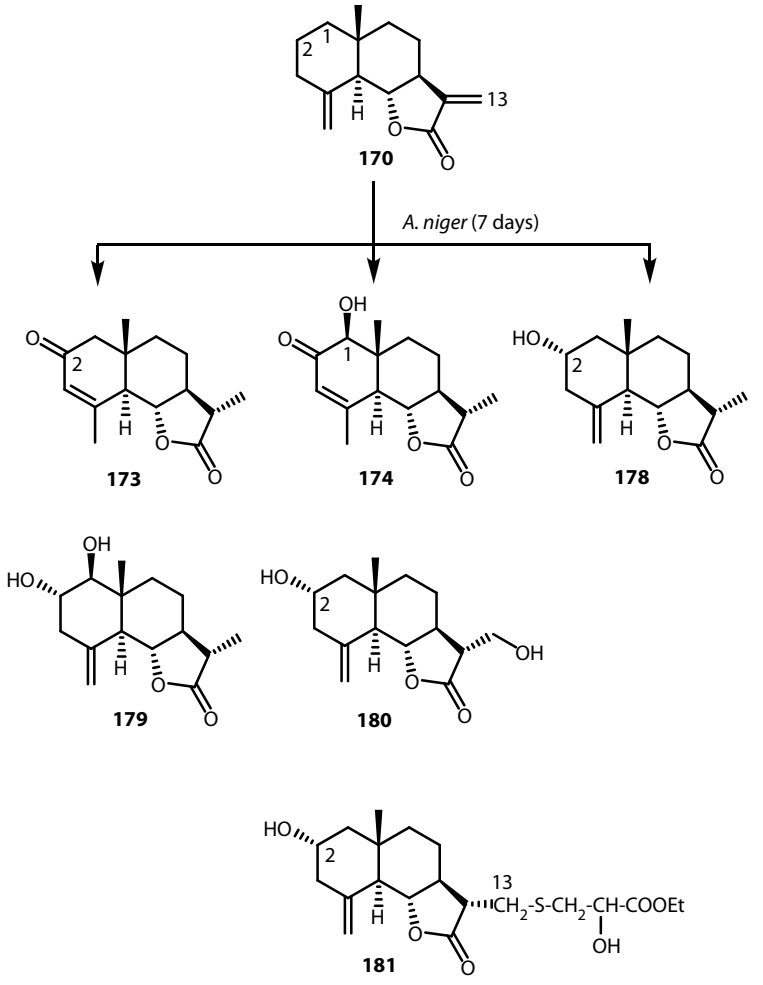


FIGURE 15.56 Biotransformation of β-cyclocostunolide (**170**) by *Aspergillus niger*.

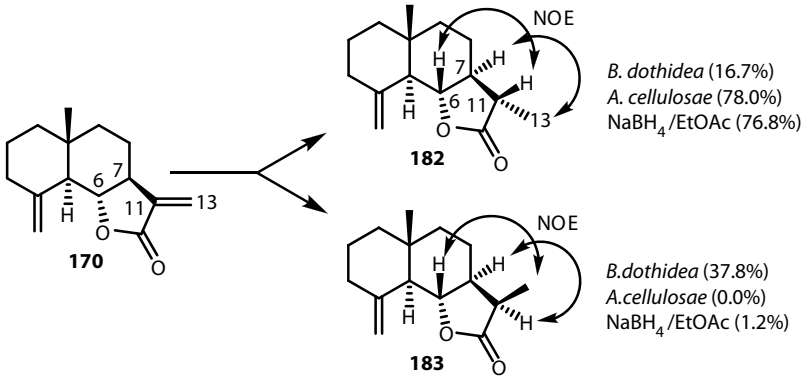


FIGURE 15.57 Biotransformation of β-cyclocostunolide (**170**) by *Aspergillus cellulosae* and *Botryosphaeria dothidea*.

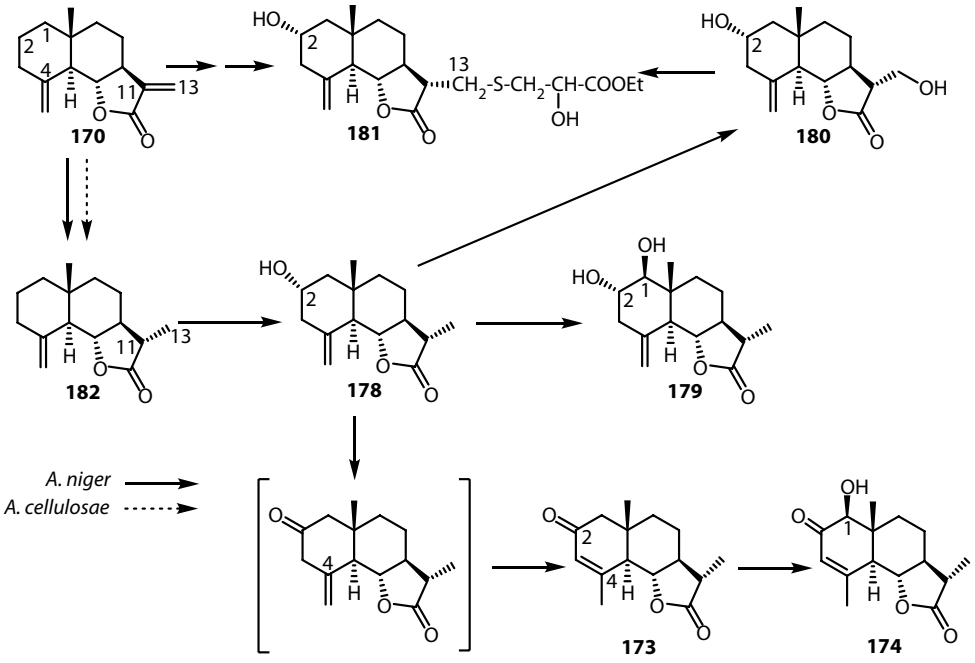


FIGURE 15.58 Possible pathway of biotransformation of β-cyclocostunolide (**170**) by *Aspergillus niger* and *Aspergillus cellulosae*.

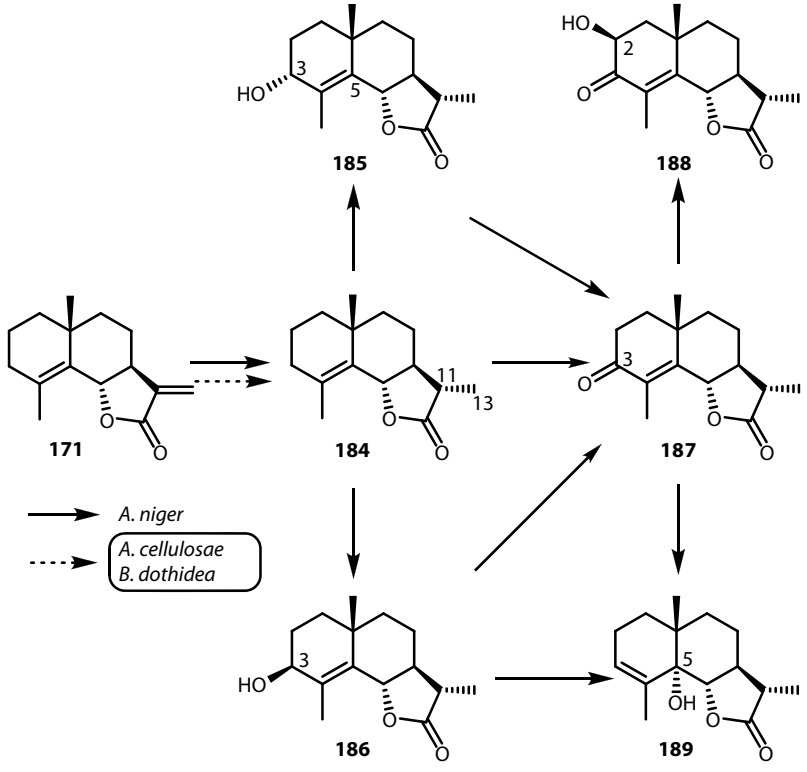


FIGURE 15.59 Biotransformation of γ-cyclocostunolide (**171**) by *Aspergillus niger*, *Aspergillus cellulosae*, and *Botryosphaeria dothidea*.

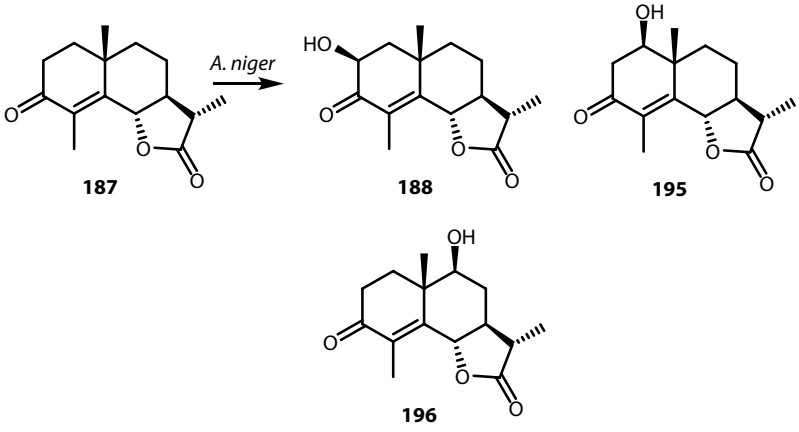


FIGURE 15.60 Biotransformation of dihydro-α-santonin (**187**) by *Aspergillus niger.*

as mentioned above to give **199** and **206**, the latter of which was obtained as a major metabolite (44.7%) (Yang et al., 2003) (Figure 15.63).

α-Santonin (**190**) was incubated in the cultured cells of *Nicotiana tabacum* and the liverwort *Marchantia polymorpha*. *Nicotiana tabacum* cells gave 1,2-dihydro-α-santonin (**187**) (50%) for 6 days. The latter cells also converted α-santonin to 1,2-dihydro-α-santonin, but conversion ratio was only 28% (Matsushima et al., 2004) (Figure 15.64).

6-*Epi*-α-santonin (**198**) and its tetrahydro analogue (**210**) were also incubated with fungus *Rhizopus nigricans* to give 2α-hydroxydihydro-α-santonin (**211**) (Amate et al., 1991), the epimer of **188** obtained from the biotransformation of dihydro-α-santonin (**187**) by *Aspergillus niger* (Hashimoto et al., 2001). The product **211** might be formed via 1,2-epoxide of **198**. Compound **210** was converted through carbonyl reduction to furnish **212** and **213** under epimerization at C4 (Amate et al., 1991) (Figure 15.65).

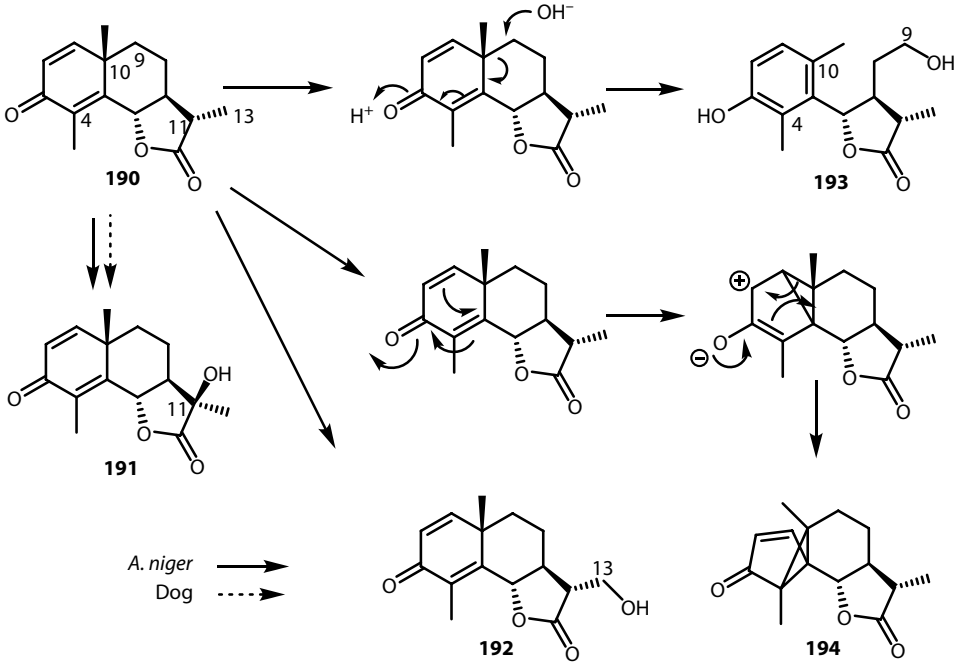


FIGURE 15.61 Biotransformation of α-santonin (**190**) by *Aspergillus niger* and dogs.

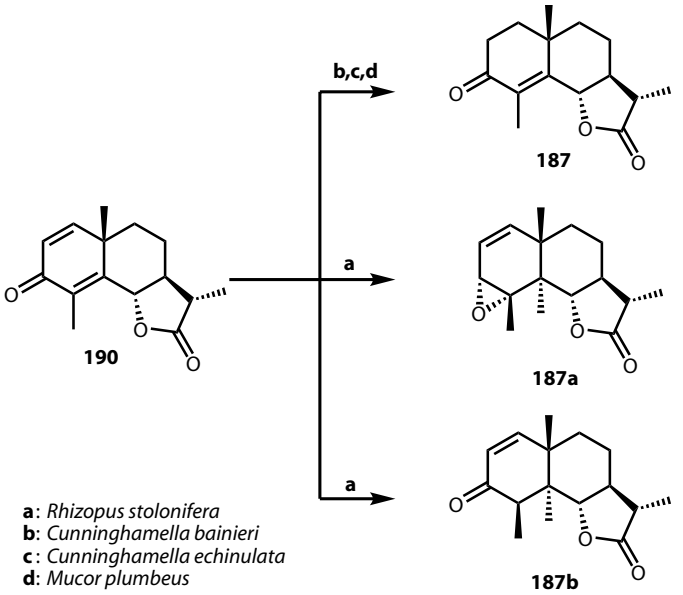


FIGURE 15.62 Biotransformation of α-santonin (**190**) by *Rhizopus stolonifera, Cunninghamella bainieri, Cunninghamella echinulata*, and *Mucor plumbeus*.

1,2,4β,5α-Tetrahydro-α-santonin (**214**) prepared from α-santonin (**190**) was treated with *Aspergillus niger* to afford six metabolites (**215**–**220**) of which **219** was the major product (21%). When the substrate (**214**) was treated with CYP450 inhibitor, 1-aminobenzotriazole, only **215** was obtained without its homologues, **216**–**220**, while the C4 epimer (**221**) of **214** was converted by the same microorganism to afford a single metabolite (**222**) (73%). Further oxidation of **222** did not occur. This reason might be considered by the steric hindrance of β (axial) methyl group at C4 (Hashimoto et al., 2001) (Figure 15.66).

7α-Hydroxyfrullanolide (**223**) possessing cytotoxicity and antitumor activity, isolated from *Sphaeranthus indicus* (Asteraceae), was bioconverted by *Aspergillus niger* to afford 13*R*-dihydro

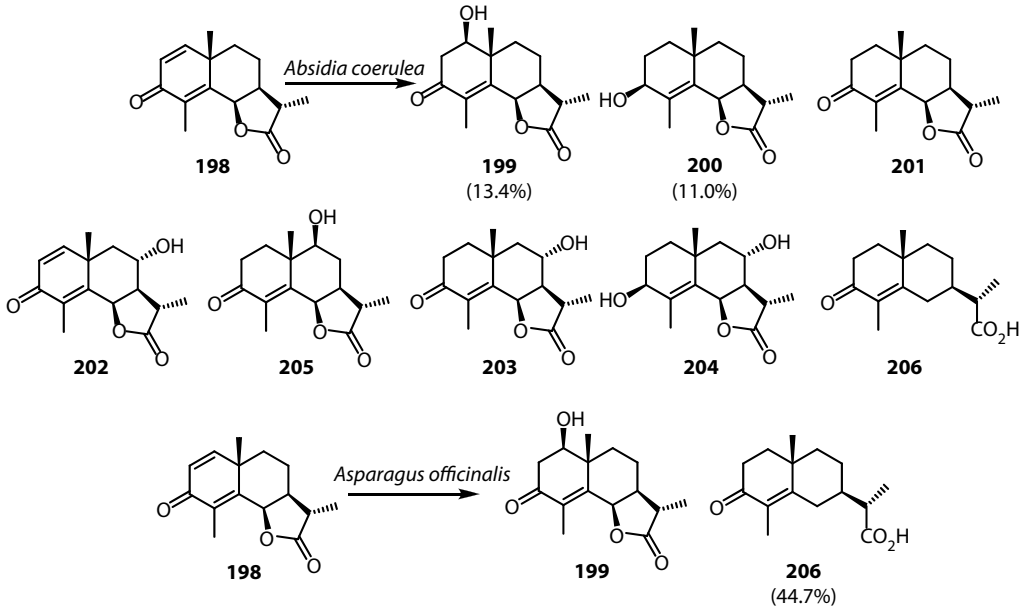


FIGURE 15.63 Biotransformation of α-epi-santonin (**198**) by *Absidia coerulea* and *Asparagus officinalis*.

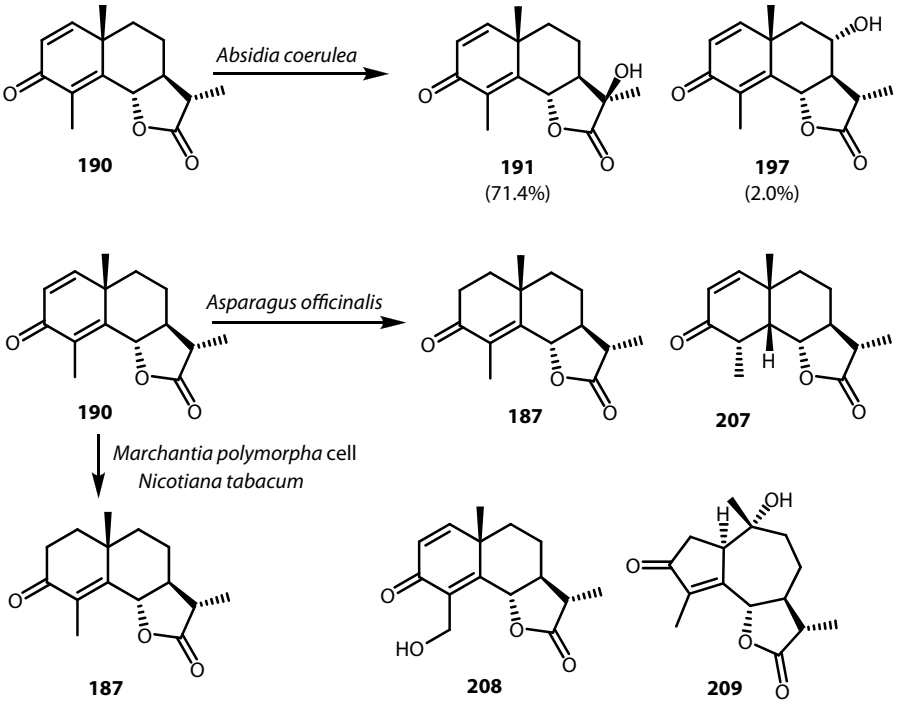


FIGURE 15.64 Biotransformation of 6-epi-α-santonin (**190**) by *Absidia coerulea, Asparagus officinalis, Marchantia polymorpha*, and *Nicotiana tabacum*.

derivative (**224**). The same substrate was also treated in *Aspergillus quardilatus* (wild type) to give 13-acetyl product (**225**) (Atta-ur Rahman et al., 1994) (Figure 15.67).

Incubation of (−)-frullanolide (**226**), obtained from the European liverwort, *Frullania tamarisci* subsp. *tamarisci* causes a potent allergenic contact dermatitis, was incubated by *Aspergillus niger* to give dihydrofrullanolide (**227**), nonallergenic compound in 31.8% yield. In this case, C11–C13 dihydro derivative was not obtained (Hashimoto et al., 2005).

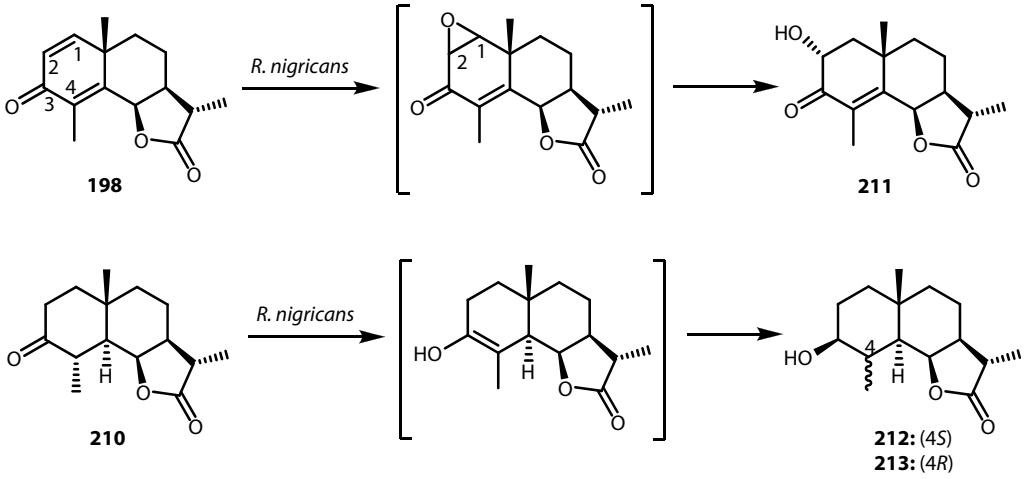


FIGURE 15.65 Biotransformation of α-episantonin (**198**) and tetrahydrosantonin (**210**) by *Rhizopus nigricans*.

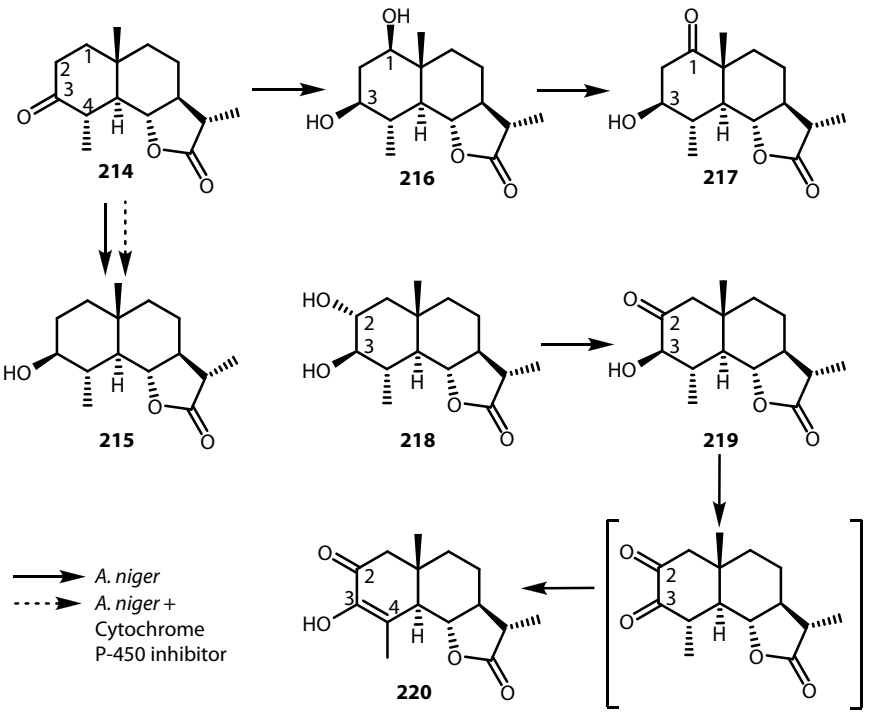


FIGURE 15.66 Biotransformation of 1,2,4β,5α-tetrahydro-α-santonin (**214**) by *Aspergillus niger.*

Guaiane-type sesquiterpene hydrocarbon, (+)-γ-gurjunene, (**228**) was treated in plant pathogenic fungus *Glomerella cingulata* to give two diols, (1*S*,4*S*,7*R*,10*R*)-5-guaien-11,13-diol (**229**), and (1*S*,4*S*,7*R*,10*S*)-5-guaien-10,11,13-triol (**230**) (Miyazawa et al., 1997, 1998) (Figure 15.68).

Glomerella cingulata converted guaiol (**231**) and bulnesol (**232**) to 5,10-dihydroxy (**233**) and 15-hydroxy derivative (**234**), respectively (Miyazawa et al., 1996) (Figure 15.69).

When *Eurotium rubrum* was used as the bioreactor of guaiene (**235**), rotunodone (**236**) was obtained (Sugawara and Miyazawa, 2004). Guaiol (**231**) was also transformed by *Aspergillus niger* to give a cylopentane derivative, pancherione (**237**) and two dihydroxy guaiols (**238**, **239**) (Morikawa et al., 2000), of which **237** was obtained from the same substrate using *Eurotium rubrum* for 10 days (Sugawara and Miyazawa, 2004; Miyazawa and Sugawara, 2006) (Figure 15.70).

Parthenolide (**240**), a germacrane-type lactone, isolated from the European feverfew (*Tanacetum parthenium*) as a major constituent shows cytotoxic, antimicrobial, and antifungal, anti-inflammatory, antirheumatic activity, apoptosis inducing, and NF-κB and DNA binding inhibitory activity. This substrate was incubated with *Aspergillus niger* in Czapek-peptone medium for 2 days to give six metabolites (**241**, 12.3%, **242**, 11.3%, **243**, 13.7%, **244**, 5.0%, **245**, 9.6%, **246**, 5.1%) (Hashimoto et al., 2005) (Figure 15.71). Compound **244** was a naturally occurring lactone from *Michelia champaca* (Jacobsson et al., 1995). The stereostructure of compound **243** was established by x-ray crystallographic analysis.

When parthenolide (**240**) was treated in *Aspergillus cellulosae* for 5 days, two new metabolites, 11β,13-dihydro-(**247**, 43.5%) and 11α,13-dihydroparthenolides (**248**, 1.6%) were obtained together with the same metabolites (**241**, 5.3%, **243**, 11.2%, **245**, 10.4%) as described above (Figure 15.72). Possible metabolic root of **240** has been shown in Figure 15.73 (Hashimoto et al., 2005).

Galal et al. (1999) reported that *Streptomyces fulvissimus* or *Rhizopus nigricans* converted parthenolide (**240**) into 11α-methylparthenolide (**247**) in 20–30% yield while metabolite 11β-hydroxyparthenolide (**248**) was obtained by incubation of **240** with *Rhizopus nigricans* and

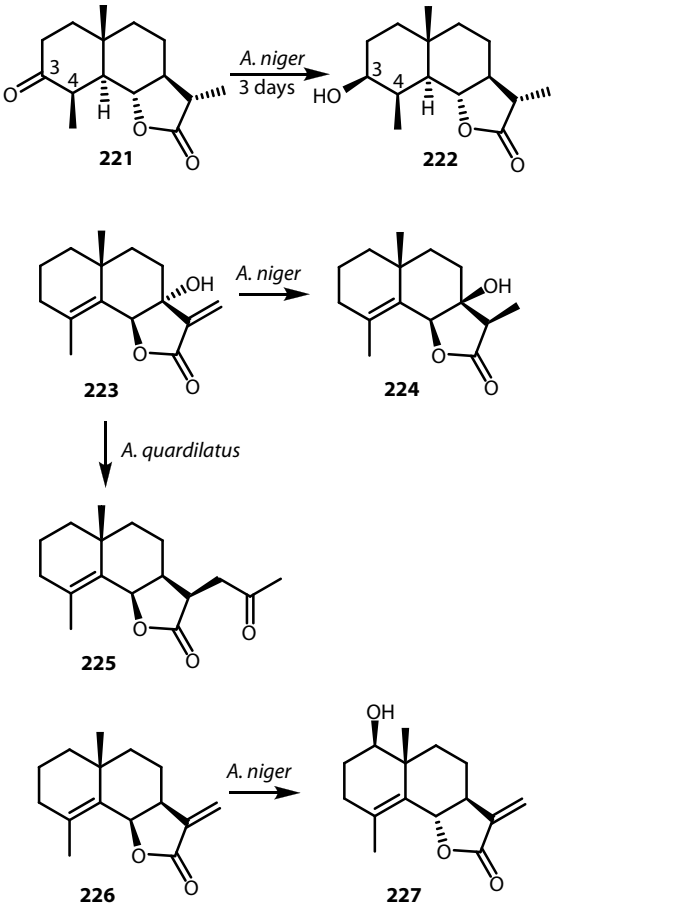


FIGURE 15.67 Biotransformation of C4-epimer (**221**) of **214**, 7α-hydroxyfrullanolide (**223**), and frullanolide (**226**) by *Aspergillus niger* and *Aspergillus quardilatus.*

Rhodotorula rubra. In addition to the metabolite **247**, *Streptomyces fulvissimus* gave minor polar metabolite, 9β-hydroxy derivative (**248a**) in low yield (3%). The same metabolite (**248a**) was obtained from **247** by fermentation of *Streptomyces fulvissimus* as a minor constituent. Furthermore, 14-hydroxyparthenolide (**248b**) was obtained from **240** and **247** as a minor component (4%) by *Rhizopus nigricans* (Figure 15.74).

Pyrethrosin (**248c**), a germacranolide, was treated in the fungus *Rhizopus nigricans* to afford five metabolites (**248d**–**248h**). Pyrethrosin itself and metabolite **248e** displayed cytotoxic activity against human malignant melanoma with IC_{50} 4.20 and 7.5 μg/mL, respectively. Metabolite **248h** showed significant *in vitro* cytotoxic activity against human epidermoid carcinoma (KB cells) and against human ovary carcinoma with $IC_{50} < 1.1$ and 8.0 μg/mL, respectively. Compounds **248f** and

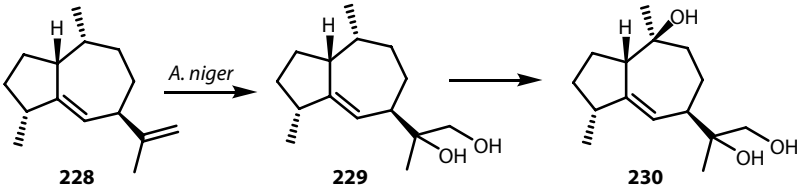


FIGURE 15.68 Biotransformation of (+)-γ-gurjunene (**228**) by *Glomerella cingulata.*

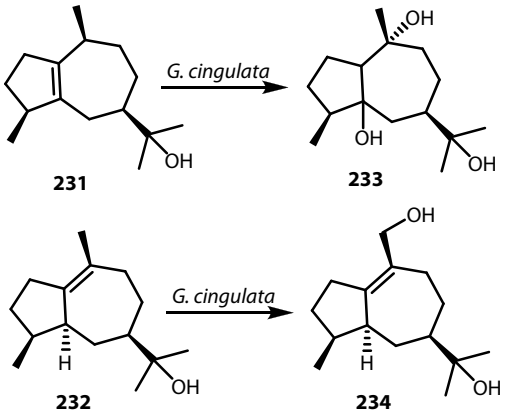


FIGURE 15.69 Biotransformation of guaiol (**221**) and bulnesol (**232**) by *Glomerella cingulata*.

248i were active against *Cryptococcus neoformans* with IC_{50} 35.0 and 25 μg/mL, respectively while **248a** and **248g** showed antifungal activity against *Candida albicans* with IC_{50} 30 and 10 μg/mL. Metabolites **248g** and its acetate (**248i**), derived from **248g** showed antiprotozoal activity against *Plasmodium falciparum* with IC_{50} 0.88 and 0.32 μg/mL, respectively without significant toxicity. Compound **248i** also exhibited pronounced activity against the chloroquine-registant strain of *Plasmodium falciparum* with IC_{50} 0.38 μg/mL (Galal, 2001) (Figure 15.75).

(–)-Dehydrocostuslactone (**249**), inhibitors of nitric oxide synthases and TNF-α, isolated from *Saussurea* radix, was incubated with *Cunninghamella echinulata* to afford (+)-11α,13-dihydrodehydrocostuslactone (**250a**). The epoxide (**251**) and a C11 reduced compound (**250**) were obtained by the above microorganisms (Galal, 2001).

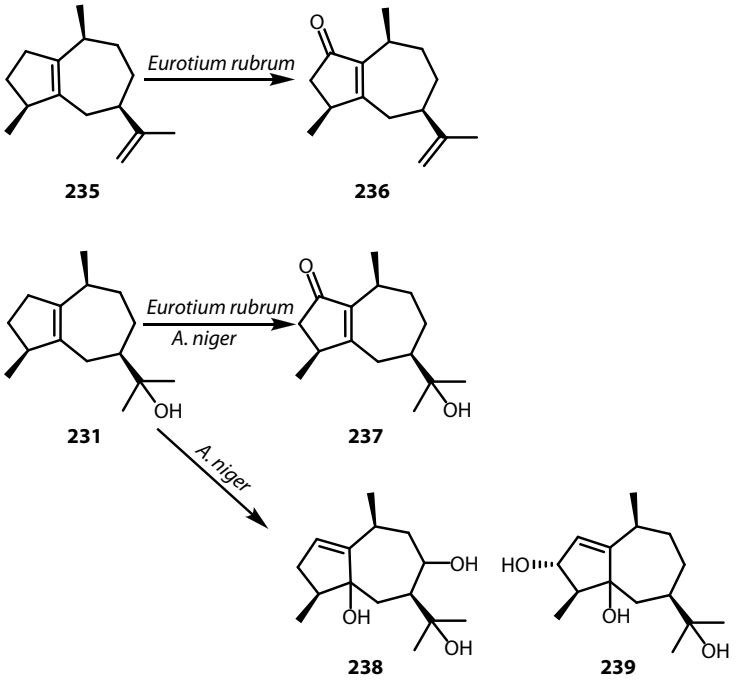


FIGURE 15.70 Biotransformation of guaiene (**235**) by *Eurotium rubrum* and guaiol (**231**) by *Aspergillus niger* and *Eurotium rubrum*.

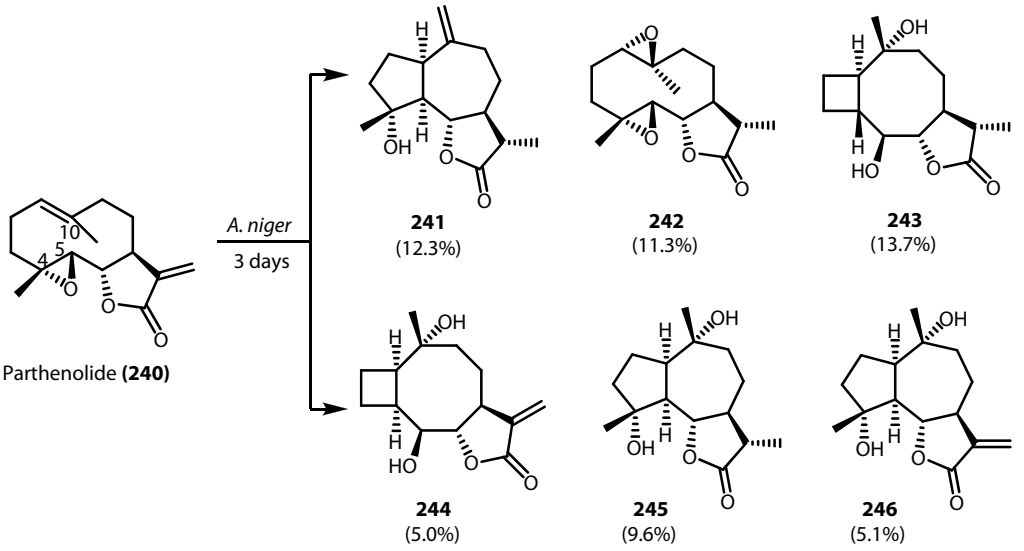


FIGURE 15.71 Biotransformation of parthenolide (**240**) by *Aspergillus niger.*

Cunninghamella echinulata and *Rhizopus oryzae* bioconverted **249** into C11/C13 dihydrogenated (**250**) and C10/C14 epoxidated product (**251**). Treatment of **252a** in *Cunninghamella echinulata* and *Rhizopus oryzae* gave (–)-16-(1-methyl-1-propenyl) eremantholide (**252b**) (Galal, 2001) (Figure 15.76).

The same substrate (**249**) was fed by *Aspergillus niger* for 7 days to afford four metabolites costuslactone (**250**), and their derivatives (**251**–**253**), of which **251** was the major product (28%) while the same substrate was cultivated with *Aspergillus niger* for 10 days, two minor metabolites (**254**, **255**) were newly obtained in addition to **252** and **253** of which the latter lactone was predominant (20.7%) (Hashimoto et al., 2001) (Figure 15.77).

When compound (**249**) was treated with *Aspergillus niger* in the presence of 1-aminobenzotriazole, **249** was completely converted into 11β,13-dihydro derivative (**250**) for 3 days; however, further biodegradation did not occur for 10 days (Hashimoto et al., 1999, 2001). The same substrate (**249**) was cultivated with *Aspergillus cellulosae* IFO to furnish 11,13-dihydro- (**250**) (82%) for only one day and then the product (**250**) slowly oxidized into 11,13-dihydro-8β-hydroxycostuslactone (**256**) (1.6%) from 8 days (Hashimoto et al., 1999, 2001) (Figure 15.78).

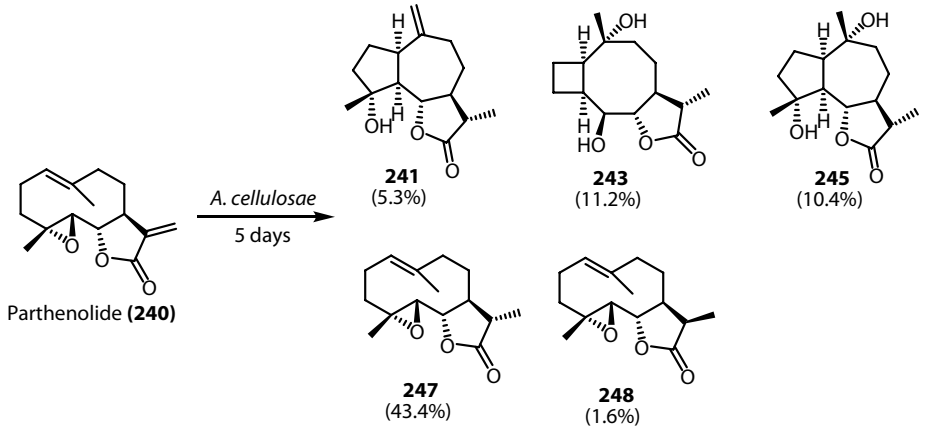


FIGURE 15.72 Biotransformation of parthenolide (**240**) by *Aspergillus cellulosae.*

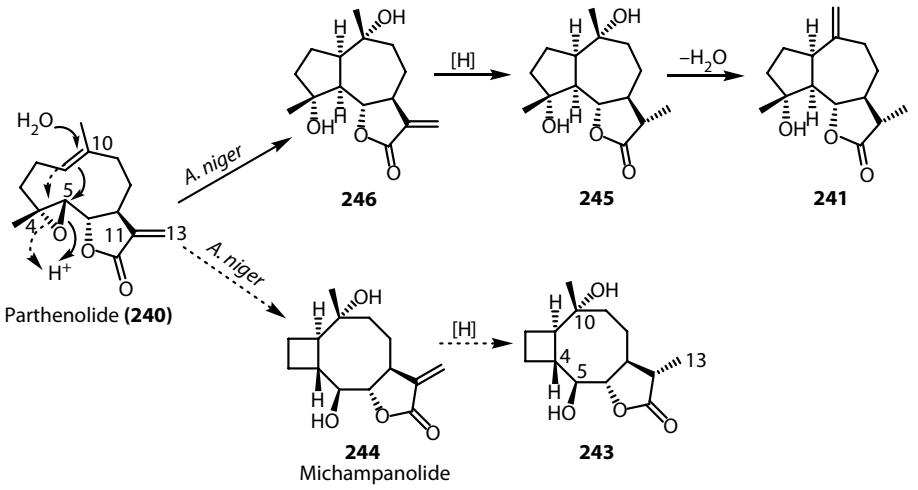


FIGURE 15.73 Possible pathway of biotransformation of parthenolide (**240**).

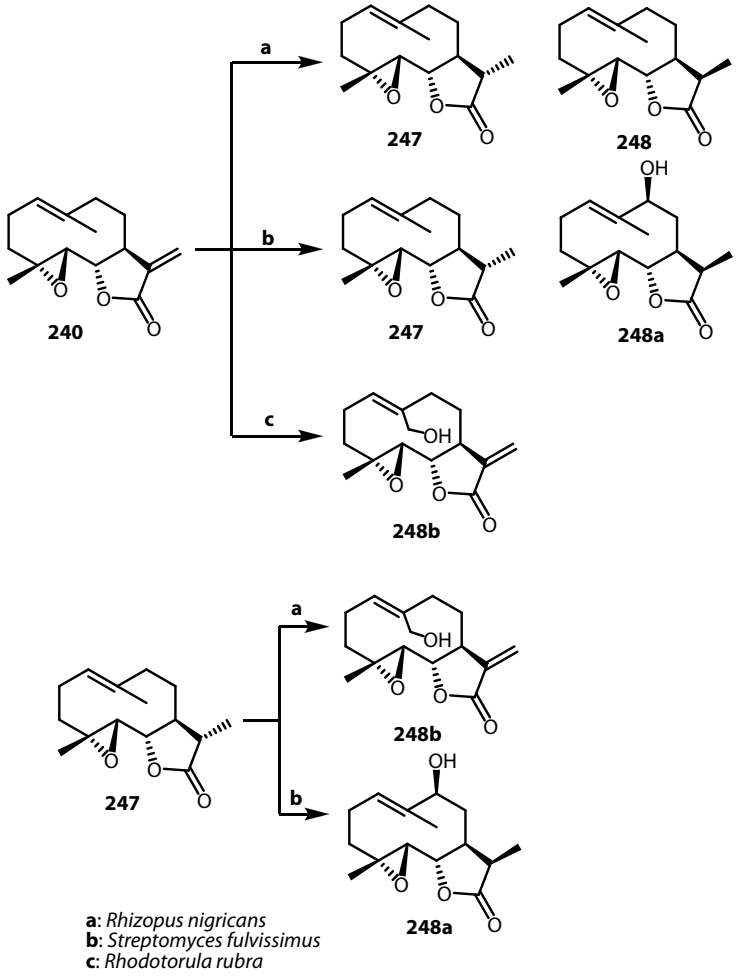


FIGURE 15.74 Biotransformation of parthenolide (**240**) and its dihydro derivative (**247**) by *Rhizopus nigricans*, *Streptomyces fulvissimus*, and *Rhodotorula rubra*.

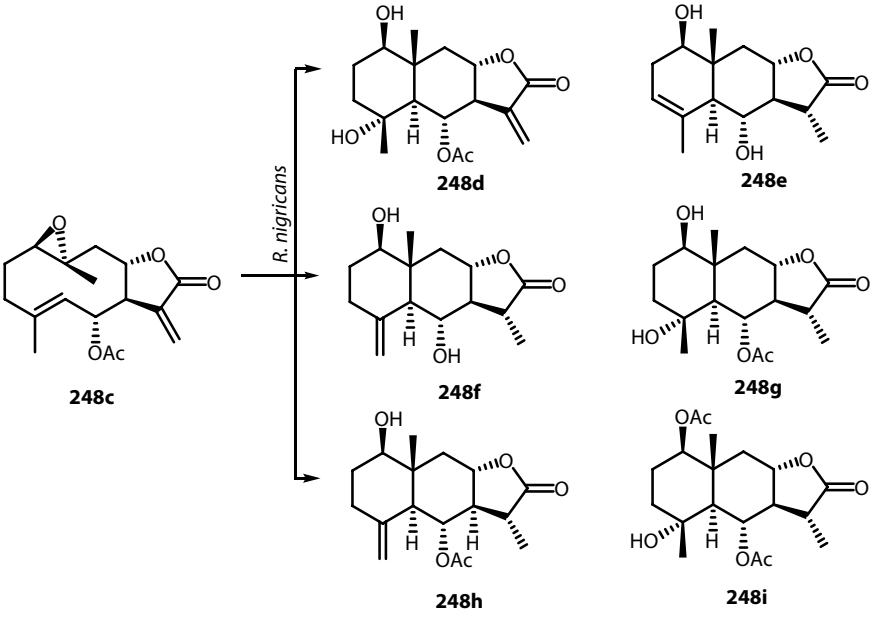


FIGURE 15.75 Biotransformation of pyrethrosin (**248c**) by *Rhizopus nigricans.*

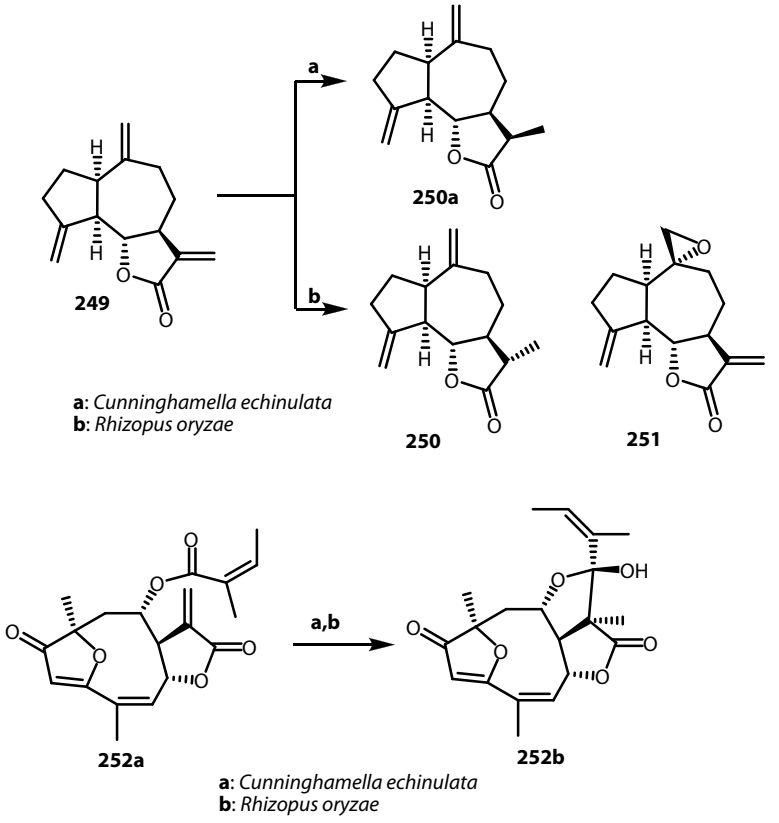


FIGURE 15.76 Biotransformation of (−)-dehydrocostuslactone (**249**) and rearranged guaianolide (**252a**) by *Cunninghamella echinulata* and *Rhizopus oryzae*.

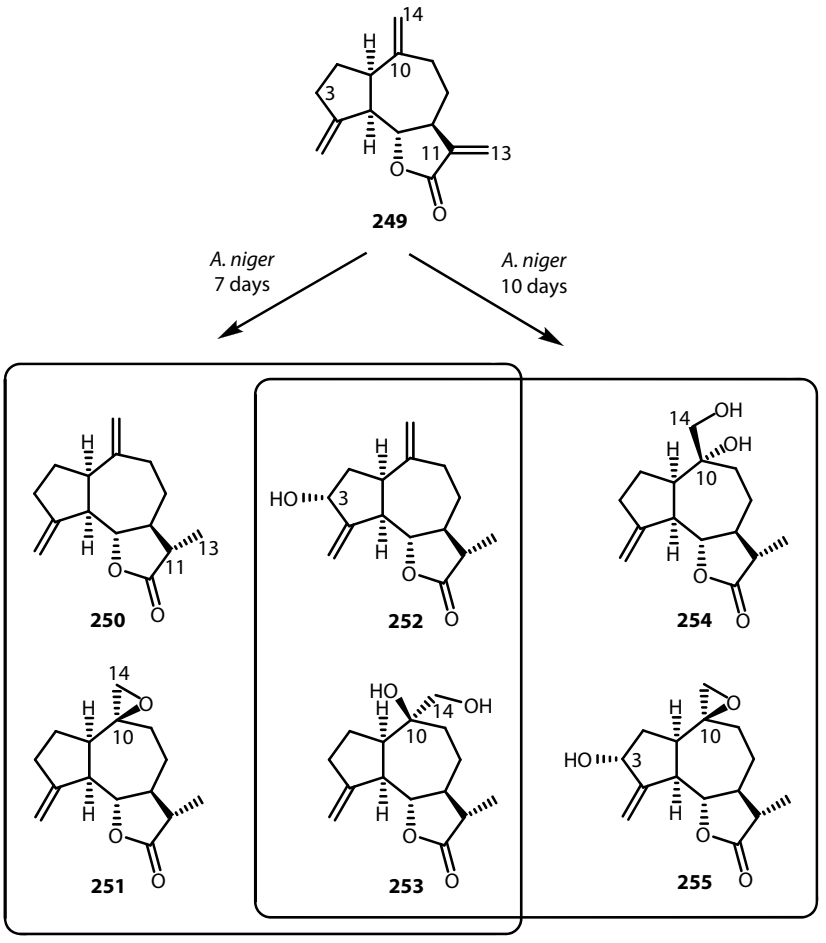


FIGURE 15.77 Biotransformation of (−)-dehydrocostuslactone (**249**) by *Aspergillus niger.*

The lactone (**249**) was biodegraded by the plant pathogen *Botryosphaeria dothidea* for 4 days to give the metabolites (**250**) (37.8%) and **257** (8.6%) while *Aspergillus niger* IFO-04049 (4 days) and *Aspergillus cellulosae* for 1 day gave only **250**. Thus *Botryosphaeria dothidea* demonstrated low stereoselectivity to reduce C11–C13 double bond (Hashimoto et al., 2001). Furthermore three *Aspergillus* species, *Aspergillus niger* IFO 4034, *Aspergillus awamori* IFO 4033, and *Aspergillus terreus* IFO6123 were used as bioreactors for compounds **249**. *Aspergillus niger* IFO 4034 gave three products (**250**–**252**), of which **252** was predominant (56% in GC-MS). *Aspergillus awamori* IFO 4033 and *Aspergillus terreus* IFO 6123 converted **249** to give **250** (56% from *Aspergillus awamori*, 43% from *Aspergillus terreus*) and **252** (43% from *Aspergillus awamori*, 57% from *Aspergillus terreus*), respectively (Hashimoto et al., 2001) (Figure 15.79).

Vernonia arborea (Asteraceae) contains zaluzanin D (**258**) in high content. Ten microorganisms were used for the biotransformation of compound **258**. *Botrytis cinerea* converted **258** into **259** and **260** (85:15%) and *Fusarium equiseti* gave **259** and **260** (33:66%). *Curvularia lunata*, *Colletotrichum lindemuthianum*, *Alternaria alternate*, and *Phyllosticta capsici* produced **259** as the sole metabolite in good yield while *Sclerotinia sclerotiorumn* and *Rhizpctonia solani* gave deactyl product (**261**) as a sole product, and **260**, **262**–**264** among which **263** and **264** are the major products, respectively. Reduction of C11–C13 exocylic double bond is the common transformation of α-methylene γ-butyrolactone (Kumari et al., 2003) (Figure 15.80).

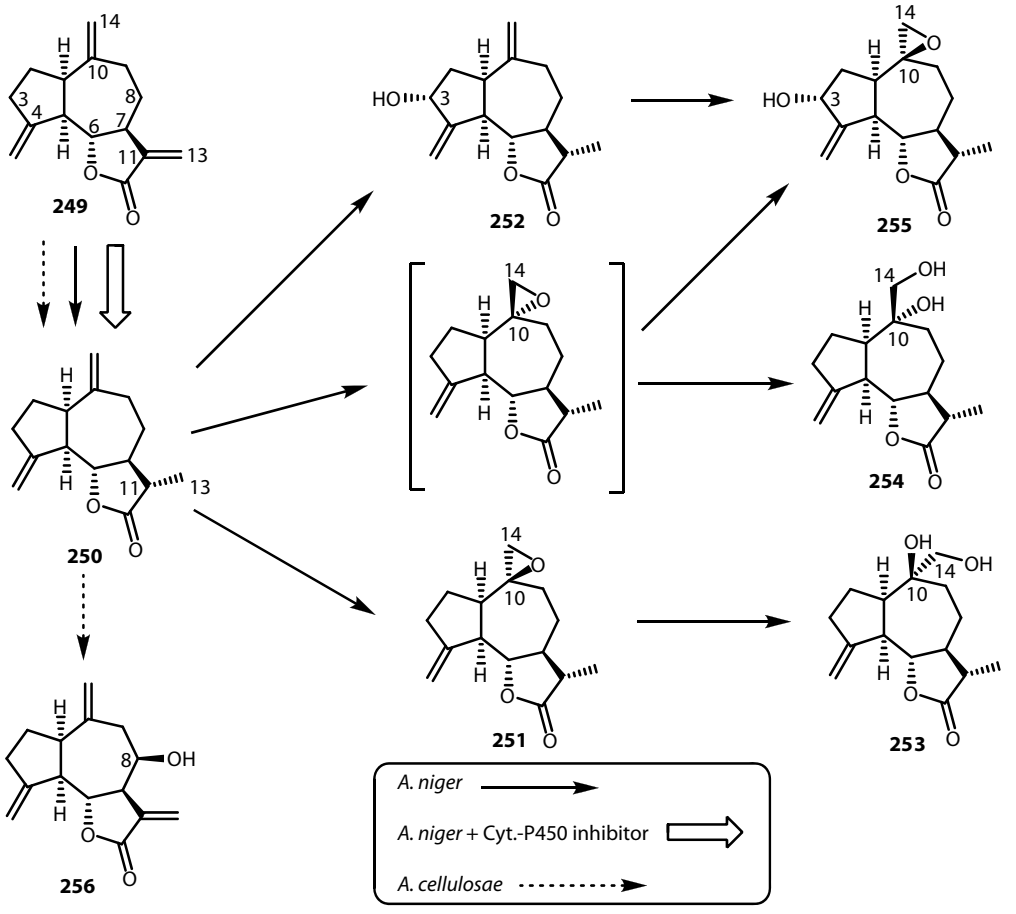


FIGURE 15.78 Possible pathway of biotransformation of (–)-dehydrocostuslactone (**249**) by *Aspergillus niger* and *Aspergillus cellulosae*.

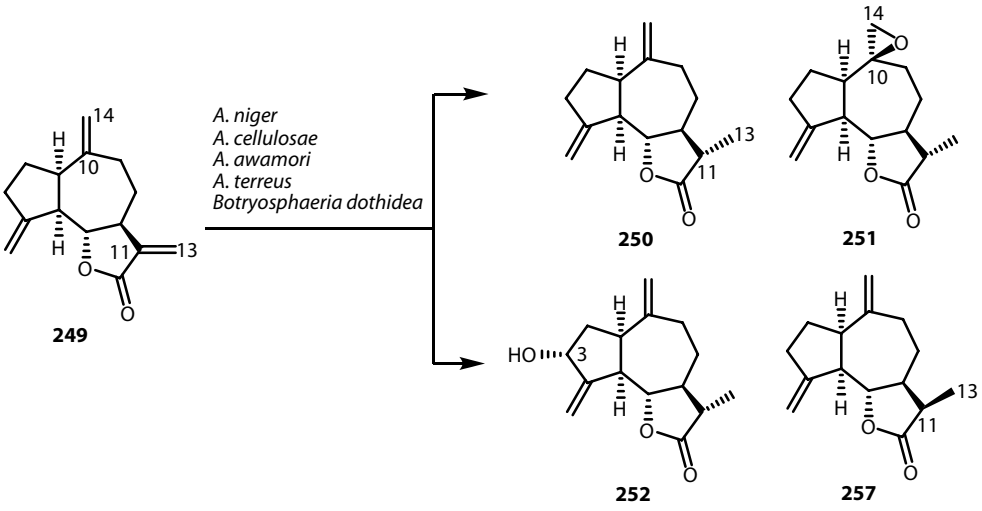


FIGURE 15.79 Biotransformation of (–)-dehydrocostuslactone (**249**) by *Aspergillus* species and *Botryosphaeria dothidea*.

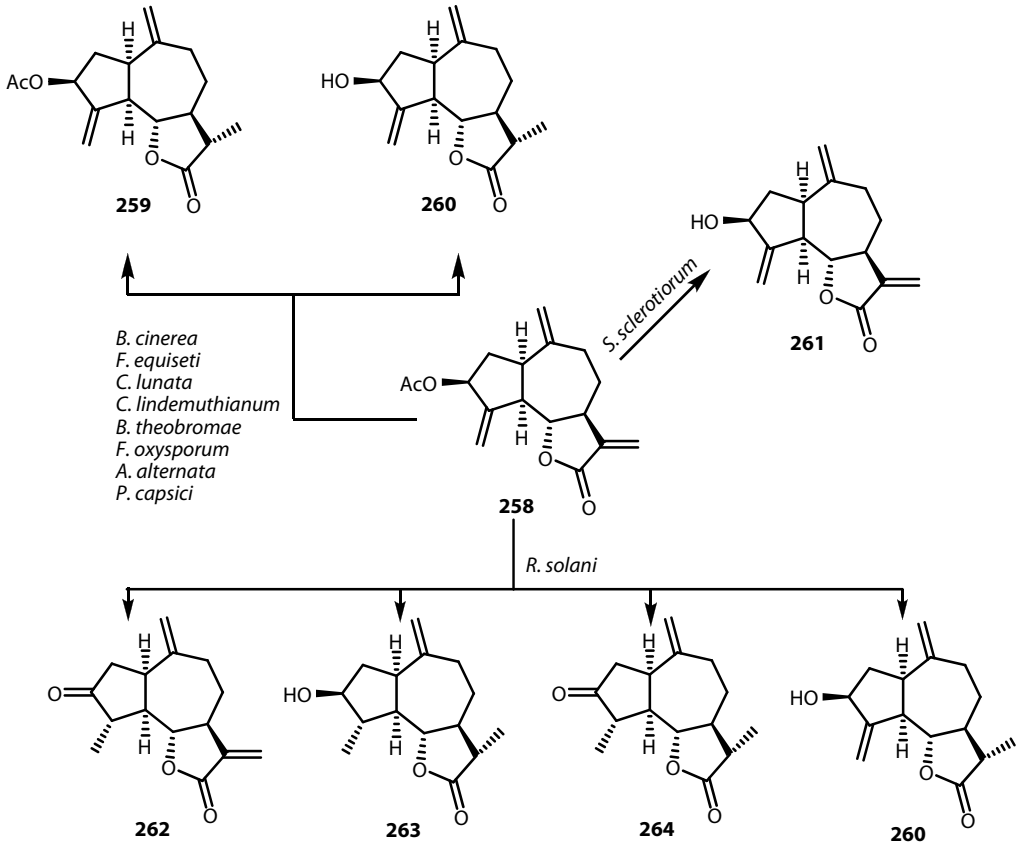


FIGURE 15.80 Biotransformation of zaluzanin D (**258**) by various fungi.

Incubation of parthenin (**264a**) with the fungus *Beauveria bassiana* in modified Richard's medium gave C11–C13 reduced product (**264b**) in 37% yield, while C11 α-hydroxylated product (**264c**) was obtained in 32% yield from the broth of the fungus *Sporotrichum pulverulentum* using the same medium (Bhutani and Thakur, 1991) (Figure 15.81).

Cadina-4,10(15)-dien-3-one (**265**) possessing insecticidal and ascaricidal activity, from Jamaican medicinal plant *Hyptis verticillata* was metabolized by *Curvularia lunat*a ATCC 12017 in potato dextrose to give its 12-hydoxy- (**266**), 3α-hydroxycadina-4,10(15)-dien (**267**), and 3α-hydroxy-4,5-dihydrocadinenes (**268**) while **265** was incubated by the same fungus in peptone, yeast, and beef extracts and glucose medium, only **267** and **268** were obtained. Compound **267** derived synthetically was treated in the same fungus *Curvularia lunata* to afford three metabolites (**269**–**271**) (Collins and Reese, 2002) (Figure 15.82).

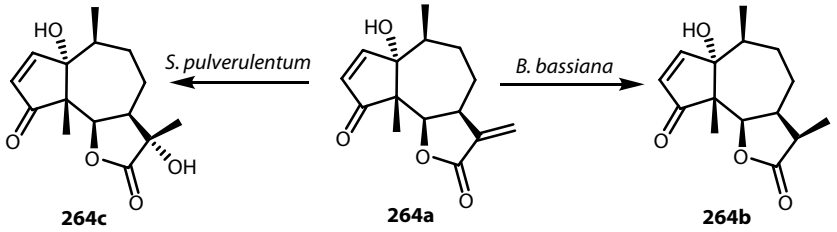


FIGURE 15.81 Biotransformation of parthenin (**264a**) by *Sporotrichum pulverulentum* and *Beauveria bassiana*.

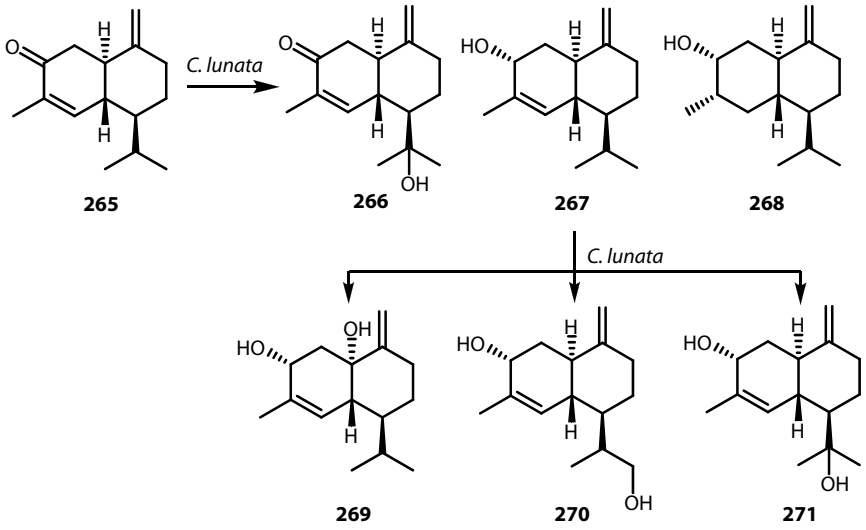


FIGURE 15.82 Biotransformation of cadina-4,10(15)-dien-3-one (**265**) by *Curvularia lunata*.

The incubation of the same substrate (**265**) in *Mucor plumbeus* ATCC 4740 in high iron-rich medium gave **270**, which was obtained from *Curvularia lunata* mentioned above, **268**, **272**, **273**, **277**, **278**, and **279**. In low iron medium, this fungus converted the same substrate **265** into three epoxides (**274**–**276**), a tetraol (**280**) with common metabolites (**268**, **273**, **277**, **278**), and **271**, which was the same metabolite used by *Curvularia lunata* (Collins, Reynold, and Reese, 2002). It is interesting to note that only epoxides were obtained from the substrate (**265**) by *Mucor* fungus in low iron medium (Figure 15.83).

The same substrate (**265**) was incubated with the deuteromycete fungus, *Beauveria bassiana,* which is responsible for the muscaridine disease in insects, in order to obtain new functionalized analogues with improved biological activity. From compound **265**, nine metabolites were obtained.

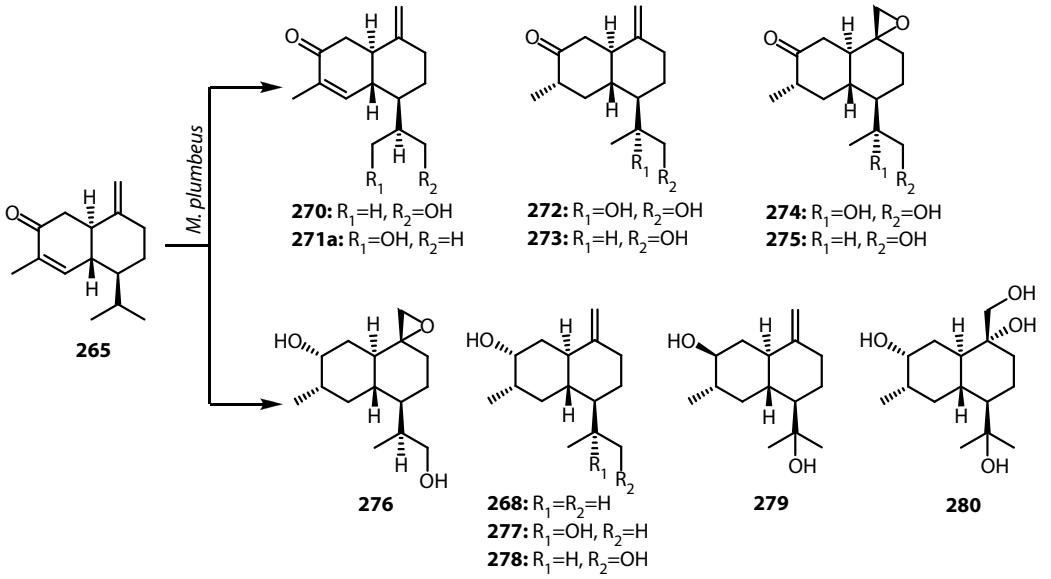


FIGURE 15.83 Biotransformation of cadina-4,10(15)-dien-3-one (**265**) by *Mucor plumbeus*.

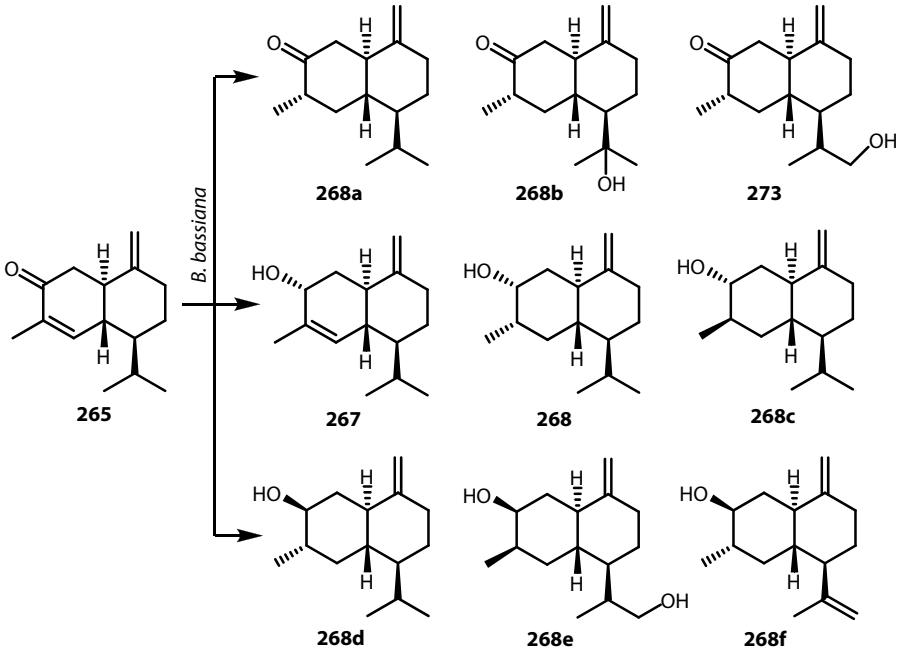


FIGURE 15.84 Biotransformation of cadina-4,10(15)-dien-3-one (**265**) by *Beauveria bassiana*.

The insecticidal potential of the metabolites (**267**, **268**, **268a**–**268f**) were evaluated against *Cylas formicarius*. The metabolites (**273**, **268**, **268d**) showed enhanced activity compared with the substrate (**265**). The plant growth regulatory activity of the metabolites against radish seeds was tested. All the compounds showed inhibitory activity; however, their activity was less than colchicine (Buchanan et al., 2000) (Figure 15.84).

Cadinane-type sesquiterpene alcohol (**281**) isolated from the liverwort *Mylia taylorii* gave a primary alcohol (**282**) by *Aspergillus niger* treatment (Morikawa et al., 2000) (Figure 15.85).

Fermentation of (−)-α-bisabolol (**282a**) possessing anti-inflammatory activity with plant pathogenic fungus *Glomerella cingulata* for 7 days yielded oxygenated products (**282b**–**282e**) of which compound **282e** was predominant. 3,4-Dihydroxy products (**282b**, **282d**, **282e**) could be formed by hydrolysis of the 3,4-epoxide from **282a** and **282c** (Miyazawa et al., 1995b) (Figure 15.86).

El Sayed et al. (2002) reported microbial and chemical transformation of (*S*)-(+)-curcuphenol (**282g**) and curcudiol (**282n**), isolated from the marine sponges, *Didiscus axeata*. Incubation of compound **282g** with *Kluyveromyces marxianus* var. *lactis* resulted in the isolation of six metabolites (3–8, **282h**–**282j**). The same substrate was incubated with *Aspergillus alliaceus* to give the metabolites (**282p**, **282q**, **282s**) (Figure 15.87).

Compounds **282g** and **282n** were treated in *Rhizopus arrhizus* and *Rhodotorula glutinus* for 6 and 8 days to afford glucosylated metabolites, 1α-D-glucosides (**282o**) and **282r**, respectively. The

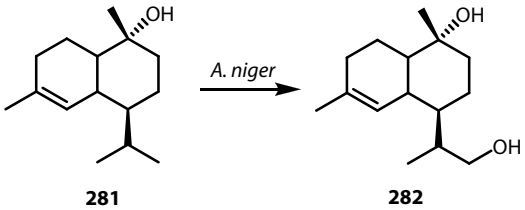


FIGURE 15.85 Biotransformation of cadinol (**281**) by *Aspergillus niger*.

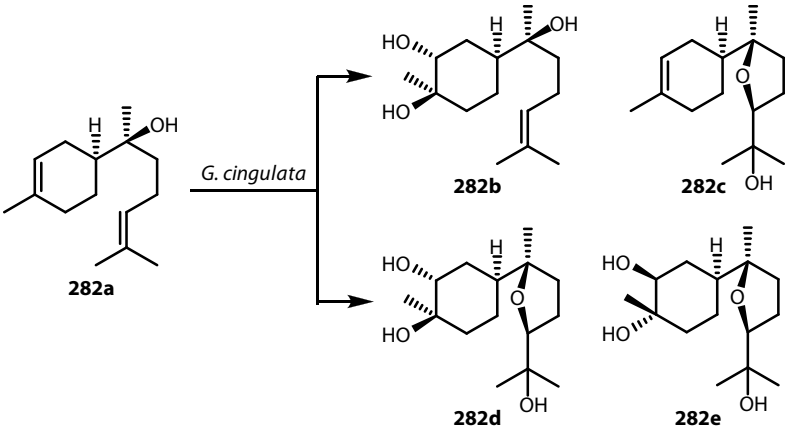


FIGURE 15.86 Biotransformation of β-bisabolol (**282a**) by *Glomerella cingulata*.

substrate itself showed antimicrobial activity against *Candia albicans, Cryptococcus neoformans*, and MRSA-resistant *Staphylococcus aureus* and *Staphylococcus aureus* with MIC and MFC/MBC ranges of 7.5–25 and 12.5–50 μg/mL, respectively. Compounds **282g** and **282h** also exhibited *in vitro* antimalarial activity against *Plasmodium falciparium* (D6 clone) and *Plasmodium falciparium* (W2 clone) of 3600 and 3800 ng/mL (selective index (S.I.) > 1.3), and 1800 (S.I. > 2.6), and 2900 (S.I. > 1.6), respectively (El Sayed et al., 2002) (Figure 15.87).

Artemisia annua is one of the most important Asteraceae species as antimalarial plant. There are many reports of microbial biotransformation of artemisinin (**283**), which is active antimalarial rearranged cadinane sesquiterpene endoperoxide, and its derivatives to give novel antimalarials with increased activities or differing pharmacological characteristics.

Lee et al. (1989) reported that deoxoartemisinin (**284**) and its 3α-hydroxy derivative (**285**) were obtained from the metabolites of artemisinin (**283**) incubated with *Nocardia corallina* and *Penicillium chrysogenum* (Figure 15.88).

Zhan et al. (2002) reported that incubation of artemisinin (**283**) with *Cunninghamella echinulata* and *Aspergillus niger* for 4 days at 28°C resulted in the isolation of two metabolites, 10β-hydroxyartemisinin (**287a**) and 3α-hydroxydeoxyartemisinin (**285**), respectively.

Compound **283** was also biotransformed by *Aspergillus niger* to give four metabolites, deoxyartemisinin (**284**, 38%), 3α-hydroxydeoxyartemisinin (**285**, 15%), and two minor products (**286**, 8% and **287**, 5%) (Hashimoto et al., 2003b).

Artemisinin (**283**) was also bioconverted by *Cunninghamella elegans*. During this process, 9β-hydroxyartemisinin (**287b**, 78.6%), 9β-hydroxy-8α-artemisinin (**287c**, 6.0%), 3α- hydroxydeoxoartemisinin (**285**, 5.4%), and 10β-hydroxyartemisinin (**287d**, 6.5%) have been formed. On the basis of quantitative structure-activity relationship (QSAR) and molecular modeling investigations, 9β-hydoxy derivatization of artemisinin skeleton may yield improvement in antimalarial activity and may potentially serve as an efficient means of increasing water solubility (Parshikov et al., 2004) (Figure 15.89).

Albicanal (**288**) and (–)-drimenol (**289**) are simple drimane sesquiterpenoids isolated from the liverwort, *Diplophyllum serrulatum*, and many other liverworts and higher plants. The latter compound was incubated with *Mucor plumbeus* and *Rhizopus arrhizus*. The former microorganism converted **289** to 6,7α-epoxy- (**290**), 3β-hydroxy- (**291**), and 6α-drimenol (**292**) in the yields of 2%, 7%, and 50%, respectively. On the other hand, the latter species produced only 3β-hydroxy derivative (**291**) in 60% yield (Aranda et al., 1992) (Figure 15.90).

(–)-Polygodial (**293**) possessing piscicidal, antimicrobial, and mosquito-repellant activity is the major pungent sesquitepene dial isolated from *Polygonum hydropiper* and the liverwort, *Porella vernicosa* complex. Polygodial was incubated with *Aspergillus niger*, however, because of its

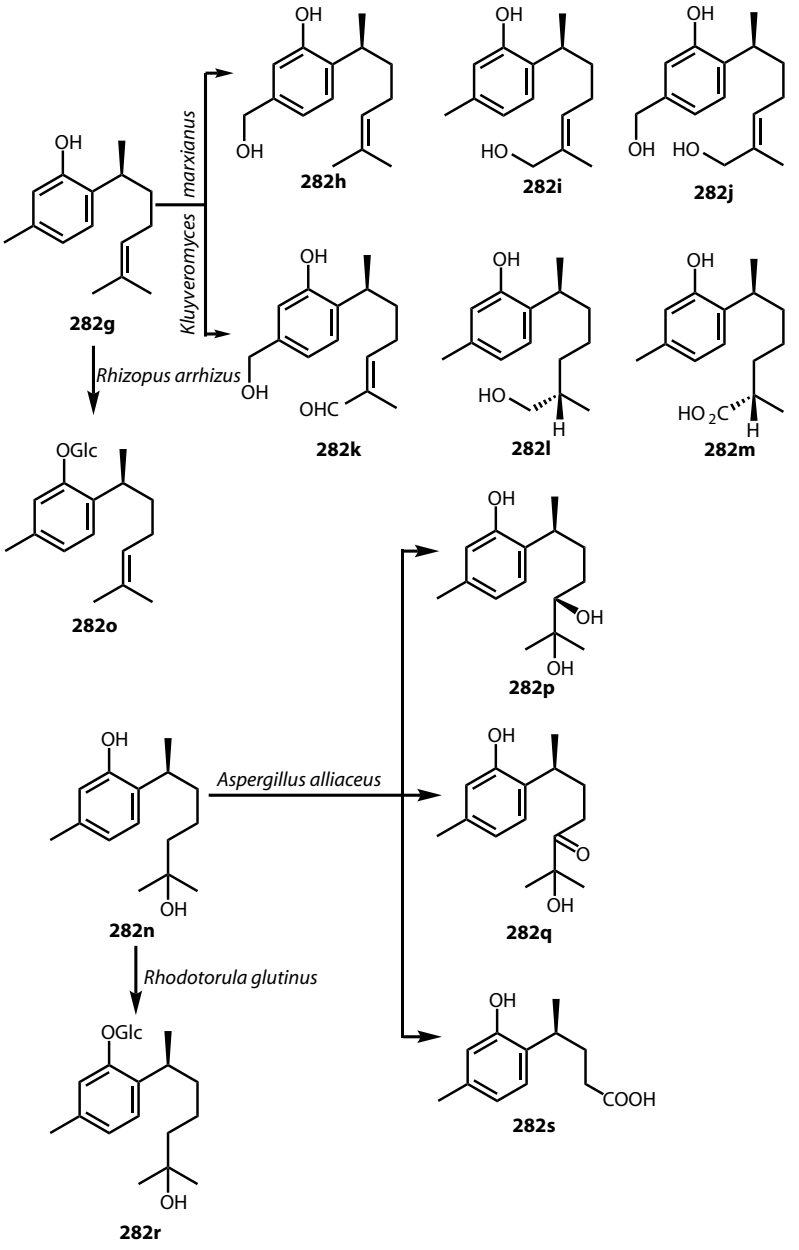


FIGURE 15.87 Biotransformation of (*S*)-(+)-curcuphenol (**282g**) by *Kluyveromyces marxianus* and *Rhizopus arrhizus* and curcudiol (**282n**) by *Aspergillus alliaceus* and *Rhodotorula glutinus*.

antimicrobial activity, nothing metabolite was obtained (Sekita et al., 2005). Polygodiol (**295**) prepared from polygodial (**293**) was also treated in the same manner as described above to afford 3β-hyrdoxy- (**297**), which was isolated from *Marasmius oreades* as antimicrobial activity (Ayer and Craw, 1989) and 6α-hydroxypolygodiol (**298**) in 66–70% and 5–10% yields, respectively (Aranda et al., 1992). The same metabolite (**297**) was also obtained from polygodiol (**295**) as a sole metabolite from the culture broth of *Aspergillus niger* in Czapek-peptone medium for 3 days in 70.5% yield (Sekita et al., 2005), while the C9 epimeric product (**296**) from isopolygodial (**294**) was incubated with *Mucor plumbeus* to afford 3β-hydroxy- (**299**) and 6α-hydroxy derivative (**300**) in low yields,

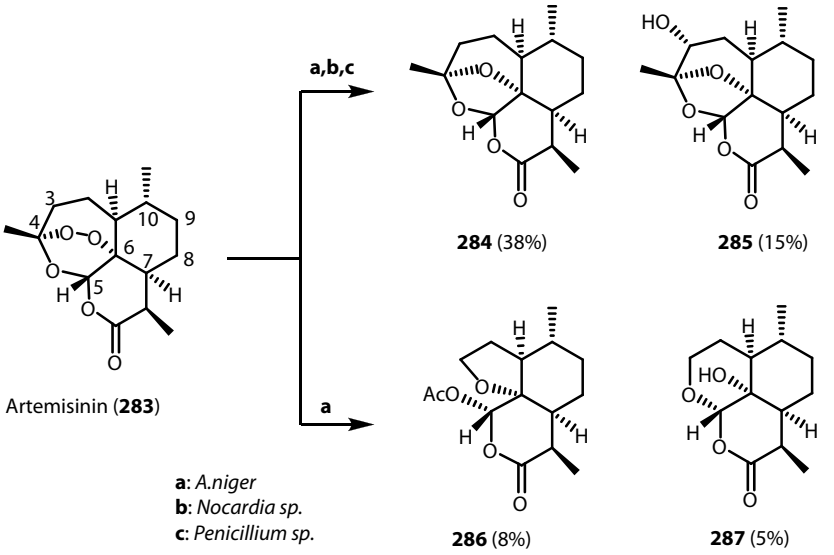


FIGURE 15.88 Biotransformation of artemisinin (**283**) by *Aspergillus niger*, *Nocardia corallina*, and *Penicillium chrysogenum*.

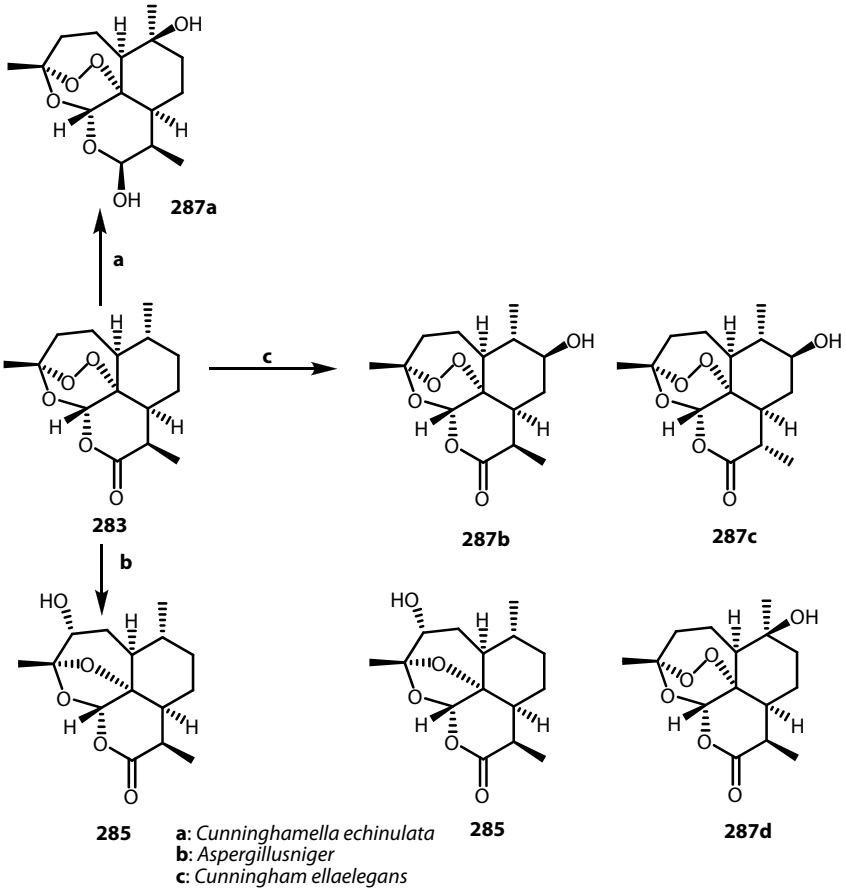


FIGURE 15.89 Biotransformation of artemisinin (**283**) by *Cunninghamella echinulata*, *Cunninghamella elegans*, and *Aspergillus niger*.

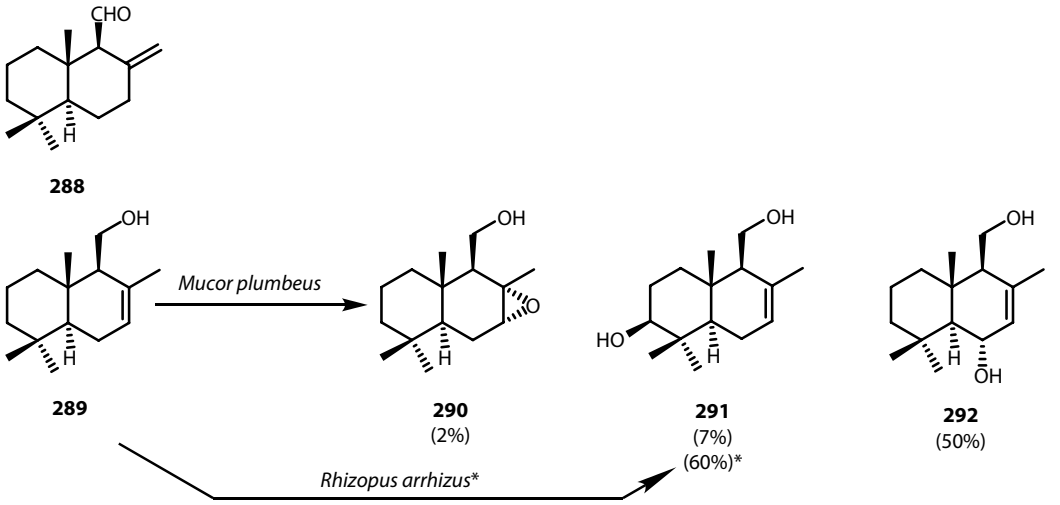


FIGURE 15.90 Biotransformation of drimenol (**289**) by *Mucor plumbeus* and *Rhizopus arrhizus*.

7% and 13% (Aranda et al., 1992). Drim-9α-hydroxy-11β,12-diacetoxy-7-ene (**301**) derived from polygodiol (**295**) was treated in the same manner as described above to yield its 3β-hydroxy derivative (**302**, 42%) (Sekita et al., 2005) (Figures 15.91 and 15.92).

Cinnamodial (**303**) from the Malagasy medicinal plant, *Cinnamosma fragrans*, was also treated in the same medium including *Aspergillus niger* to furnish three metabolites, respectively in very law yields (**304**, 2.2%; **305**, 0.05%; and **306**, 0.62%). Compound **305** and **306** are naturally occurring cinnamosmolide, possessing cytotoxicity and antimicrobial activity, and fragrolide. In this case, the introduction of 3β-hydroxy group was not observed (Sekita et al., 2006) (Figure 15.93).

Naturally occurring rare drimane sesquiterpenoids (**307–314**) were biosynthesized by the fungus *Cryptoporus volvatus* with isocitric acids. Among these compounds, in particular, cryptoporic acid E (**312**) possesses antitumor promoter, anticolon cancer, and very strong super oxide anion radical scavenging activities (Asakawa et al., 1992). When the fresh fungus allowed standing in moisture condition, olive fungus *Paecilomyces varioti* grows on the surface of the fruit body of this

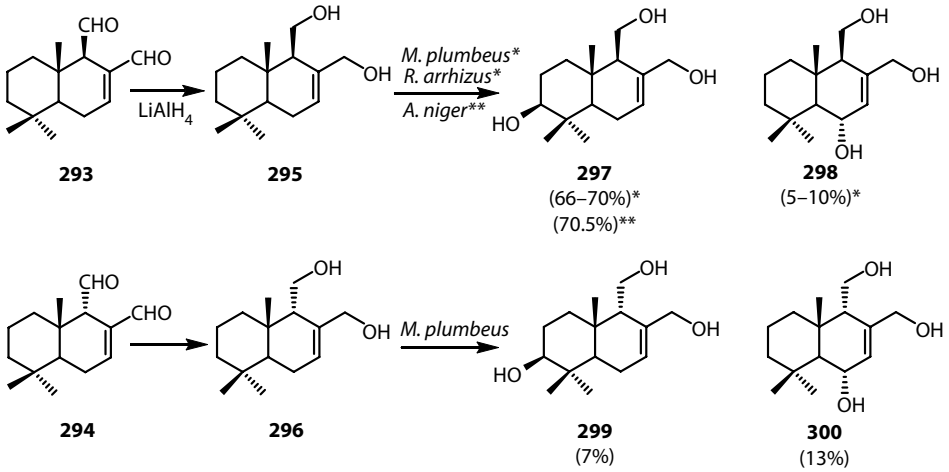
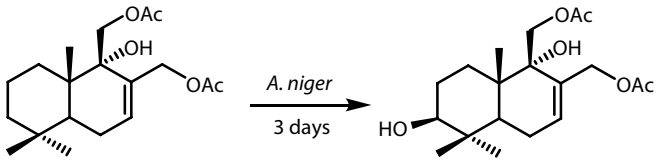


FIGURE 15.91 Biotransformation of polygodiol (**295**) by *Mucor plumbeus*, *Rhizopus arrhizus*, and *Aspergillus niger*.



301 **302** (47.2%)

FIGURE 15.92 Biotransformation of drim-9α-hydroxy-11,12-diacetoxy-7-ene (**301**) by *Aspergillus niger.*

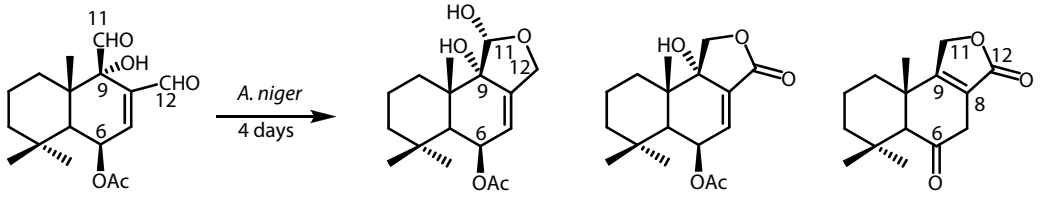
fungus. 2 kg of the fresh fungus was infected by *Cryptoporus volvatus* for 1 month, followed by the extraction of methanol to give the crude extract, then purification using silica gel and Sephadex LH-20 to give five metabolites (**316**, **318**–**321**), which were not found in the fresh fungus (Takahashi et al., 1993a). Compound **318** was also isolated from the liverworts, *Bazzania* and *Diplophyllum* species (Asakawa, 1982, 1995) (Figure 15.94).

Liverworts produce a large number of enantiomeric mono-, sesqui-, and diterpenoids to those found in higher plants and lipophilic aromatic compounds. It is also noteworthy that some liverworts produce both normal and its enantiomers. The more interesting phenomenon in the chemistry of liverworts is that the different species in the same genus, for example, *Frullania tamarisci* subsp. *tamarisci* and *Frullania dilatata* produce totally enantiomeric terpenoids. Various sesqui- and diterpenoids, bibenzyls, and bisbibenzyls isolated from several liverworts show characteristic fragrant odor, intensely hot and bitter taste, muscle relaxing, antimicrobial, antifungal, allergenic contact dermatitis, antitumor, insect antifeedant, superoxide anion release inhibitory, piscicidal, and neurotrophic activity (Asakawa, 1982, 1990, 1995, 1999, 2007, 2008; Asakawa and Ludwiczuk, 2008). In order to obtain the different kind of biologically active products and to compare the metabolites of both normal and enantiomers of terpenoids, several secondary metabolites of specific liverworts were biotransformed by *Penicillium sclerotiorum*, *Aspergillus niger*, and *Aspergillus cellulosae.*

(−)-Cuparene (**322**) and (−)-2-hydroxycuparene (**323**) have been isolated from the liverworts, *Bazzania pompeana* and *Marchantia polymorpha*, while its enantiomer (+)-cuparene (**324**) and (+)-2-hydroxycuparene (**325**) from the higher plant, *Biota orientalis* and the liverwort *Jungermannia rosulans.* (*R*)-(−)-α-Cuparenone (**326**) and grimaldone (**327**) demonstrate intense flagrance. In order to obtain such compounds from both cuparene and its hydroxy compounds, both enatiomers mentioned above were cultivated with *Aspergillus niger* (Hashimoto et al., 2001a) (Figure 15.95).

From (−)-cuparene (**322**), five metabolites (**328**–**332**) all of which contained cyclopentanediols or hydroxycyclopentanones were obtained. An aryl methyl group was also oxidized to give primary alcohol, which was further oxidized to afford carboxylic acids (**329**–**331**) (Hashimoto et al., 2001a) (Figure 15.96).

From (+)-cuparene, six metabolites (**333**–**338**) were obtained. These are structurally very similar to those found in the metabolites of (−)-cuparene, except for the presence of an acetonide (**336**), but they are not identical. All metabolites possess benzoic acid moiety.



Cinnamodial (**303**) **304** (2.2%) **305** (0.05%) Cinnamosmolide **306** (0.20%) Fragrolide

FIGURE 15.93 Biotransformation of cinnamodial (**303**) by *Aspergillus niger.*

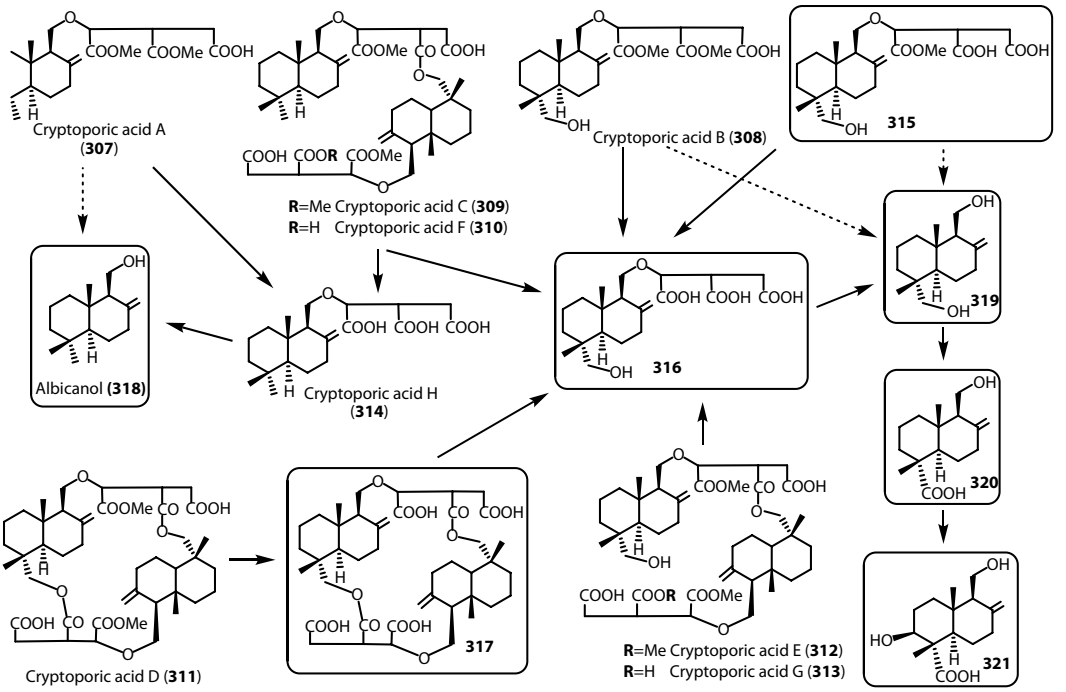


FIGURE 15.94 Biotransformation of cryptoporic acids (**307**–**317**, **316**) by *Paecilomyces varioti.*

The possible biogenetic pathways of (+)-cuparene (**324**) has been proposed in Figure 15.97. Unfortunately, none of the metabolites show strong mossy odor (Hashimoto et al., 2006). The presence of an acetonide in the metabolites has also been seen in those of dehydronootkatone (**25**) (Furusawa et al., 2003) (Figure 15.98).

The liverwort *Herbertus adancus*, *Herbertus sakuraii*, and *Mastigophora diclados* produce (−)-herbertene, the C3 methyl isomer of cuparene, with its hydroxy derivatives, for example, herbertanediol (**339**), which shows NO production inhibitory activity (Harinantenaina et al., 2007) and herbertenol (**342**). Treatment of compound (**339**) in *Penicillium sclerotiorum* in Czapek-polypeptone medium gave two dimeric products, mastigophorene A (**340**) and mastigophorene B (**341**), which showed neurotrophic activity (Harinantenaina et al., 2005).

When (−)-herbertenol (**342**) was biotransformed for 1 week by the same fungus, no metabolic product was obtained; however, five oxygenated metabolites (**344**–**348**) were obtained from its methyl ether (**343**). The possible metabolic pathway is shown in Figure 15.99. Except for the presence of the ether (**348**), the metabolites from **342** resemble those found in (−)- and (+)-cuparene (Hashimoto et al., 2006) (Figures 15.100 and 15.101).

Maalioxide (**349**), mp 65–66°, $[\alpha]_D^{21}$–34.4°, obtained from the liverwort, *Plagiochila sciophila* was inoculated and cultivated rotatory (100 rpm) in Czapek-peptone medium (pH 7.0) at 30°C for 2 days. (−)-Maalioxide (**349**) (100 mg/200 mL) was added to the medium and further cultivated for 2 days to afford three metabolites, 1β-hydroxy-(**350**), 1β,9β-dihydroxy- (**351**), and 1β,12-dihydroxymaalioxides (**352**), of which **351** was predominant (53.6%). When the same substrate was cultured with *Aspergillus cellulosae* in the same medium for 9 days, 7β-hydroxymaalioxide (**353**) was obtained as a sole product in 30% yield. The same substrate (**349**) was also incubated with the fungus *Mucor plumbeus* to obtain a new metabolite, 9β-hydroxymaalioxide (**354**), together with two known hydroxylated products (**350**, **353**) (Wang et al., 2006).

Maalioxide (**349**) was oxidized by *m*-chloroperbenzoic acid to give a very small amount of **353** (1.2%), together with 2α-hydroxy- (**355**, 2%) and 8α-hydroxymaalioxide (**356**, 1.5%), which

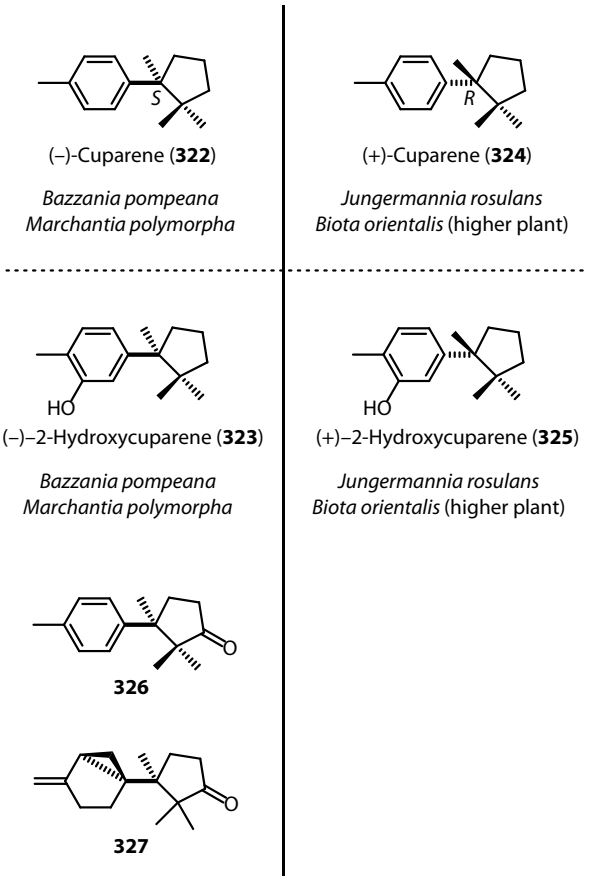


FIGURE 15.95 Naturally occurring cuparene sesquiterpenoids (**322**–**327**).

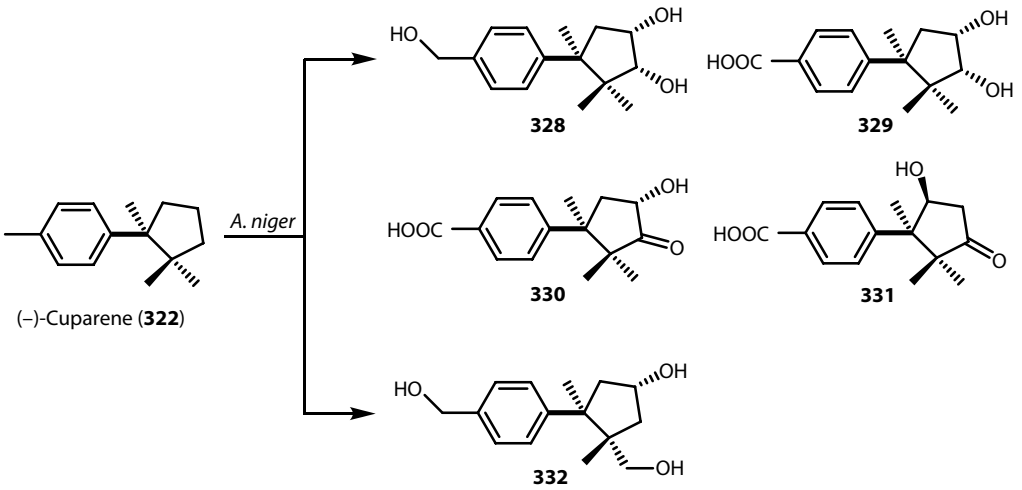


FIGURE 15.96 Biotransformation of (–)-cuparene (**322**) by *Aspergillus niger.*

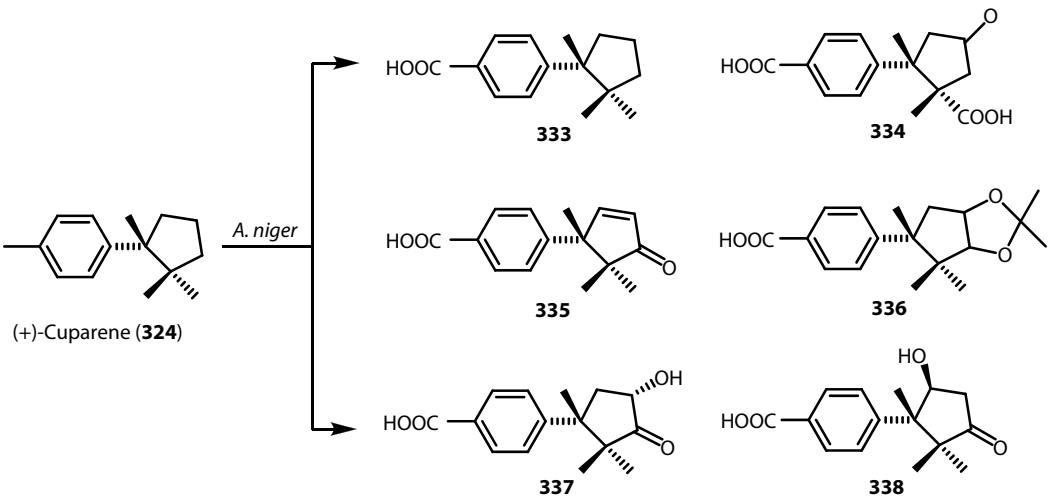


FIGURE 15.97 Biotransformation of (+)-cuparene (**324**) by *Aspergillus niger.*

have not been obtained in the metabolite of **349** in *Aspergillus niger* and *Aspergillus cellulosae* (Tori et al., 1990) (Figure 15.102).

Plagiochila sciophila is one of the most important liverworts, since it produces bicyclohumulenone (**357**), which possesses strong mossy note and is expected to manufacture compounding perfume. In order to obtain much more strong scent, **357** was treated in *Aspergillus niger* for 4 days to give 4α,10β-dihydroxybicyclohumulenone (**358**, 27.4%) and bicyclohumurenone-12-oic acid (**359**). An epoxide (**360**) prepared by *m*-chloroperbenzoic acid was further treated in the same fungus as described above to give 10β-hydoxy derivative (**361**, 23.4%). Unfortunately, these metabolites possess only faint mossy odor (Hashimoto et al., 2003c) (Figure 15.103).

The liverwort *Reboulia hemisphaerica* biosynthesizes cyclomyltaylanoids like **362** and also *ent*-1α-hydroxy-β-chamigrene (**367**). Biotransformation of cyclomyltaylan-5-ol (**362**) in the same

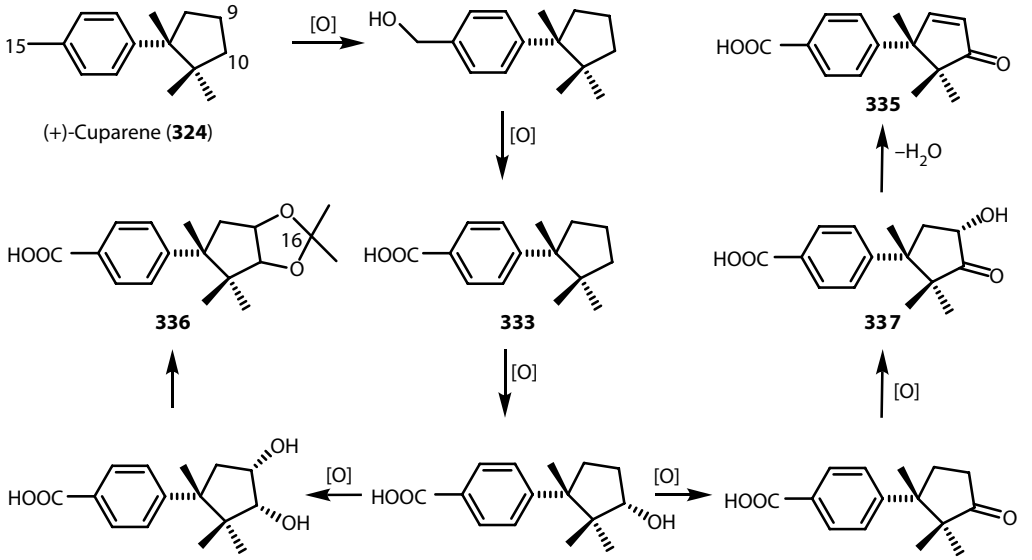


FIGURE 15.98 Possible pathway of biotransformation of (+)-cuparene (**324**) by *Aspergillus niger.*

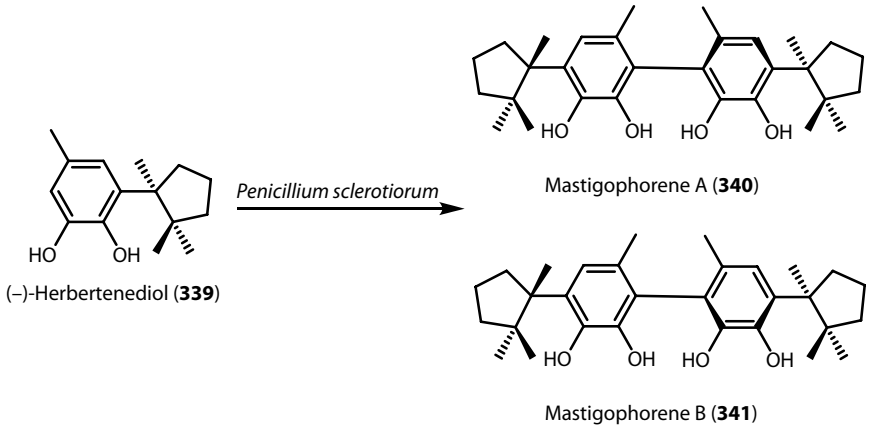


FIGURE 15.99 Biotransformation of (–)-herbertenediol (**339**) by *Penicillium sclerotiorum*.

medium including *Aspergillus niger* gave four metabolites, 9β-hydroxy- (**363**, 27%), 9β,15-dihydroxy- (**364**, 1.7%), 10β-hydroxy- (**365**, 10.3%), and 9β,15-dihydroxy derivative (**366**, 12.6%). In this case, the stereospecificity of alcohol was observed, but the regiospecificity of alcohol moiety was not seen in this substrate (Furusawa et al., 2005b, 2006b) (Figure 15.104).

The biotransformation of spirostructural terpenoids was not carried out. *Ent*-1α-hydroxy-β-chamigrene (**367**) was inoculated in the same manner as described above to give three new metabolites (**368**–**370**), of which **370** was the major product (46.2% in isolated yield). The hydroxylation of vinyl methyl group has been known to be very common in the case of microbial and mammalian biotransformation (Furusawa et al., 2005, 2006) (Figure 15.105).

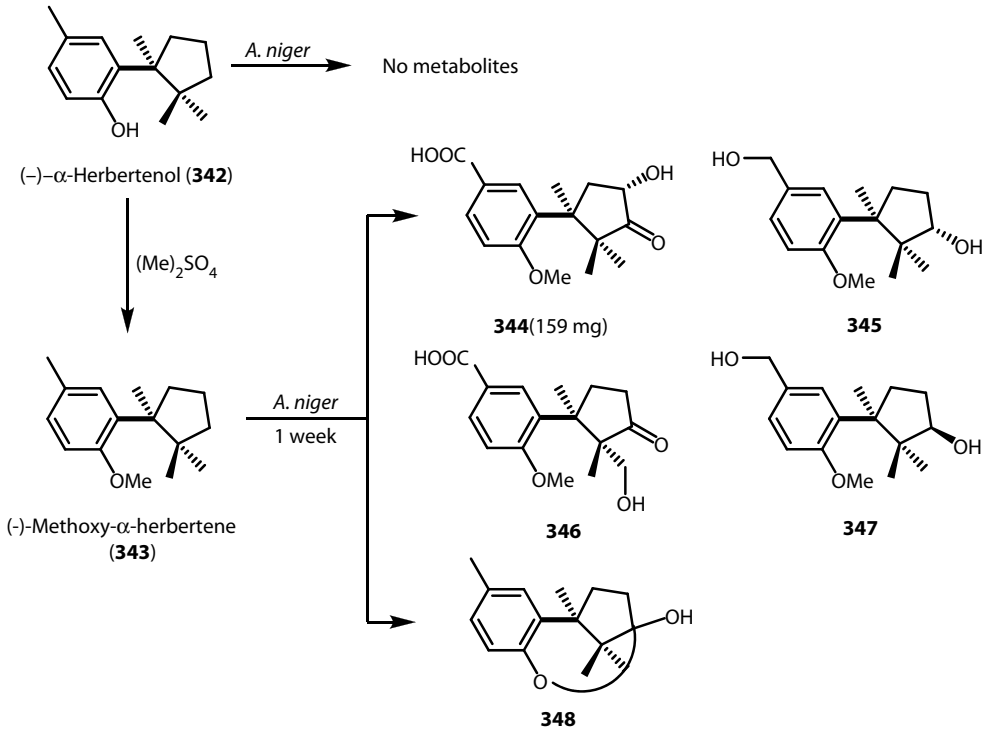


FIGURE 15.100 Biotransformation of (–)-methoxy-α-herbertene (**343**) by *Aspergillus niger*.

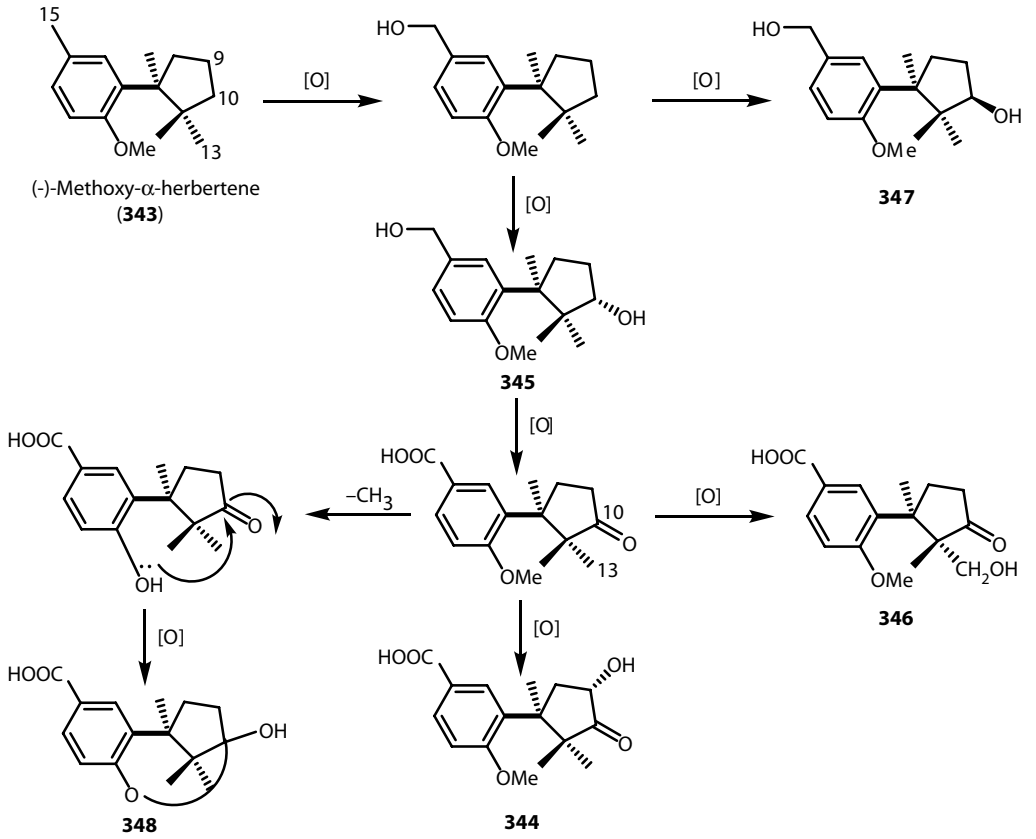


FIGURE 15.101 Possible pathway of biotransformation of (–)-methoxy-α-herbertene (**343**) by *Aspergillus niger.*

β-Barbatene (= gymnomitrene) (**4**), a ubiquitous sesquiterpene hydrocarbon, from liverwort like *Plagiochila sciophila* and many others. Jungermanniales liverworts was treated in the same manner using *Aspergillus niger* for 1 day gave a triol, 4β,9β,10β-trihydoxy-β-barbatene (**27**, 8%) (Hashimoto et al., 2003c).

Pinguisane sesquiterpenoids have been isolated from the Jungermanniales, Metzgeriales, and Marchantiales. In particular, the Lejeuneaceae and Porellaceae are rich sources of this unique type of sesquiterpenoids. One of the major furanosesquitepene (**373**) was biodegradated by *Aspergillus niger* to afford primary alcohol (**375**), which might be formed from **374** as shown in Figure 15.106 (Lahlou et al., 2000) (Figure 15.107).

In order to obtain more pharmacologically active compounds, the secondary metabolites from crude drugs and animals, for example, nardosinone (**376**) isolated from the crude drug, *Nardostachys chinensis*, which has been used for headache, stomachache, and diuresis possesses antimalarial activity. Hinesol (**384**), possessing spasmolytic activity, obtained from *Atractylodis lanceae* rhizoma, animal perfume (–)-ambrox (**391**) from ambergris were biotransformed by *Aspergillus niger, Aspergillus cellulosae*, *Botryosphaeria dothidea*, and so on.

Nardosinone (**376**) was incubated in the same medium including *Aspergillus niger* as described above for 1 day to give six metabolites (**377**, 45%; **378**, 3%; **379**, 2%; **380**, 5%; **381**, 6%; and **382**, 3%). Compounds **380**–**382** are unique trinorsesquiterpenoids although their yields are very poor. Compound **380** might be formed by the similar manner to that of phenol from cumene (**383**) (Figure 15.108) (Hashimoto et al., 2003b) (Figure 15.109).

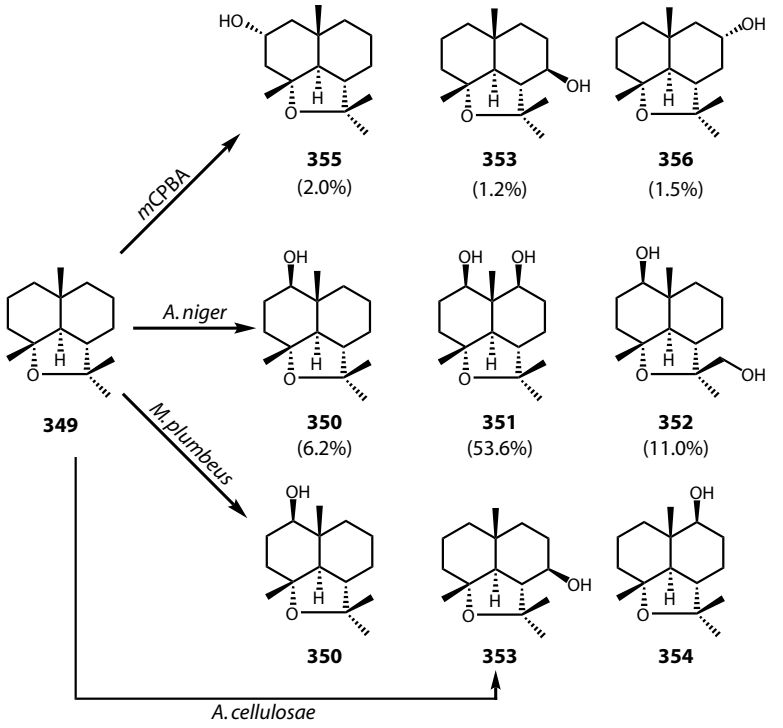


FIGURE 15.102 Biotransformation of maalioxide (**349**) by *Aspergillus niger*, *Aspergillus cellulosae*, and *Mucor plumbeus*.

From hinesol (**384**), two allylic alcohols (**386**, **387)** and their oxygenated derivative (**385**), and three unique metabolites (**388**–**390**) having oxirane ring were obtained. The metabolic pathway is very similar to that of oral administration of hinesol since the same metabolites (**395**–**387**) were obtained from the urine of rabbits (Hashimoto et al., 1998, 1999b, 2001) (Figure 15.110).

To obtain a large amount of ambrox (**391**), a deterrence, labda-12,14-dien-7α,8-diol obtained from the liverwort, *Porella pettottetiana* as a major component, was chemically converted into

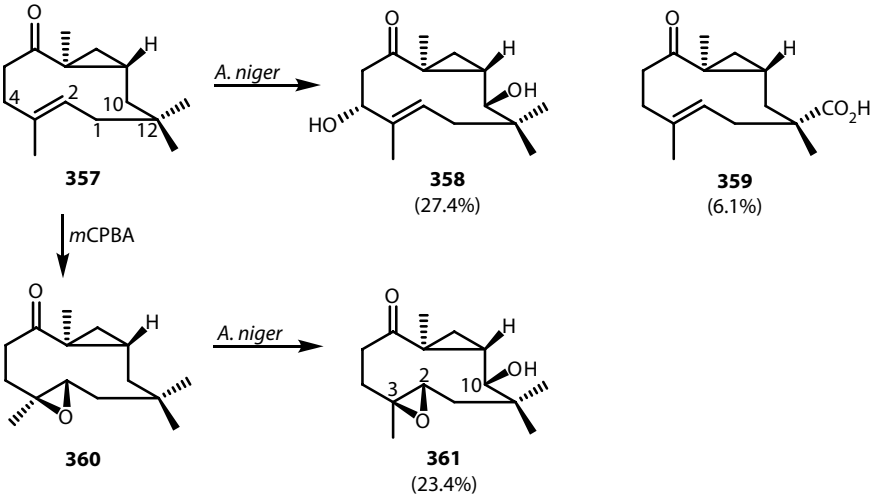


FIGURE 15.103 Biotransformation of bicyclohumulenone (**357**) by *Aspergillus niger*.

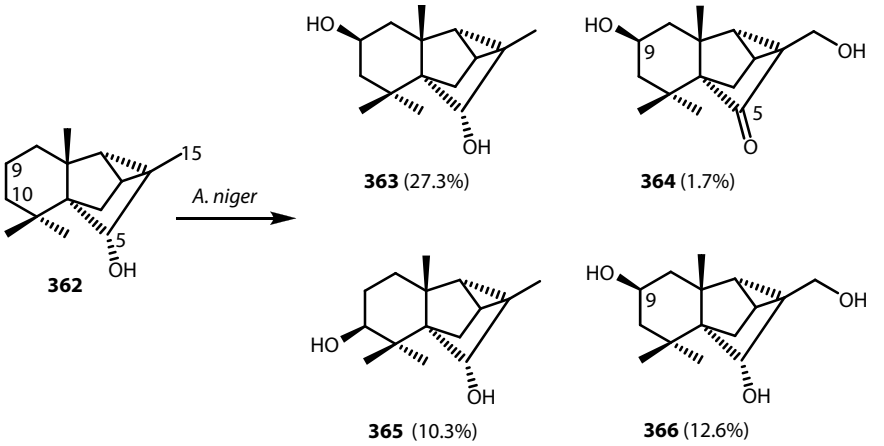


FIGURE 15.104 Biotransformation of cyclomyltaylan-5-ol (**362**) by *Aspergillus niger*.

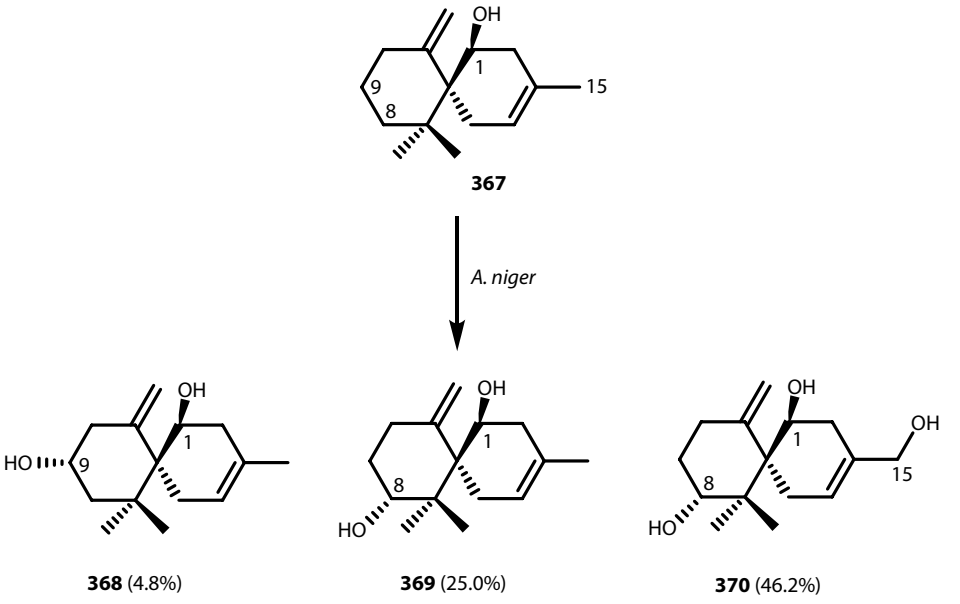


FIGURE 15.105 Biotransformation of *ent*-1α-hydroxy-β-chamigrene (**367**) by *Aspergillus niger*.

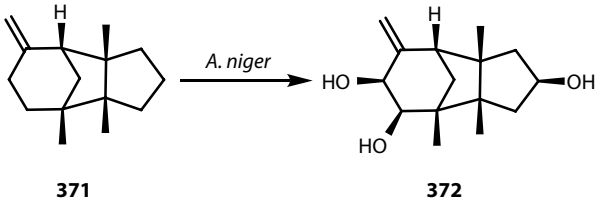


FIGURE 15.106 Biotransformation of β-barbatene (**371**) by *Aspergillus niger*.

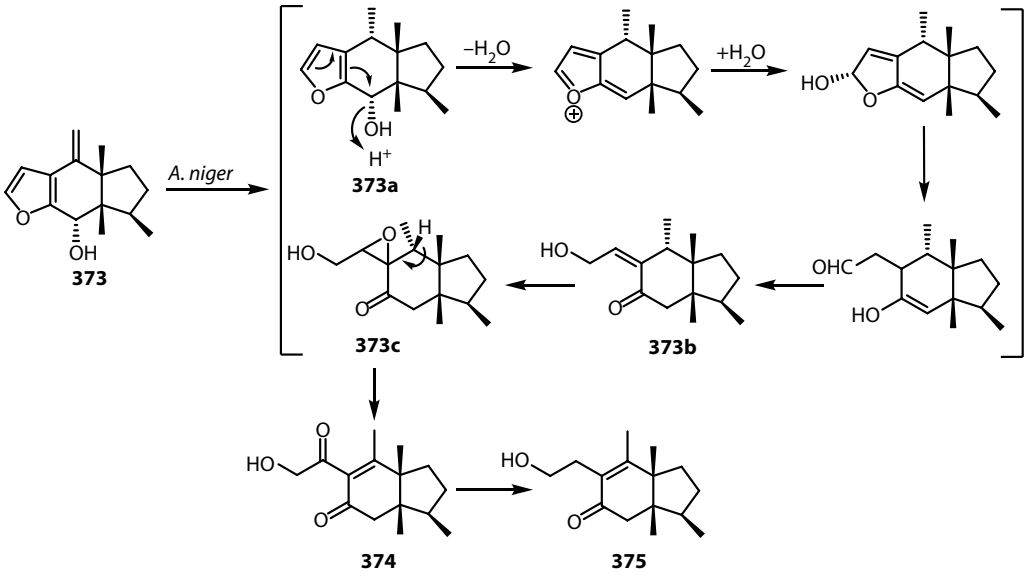


FIGURE 15.107 Biotransformation of pinguisanol (**373**) by *Aspergillus niger.*

(–)-ambrox via six steps in relatively high yield (Hashimoto et al., 1998a). Ambrox was added to Czapek-peptone medium including *Aspergillus niger*, for 4 days, followed by chromatography of the crude extract to afford four oxygenated products (**392**–**395**), among which the carboxylic acid (**393**, 52.4%) is the major product (Hashimoto et al., 2001) (Figure 15.111).

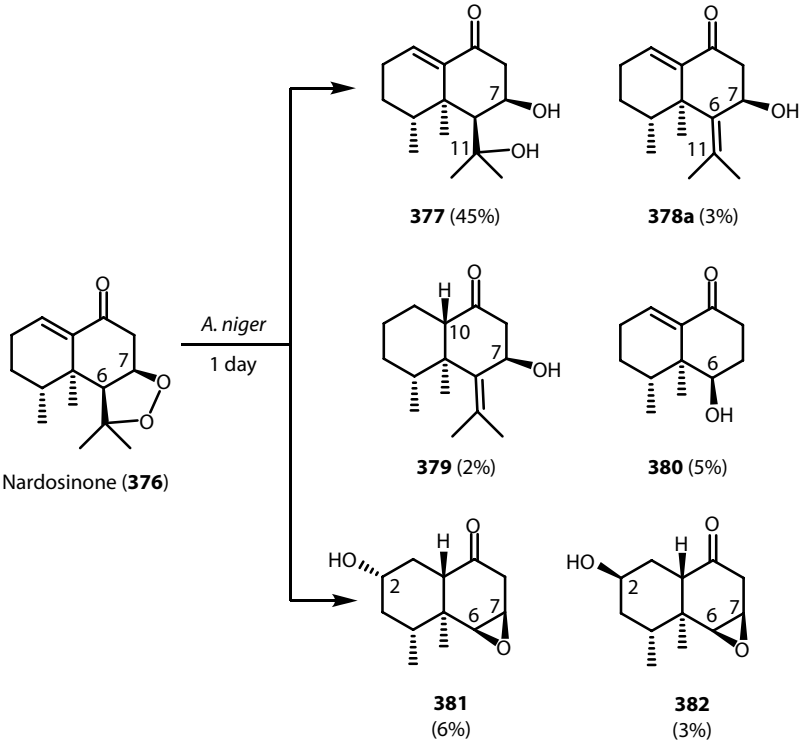


FIGURE 15.108 Biotransformation of nardosinone (**376**) by *Aspergillus niger.*

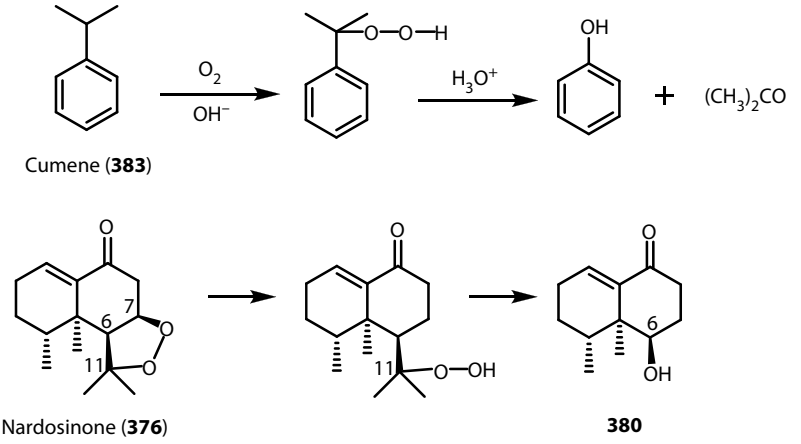


FIGURE 15.109 Possible pathway of biotransformation of nardosinone (**376**) to trinornardosinone (**380**) by *Aspergillus niger.*

When ambrox (**391**) was biotransformed by *Aspergillus niger* for 9 days in the presence of 1-aminobenzotriazole, an inhibitor of CYP450, compounds **396** and **397** were obtained instead of the metabolites (**392**–**395**), which were obtained by incubation of ambrox in the absence of the inhibitor. Ambrox was cultivated by *Aspergillus cellulosae* for 4 days in the same medium to afford

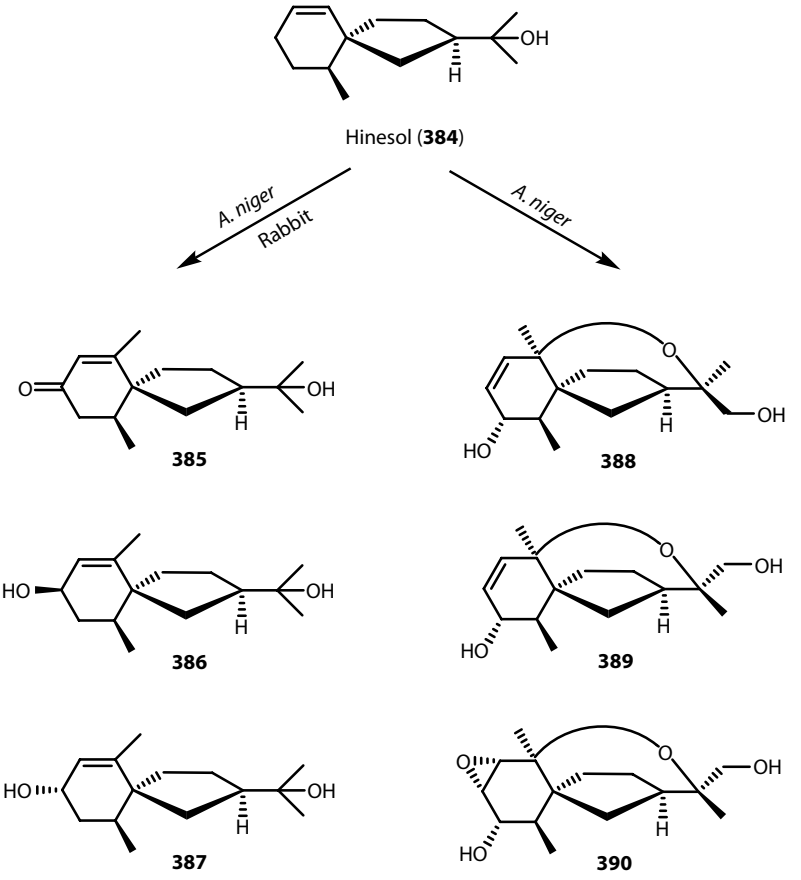


FIGURE 15.110 Biotransformation of hinesol (**384**) by *Aspergillus niger.*

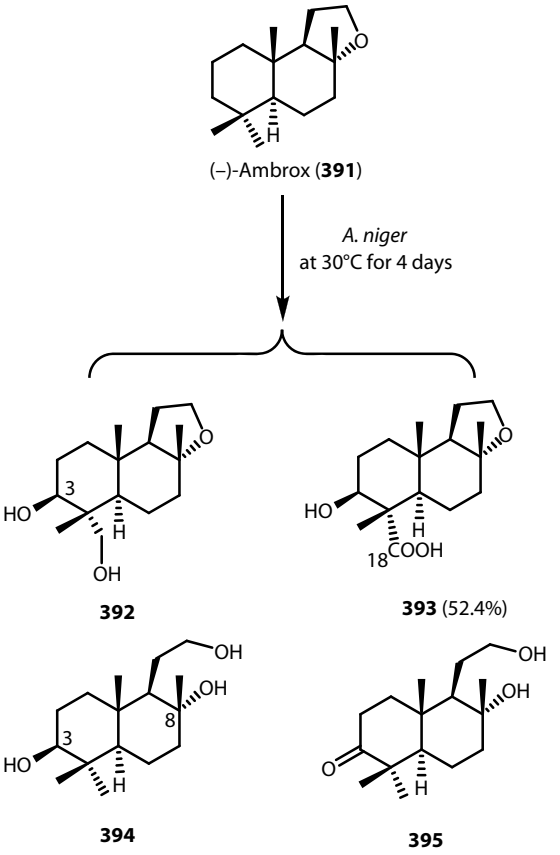


FIGURE 15.111 Biotransformation of (–)-ambrox (**391**) by *Aspergillus niger.*

C1 oxygenated products (**398** and **399**), the former of which was the major product (41.3%) (Hashimoto et al., 2001) (Figure 15.112).

The metabolite pathways of ambrox are quite different between *Aspergillus niger* and *Aspergillus cellulosae*. Oxidation at C1 occurred in *Aspergillus cellulosae* to afford **398** and **399**, which was also afforded by John's oxidation of **398**, while oxidation at C3 and C18 and ether cleavage between C8 and C12 occurred in *Aspergillus niger* to give **392**–**395**. Ether cleavage seen in *Aspergillus niger* is very rare.

Fragrance of the metabolites (**392**–**395**) and 7α-hydroxy-(–)-ambrox (**400**) and 7-oxo-(–)-ambrox (**401**) obtained from labdane diterpene diol were estimated. Only **399** demonstrated a similar odor to ambrox (**391**) (Hashimoto et al., 2001) (Figure 15.113).

(–)-Ambrox (**391**) was also microbiotransformed with *Fusarium* lini to give mono-, di-, and trihydroxylated metabolites (**401a**–**401d**), while incubation of the same substrate with *Rhizopus stolonifera* afforded two metabolites (**394**, **396**), which were obtained from **391** by *Aspergillus niger* as mentioned above, together with **397** and **401e** (Choudhary et al., 2004) (Figure 15.114).

The sclareolide (**402**) which is C12 oxo derivative of ambrox was incubated with *Mucor plumbeus* to afford three metabolites 3β-hydroxy- (**403**, 7.9%), 1β-hydroxy- (**404**, 2.5%), and 3-ketosclareolide (**405**, 7.9%) (Aranda et al., 1991) (Figure 15.115).

Aspergillus niger in the same medium as mentioned above converted sclareolide (**402**) into two new metabolites (**406**, **407**), together with known compounds (**403**, **405**), of which 3β-hydroxysclareolide (**403**) is preferentially obtained (Hashimoto et al., 2007) (Figure 15.116).

From the metabolites of sclareolide (**402**) incubated with *Curvularia lunata* and *Aspergillus niger*, five oxidized compounds, (**403**, **404**, **405**, **405a**, **405b**) were obtained. Fermentation of **402** with *Gibberella fujikuroii* afforded (**403**, **404**, **405**, **405a**). The metabolites, **403** and **405a** were

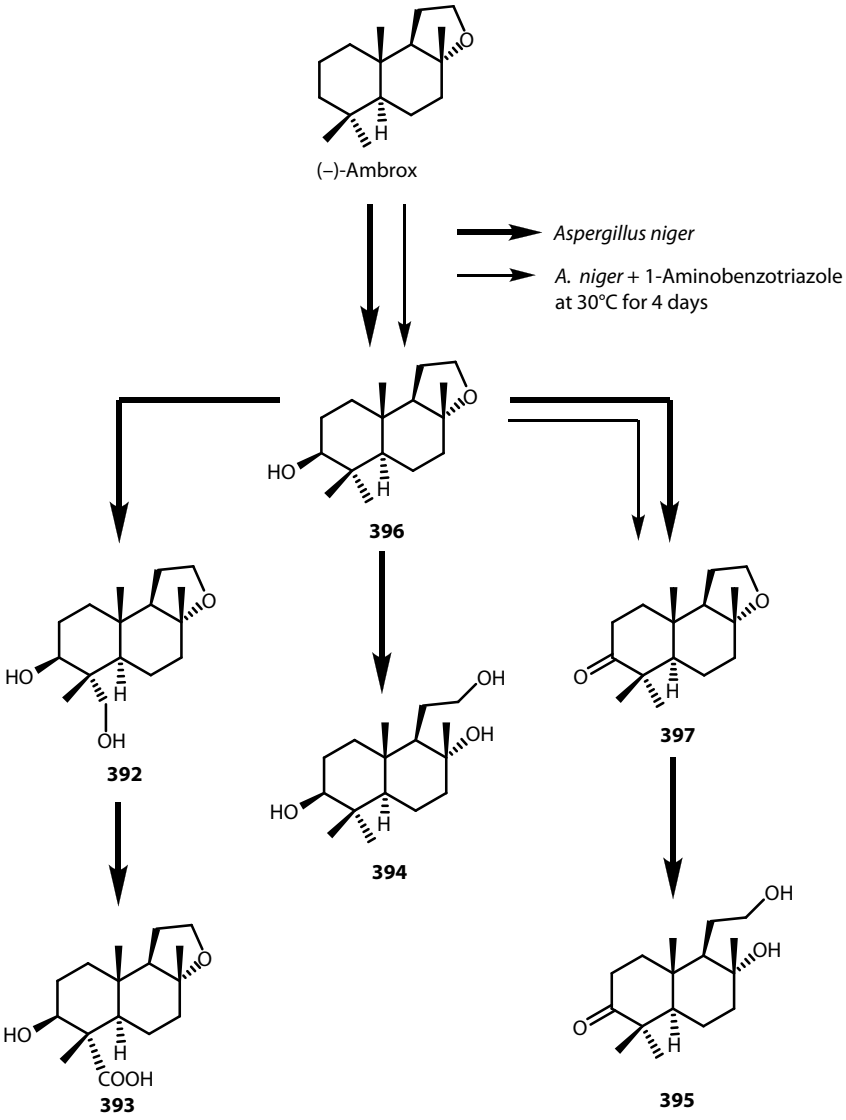


FIGURE 15.112 Possible pathway of biotransformation of (–)-ambrox (**391**) by *Aspergillus niger.*

formed from the same substrate by the incubation of *Fusarium lini*. No microbial transformation of **402** was observed with *Pleurotus ostreatus* (Atta-ur Rahman et al., 1997) (Figure 15.117).

Compound **391** treated in *Curvularia lunata* gave metabolites **401e** and **396**, while *Cunninghamella elegans* yielded compounds **401e** and **396** and (+)-sclareolide (**402**) (Figure 15.113). The metabolites (**401a**–**401e**, **396**) from **391** do not release any effective aroma when compared to **391.** Compound **394** showed a strong sweet odor quite different from the amber-like odor (Choudhary et al., 2004).

Sclareolide (**402**) exhibited phytotoxic and cytotoxic activity against several human cancer cell lines. *Cunninghamella elegans* gave new oxidized metabolites (**403**, **404**, **405a**, **405c**, **405d**, **405e**), resulting from the enantioselective hydroxylation. Metabolites **403**, **404**, and **405a** have been known as earlier as biotransformed products of **402** by many different fungi and have shown cytotoxicity against various human cancer cell lines. The metabolites (**403**, **404**, and **405a**) indicated significant phytotoxicity at higher dose against *Lemna minior* L. (Choudhary et al., 2004) (Figure 15.117).

Ambrox (**391**) and sclareolide (**402**) were incubated with the fungus *Cephalosporium aphidicola* for 10 days in shake culture to give 3β-hydroxy- (**396**), 3β,6β-dihydroxy- (**401g**), 3β,12-dihydroxy- (**401h**),

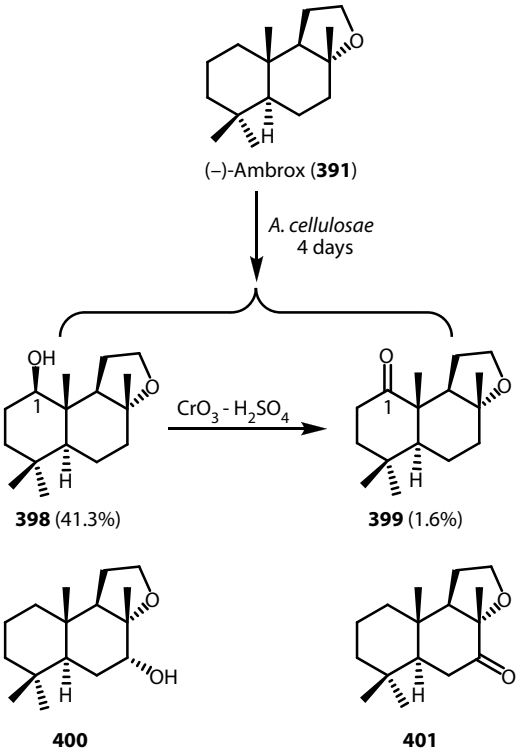


FIGURE 15.113 Biotransformation of (–)-ambrox (**391**) by *Aspergillus cellulosae*.

and sclareolide 3β,6β-diol (**401f**), and 3β-hydroxy- (**403**), 3-keto- (**405**), and sclareolide 3β,6β-diol (**401f**), respectively (Hanson and Truneh, 1996) (Figure 15.118).

Zerumbone (**408**), which is easily isolated from the wild ginger, *Zingiber zerumbet* and its epoxide (**409**) were incubated with *Fusarium culmorum* and *Aspergillus niger* in Czapek-peptone medium, respectively. The former fungus gave (1R,2R)-(+)-2,3-dihydrozerumbol (**410**) stereospecifically via either 2,3-dihydrozerumbone (**408a**) or zerumbol (**408b**) or both and accumulated **410** in the mycelium. The facile production of optically active **410** will lead a useful material of woody fragrance, namely 2,3-dihydrozerumbone. *Aspergillus niger* biotransformed **408** via epoxide (**409**) to several metabolites containing zerumbone-6,7-diol as a main product. The same fungus converted the epoxide (**409**) into three major metabolites containing (2R,6S,7S,10R,11S)-1-oxo7,9-dihydroxyisodaucane (**413**) via dihydro derivatives (**411**, **412**). However, *Aspergillus niger* biotransformed **409** only into **412** in the presence of CYP450 inhibitor, 1-aminobenzotriazole (Noma et al., 2002).

The same substrate was incubated in the *Aspergillus niger*, *Aspergillus orysae*, *Candia rugosa*, *Candia tropicalis, Mucor mucedo, Bacillus subtilis*, and *Schizosaccharomyces pombe*; however, any metabolites have been obtained. All microbes except for the last organism, zerumbone epoxide (**409**), prepared by *m*CPBA, bioconverted into two diastereoisomers, 2R,6S,7S-dihydro- (**411**) and 2R,6R,7R-derivative (**412**), whose ratio was determined by gas chromatography (GC) and their enantio-excess was over 99% (Nishida and Kawai, 2007) (Figure 15.119).

Several microorganisms and a few mammals (see later) for the biotransformation of (+)-cedrol (**414**) which is widely distributed in the cedar essential oils were used. Plant pathogenic fungus *Glomerella cingulata* converted cedrol (**414**) into three diols (**415**–**417**) and 2α-hydroxycedrene (**418**) (Miyazawa et al., 1995). The same substrate (**414**) was incubated with *Aspergillus niger* to give **416** and **417** together with a cyclopentanone derivative (**419**) (Higuchi et al., 2001). Human skin microbial flora, *Staphylococcus epidermidis* also converted (+)-cedrol into 2α-hydroxycedrol (**415**) (Itsuzaki et al., 2002) (Figure 15.120).

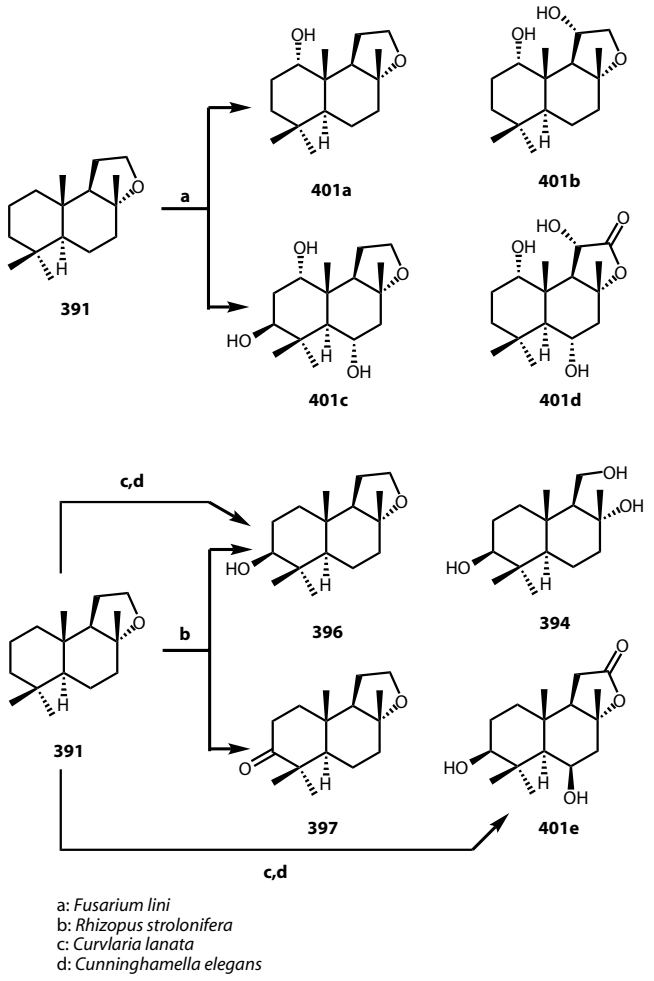


FIGURE 15.114 Biotransformation of (–)-ambrox (**391**) by *Fusarium lini* and *Rhizopus stronifera*.

Cephalosporium aphidicola bioconverted cedrol (**414**) into **417** (Hanson and Nasir, 1993). On the other hand, *Corynesphora cassiicola* produced **419** in addition to **417** (Abraham et al., 1987). It is noteworthy that *Botrytis cinerea* that damages many flowers, fruits and vegetables biotransformed cedrol into different metabolites (**420**–**422**) from those mentioned above (Aleu et al., 1999).

4α-Hydroxycedrol (**424**) was obtained from the metabolite of cedrol acetate (**423**) by using *Glomerella cingulata* (Matsui et al., 1999) (Figure 15.121).

Patchouli alcohol (**425**) was treated in *Botrytis cinerea* to give three metabolites two tertiary alcohols (**426**, **427**), four secondary alcohols (**428**, **430**, **430a**), and two primary alcohols (**430b**,

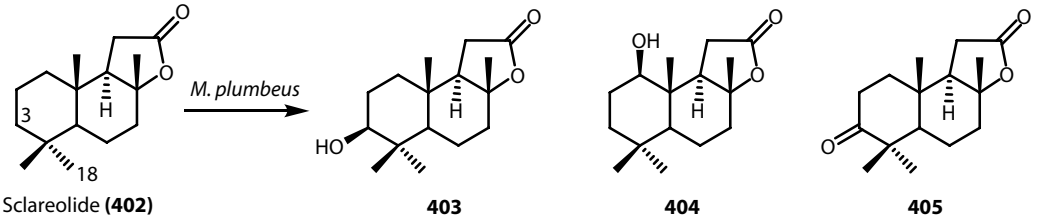


FIGURE 15.115 Biotransformation of (+)-sclareolide (**402**) by *Mucor plumbeus*.

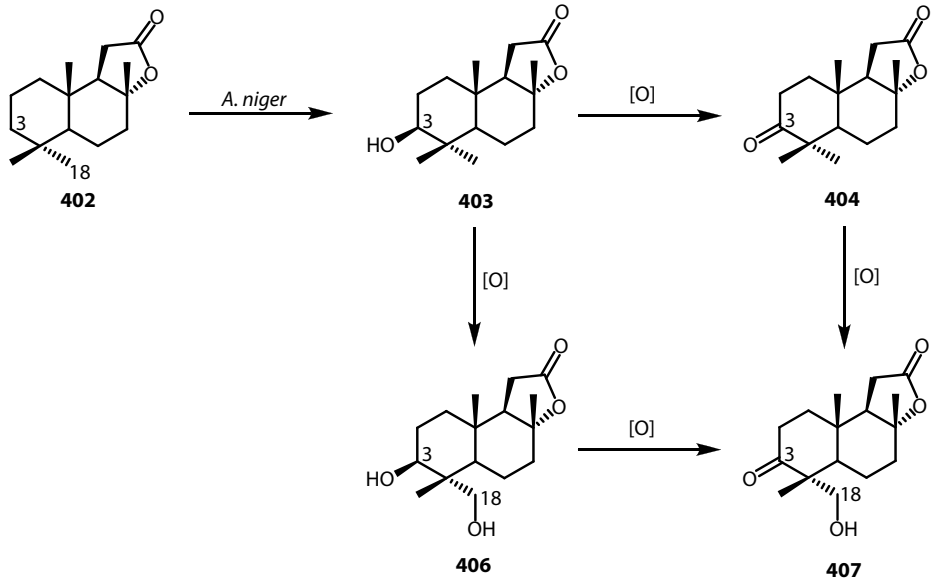


FIGURE 15.116 Biotransformation of (+)-sclareolide (**402**) by *Aspergillus niger.*

430c) of which compounds **425**, **427**, and **428** are the major metabolites (Aleu et al., 1999) while plant pathogenic fungus *Glomerella cingulata* converted the same substrate to 5-hydroxy- (**426**) and 5,8-dihydroxy derivative (**429**) (Figure 15.122).

In order to confirm the formation of **429** from **426**, the latter product was reincubated in the same medium including *Glomerella cingulata* to afford **429** (Miyazawa et al., 1997b) (Figure 15.123).

Patchouli acetate (**431**) was also treated in the same medium to give **426** and **429** (Matsui and Miyazawa, 2000). 5-Hydroxy-α-patchoulene (**432**) was incubated with *Glomerella cingulata* to afford 1α-hydroxy derivative (**426**) (Miyazawa et al., 1998a).

(−)-α-Longipinene (**433**) was treated with *Aspergillus niger* to afford 12-hydroxylated product (**434**) (Sakata et al., 2007).

Ginsenol (**435**), which was obtained from the essential oil of *Panax ginseng,* was incubated with *Botrytis cinerea* to afford four secondary alcohols (**436**–**439**) and two cyclohexanone derivatives (**440**) from **437** and **441** from **438** or **439**. Some of the oxygenated products were considered as potential antifungal agents to control *Botrytis cinerea* (Aleu et al., 1999a) (Figures 15.124 and 15.125).

(+)-Isolongifolene-9-one (**442**), which was isolated from some cedar trees was treated in *Glomerella cingulata* for 15 days to afford two primary alcohols (**443**, **444**) and a secondary alcohol (**445**) (Sakata and Miyazawa, 2006) (Figure 15.126).

Choudhary et al. (2005) reported that fermentation of (−)-isolongifolol (**445a**) with *Fusarium lini* resulted in the isolation of three metabolites, 10-oxo- (**445b**), 10α-hydroxy- (**445c**), and 9α-hydroxyisolngifolol (**445d**). Then the same substrate was incubated with *Aspergillus niger* to yield the products **445c** and **445d**. Both **445c** and **445d** showed inhibitory activity against butylcholinesterease enzyme in a concentration-dependent manner with IC_{50} 13.6 and 299.5 μM, respectively (Figure 15.127).

(+)-Cycloisolongifol-5β-ol (**445e**) was fermented with *Cunninghamella elegans* to afford three oxygenated metabolites: 11-oxo- (**445f**), 3β-hydroxy- (**445g**), and 3β,11α-dihydroxy derivative (**445h**) (Choudhary et al., 2006a). (Figure 15.128).

A daucane-type sesquiterpene derivative, lancerroldiol *p*-hydroxybenzoate (**446**) was hydrogenated with cultured suspension cells of the liverwort, *Marchantia polymorpha* to give 3,4-dihydrolancerodiol (**447**) (Hegazy et al., 2005) (Figure 15.129).

Widdrane sesquiterpene alcohol (**448**) was incubated with *Aspergillus niger* to give an oxo and an oxy derivatives (**449**, **450**) (Hayashi et al., 1999) (Figure 15.130).

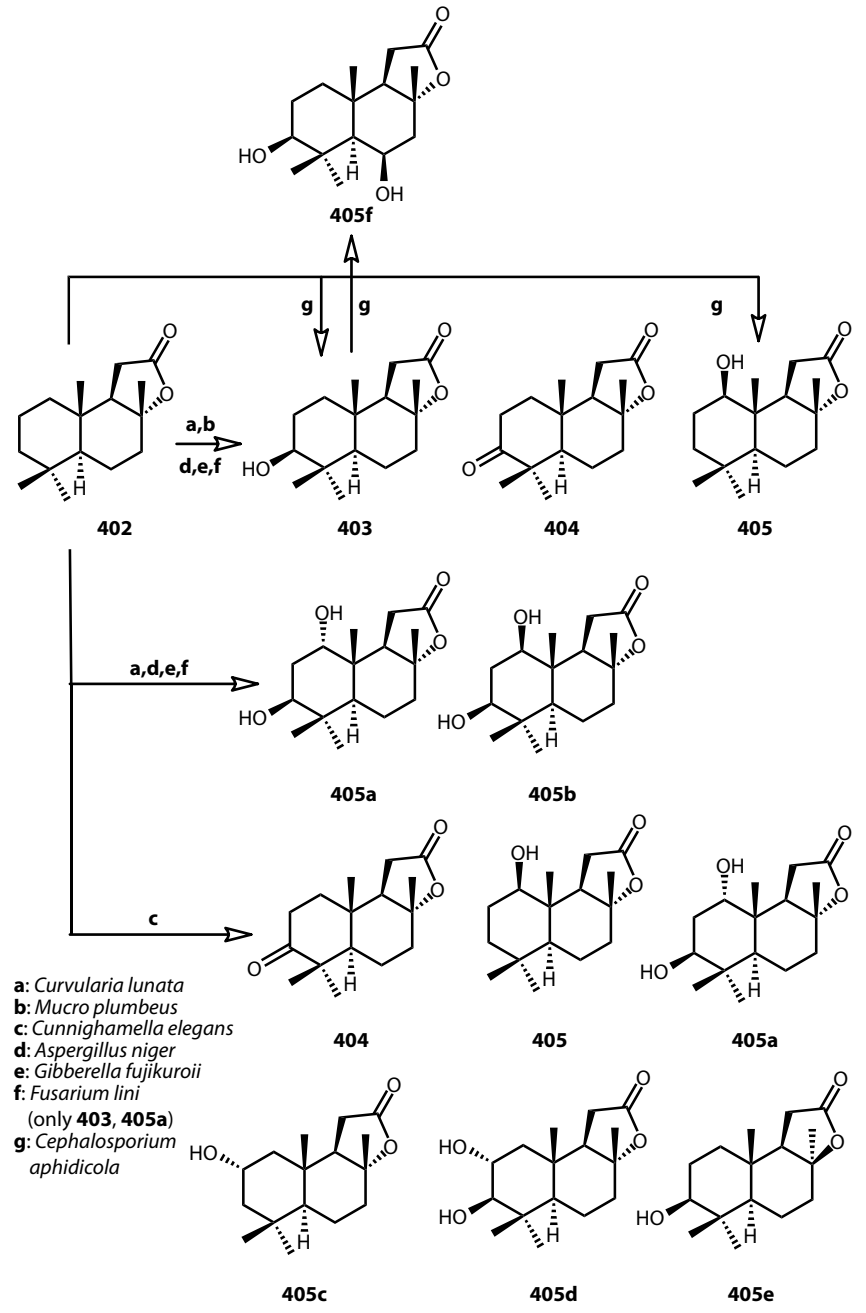


FIGURE 15.117 Biotransformation of (+)-sclareolide (**402**) by various fungi.

(–)-β-Caryophyllene (**451**), one of the ubiquitous sesquiterpene hydrocarbons found not only in higher plants but also in liverworts, was biotransformed by *Pseudomonas cruciviae*, *Diplodia gossypina*, and *Chaetomium cochlioides* (Lamare and Furstoss, 1990). *Pseudomonas cruciviae* gave a ketoalcohol (**452**) (Devi, 1979), while the latter two species produced the 14-hydroxy-5,6-epoxide (**454**), its carboxylic (**455**), and 3α-hydroxy- (**456**) and norcaryophyllene alcohol (**457**), all of which might be formed from caryophyllene C5,C6-epoxide (**453**). Oxidation pattern of (–)-β-caryophyllene by the fungi is very similar to that by mammals (see later) (Figure 15.131).

Fermentation of (–)-β-caryophyllene (**451**) with *Diplodia grossypina* afforded **14** different metabolites (**453**–**457j**), among which 14-hydroxy-5,6-epoxide (**454**) and the corresponding acid

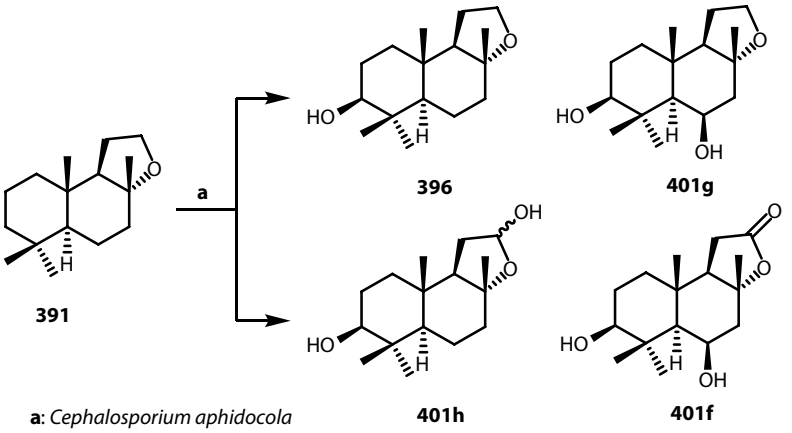


FIGURE 15.118 Biotransformation of (–)-ambrox (**391**) by *Cephalosporium aphidicola*.

(**455**) were the major metabolites. Compound **457j** is structurally very rare and found in *Poronia punctata*. The main reaction path is epoxidation at C5, C6 as mentioned above and selective hydroxylation at C4 (Abraham et al., 1990) (Figure 15.132).

(–)-β-Caryophyllene epoxide (**453**) was incubated with *Cephalosporium aphidicola* for 6 days to afford two metabolites (**457l**, **457m**) while *Macrophomina phaseolina* biotransformed the same substrate to 14- (**454**) and 15-hydroxy derivatives (**457k**). The same substrate was treated in *Aspergillus niger*, *Gibberella fujikuroii*, and *Rhizopus stolonifera*, for 8 days and *Fusarium lini* for 10 days to afford the metabolites **457n**, **457o**, **457p** and **457q**, and **457r**, respectively. All metabolites were estimated for butyrylcholine esterase inhibitory activity and compound **457k** was found to show potency similar activity to galanthamine HBr (IC_{50} 10.9 versus 8.5 μM) (Choudhary et al., 2006) (Figure 15.133).

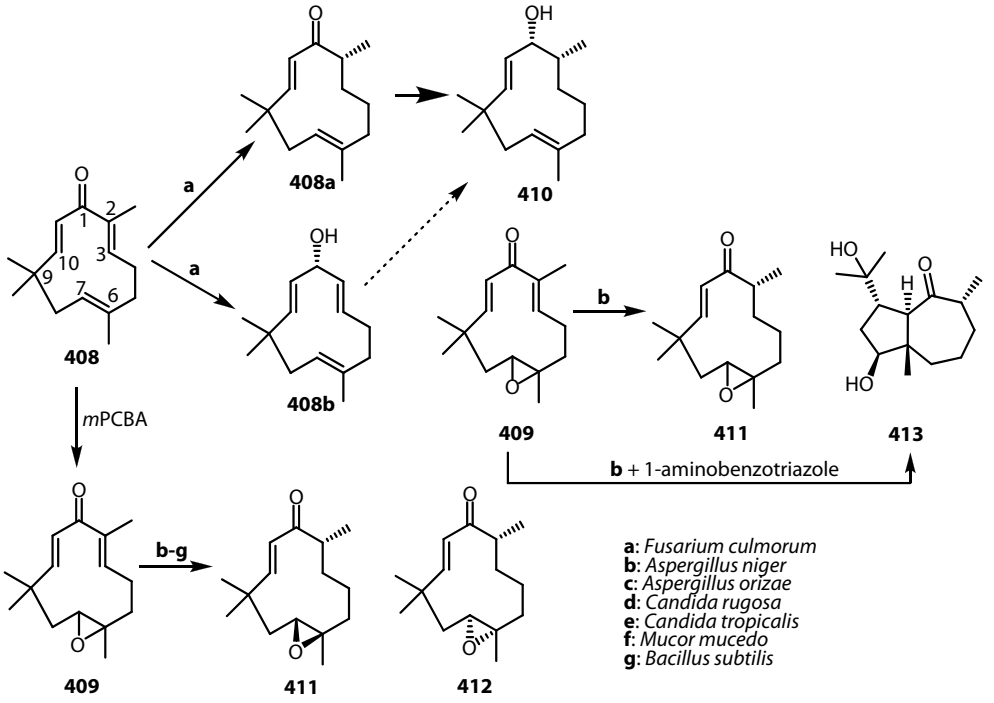


FIGURE 15.119 Biotransformation of zerumbone (**408**) by various fungi.

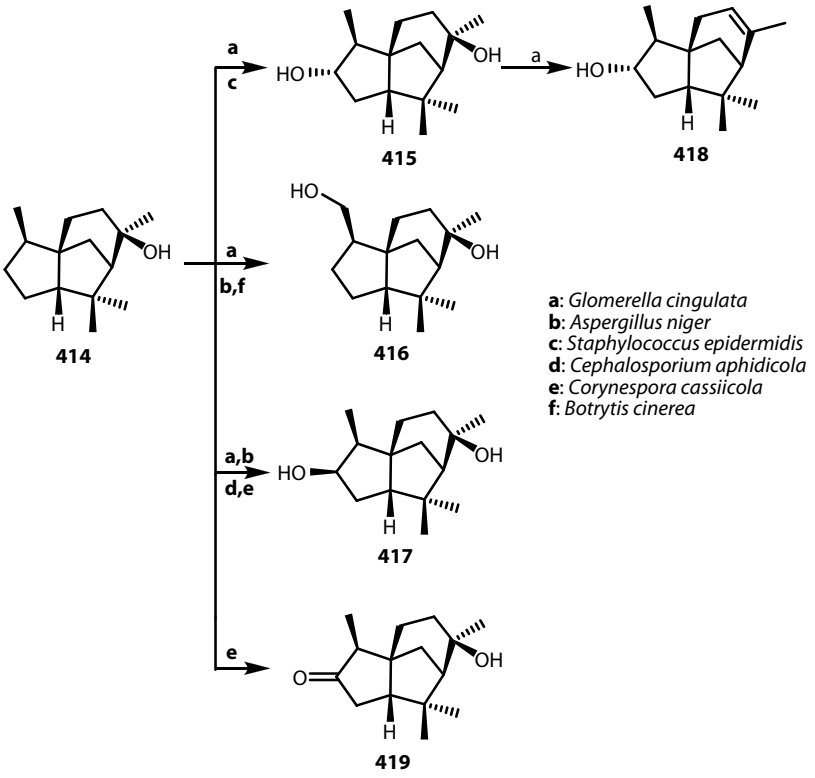


FIGURE 15.120 Biotransformation of cedrol (**414**) by various fungi.

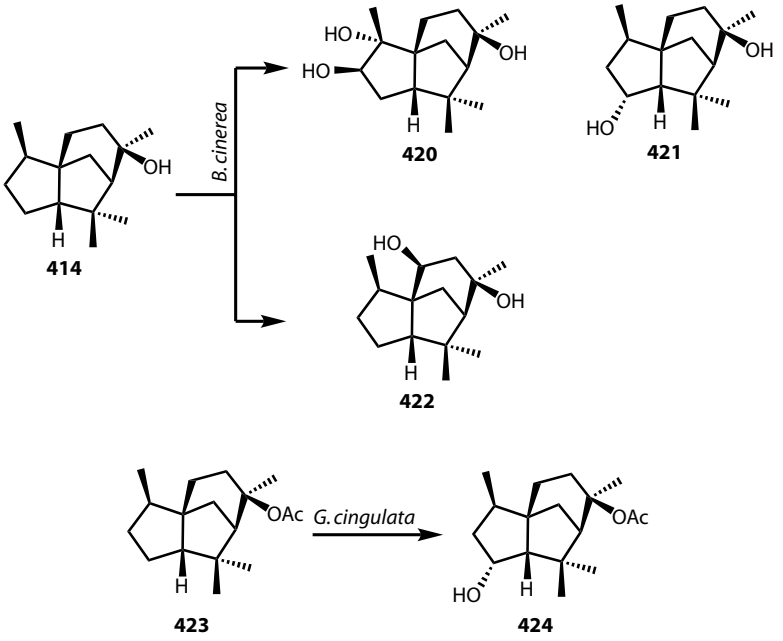


FIGURE 15.121 Biotransformation of cedrol (**414**) by *Botrytis cinerea and Glomerella cingulata*.

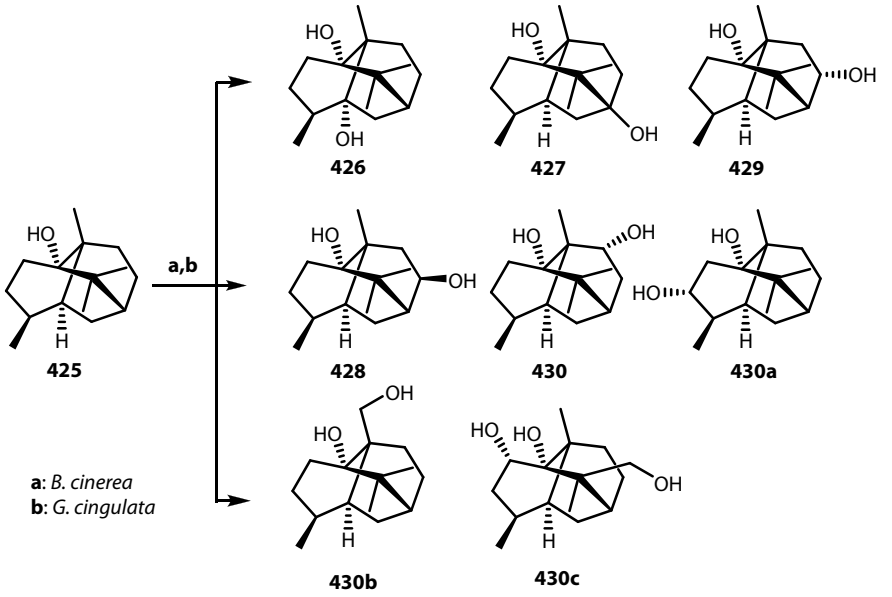


FIGURE 15.122 Biotransformation of patchoulol (**425**) by *Botrytis cinerea*.

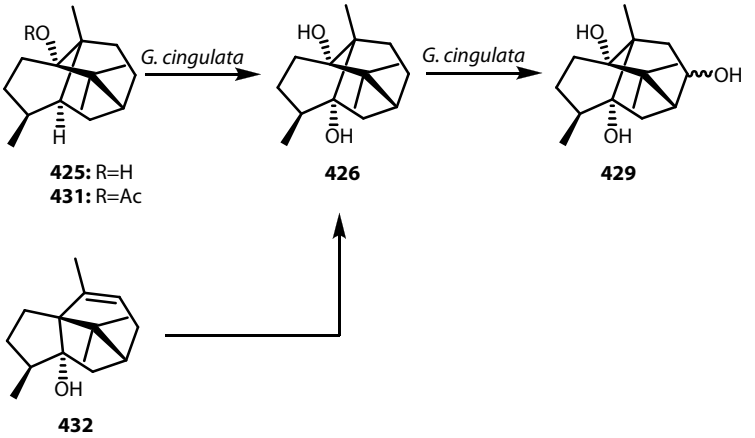


FIGURE 15.123 Biotransformation of patchoulol (**425**) by *Glomerella cingulata*.

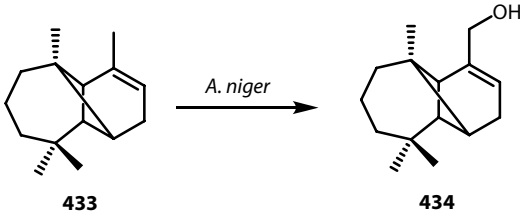


FIGURE 15.124 Biotransformation of α-longipinene (**433**) by *Aspergillus niger*.

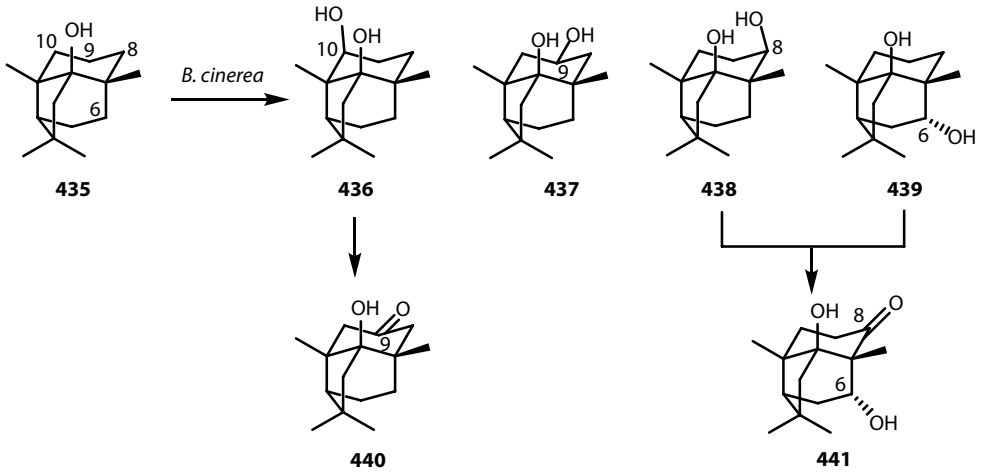


FIGURE 15.125 Biotransformation of ginsenol (**435**) by *Botrytis cinerea*.

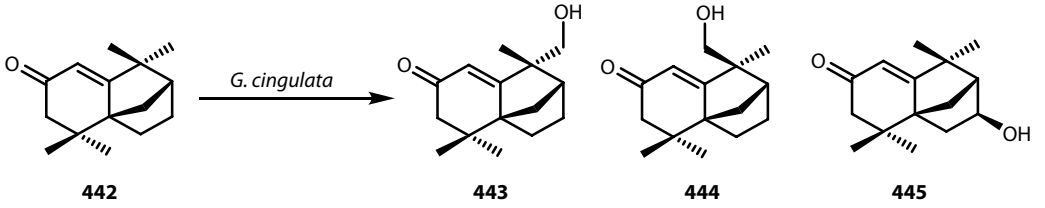


FIGURE 15.126 Biotransformation of (+)-isolongifolene-9-one (**442**) by *Glomerella cingulata*.

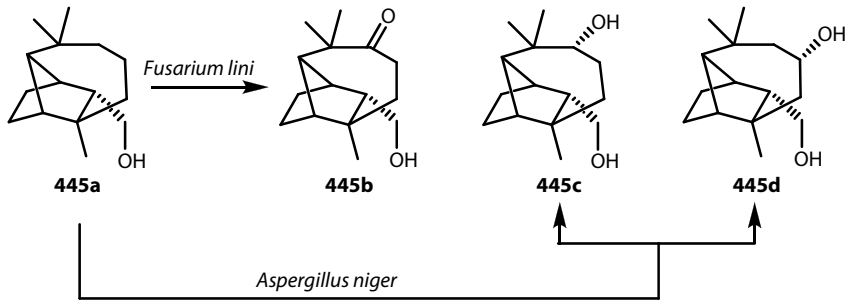


FIGURE 15.127 Biotransformation of (−)-isolongifolol (**445a**) by *Aspergillus niger* and *Fusarium lini*.

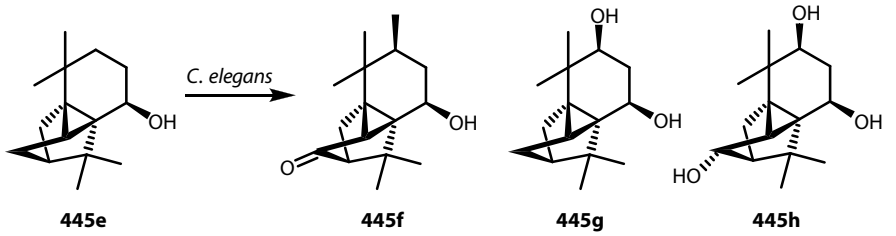


FIGURE 15.128 Biotransformation of (+)-cycloisolongifol-5β-ol (**445e**) by *Cunninghamella elegans*.

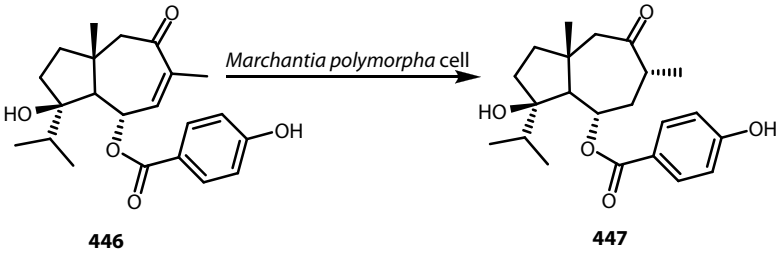


FIGURE 15.129 Biotransformation of lancerodiol *p*-hydroxybenzoate (**446**) by *Marchantia polymorpha* cells.

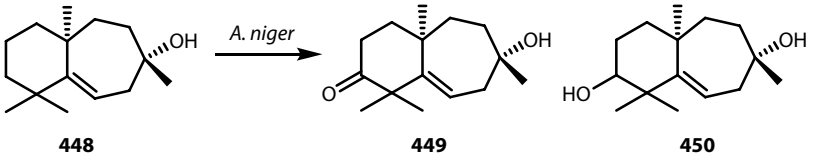


FIGURE 15.130 Biotransformation of widdrol (**448**) by *Aspergillus niger.*

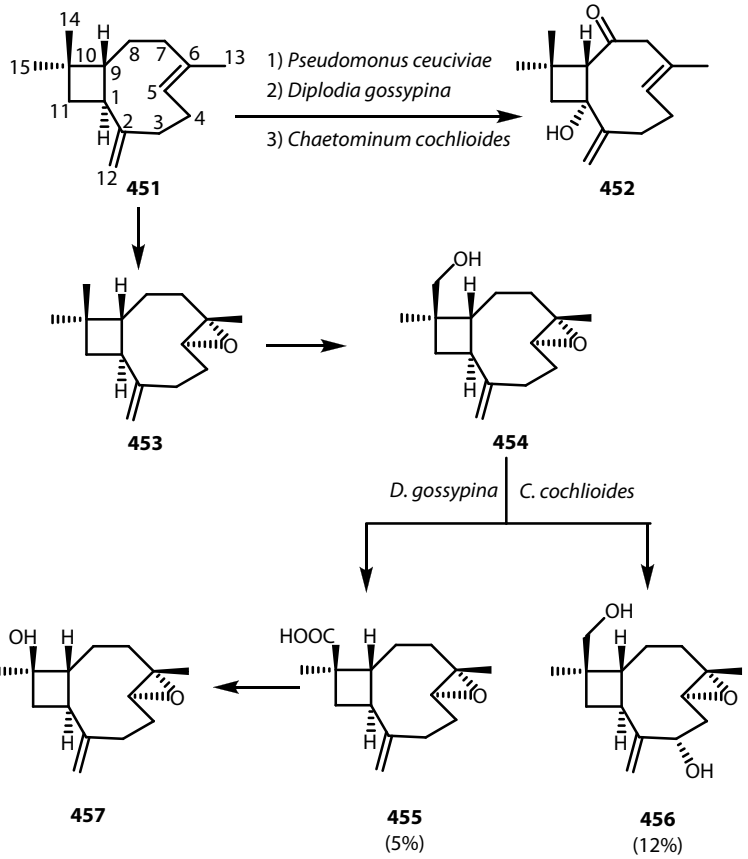


FIGURE 15.131 Biotransformation of (–)-β-caryophyllene (**451**) by *Pseudomonas ceuciviae*, *Diplodia gossypina*, and *Chaetomium cochlioides.*

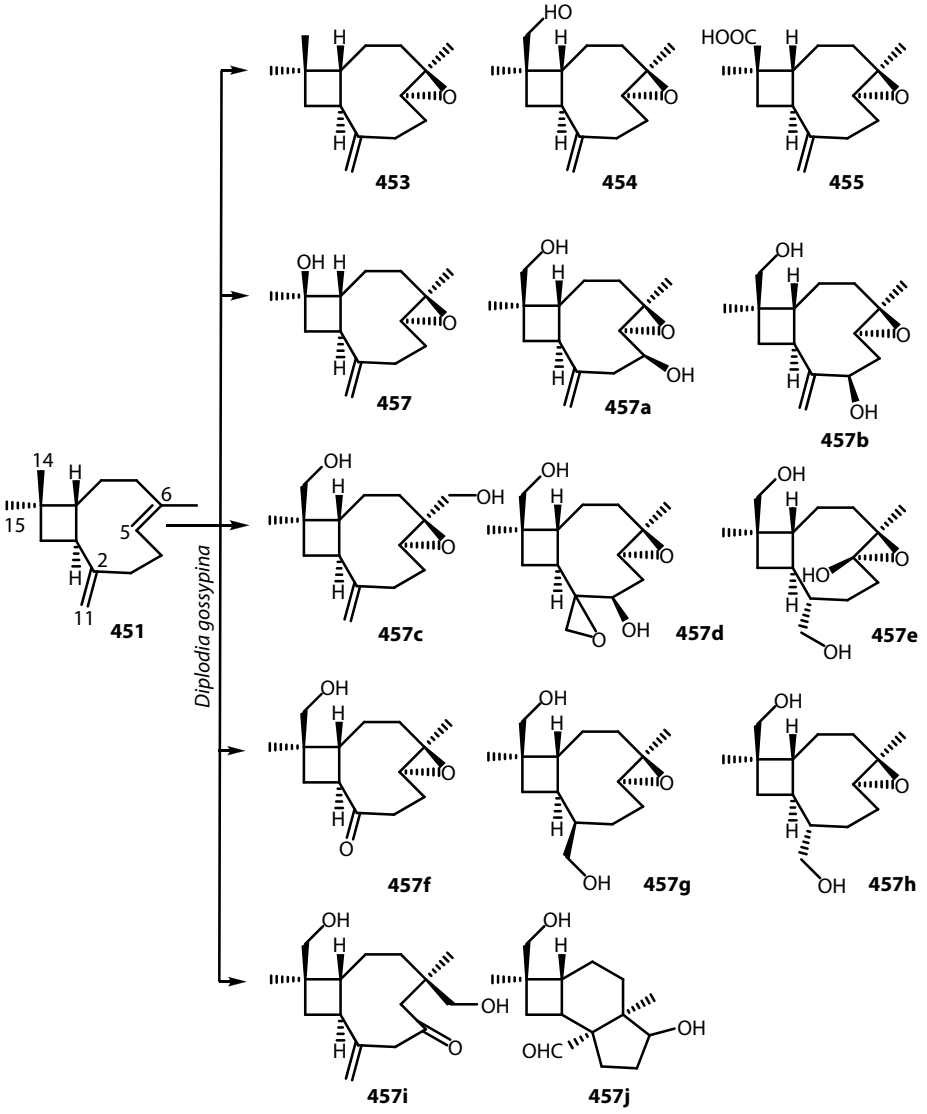


FIGURE 15.132 Biotransformation of (–)-β-caryophyllene (**451**) by *Diplodia gossypina*.

The fermentation of (–)-β-caryophyllene oxide (**453**) using *Botrytis cinerea* and the isolation of the metabolites were carried out by Duran et al. (1999) Kobuson (**457w**) was obtained with fourteen products (**457s**–**457u**, **457x**). Diepoxides **457t** and **457u** could be the precursors of epimeric alcohols **457q** and **457y** obtained through reductive opening of the C2,C11-epoxide. The major reaction paths are stereoselective epoxidation and introduction of hydroxyl group at C3. Compound **457ae** has a caryolane skeleton (Figure 15.134).

When isoprobotryan-9α-ol (**458**) produced from isocaryophyllene was incubated with *Botrytis cinerea*, it was hydroxylated at tertiary methyl groups to give three primary alcohols (**459**–**461**) (Aleu et al., 2002) (Figure 15.135).

Acyclic sesquiterpenoids, racemic *cis*-nerolidol (**462**), and nerylacetone (**463**) were treated by the plant pathogenic fungus, *Glomerella cingulata* (Miyazawa et al., 1995a). From the former substrate, a triol (**464**) was obtained as the major product. The latter was bioconverted to give the two methyl ketones (**465**, **467**) and a triol (**468**), among which **465** was the predominant. The C10,C11 diols (**464**, **465**) might be formed from both epoxides of the substrates, followed by the hydration although no C10,C11-epoxides were detected (Figure 15.136).

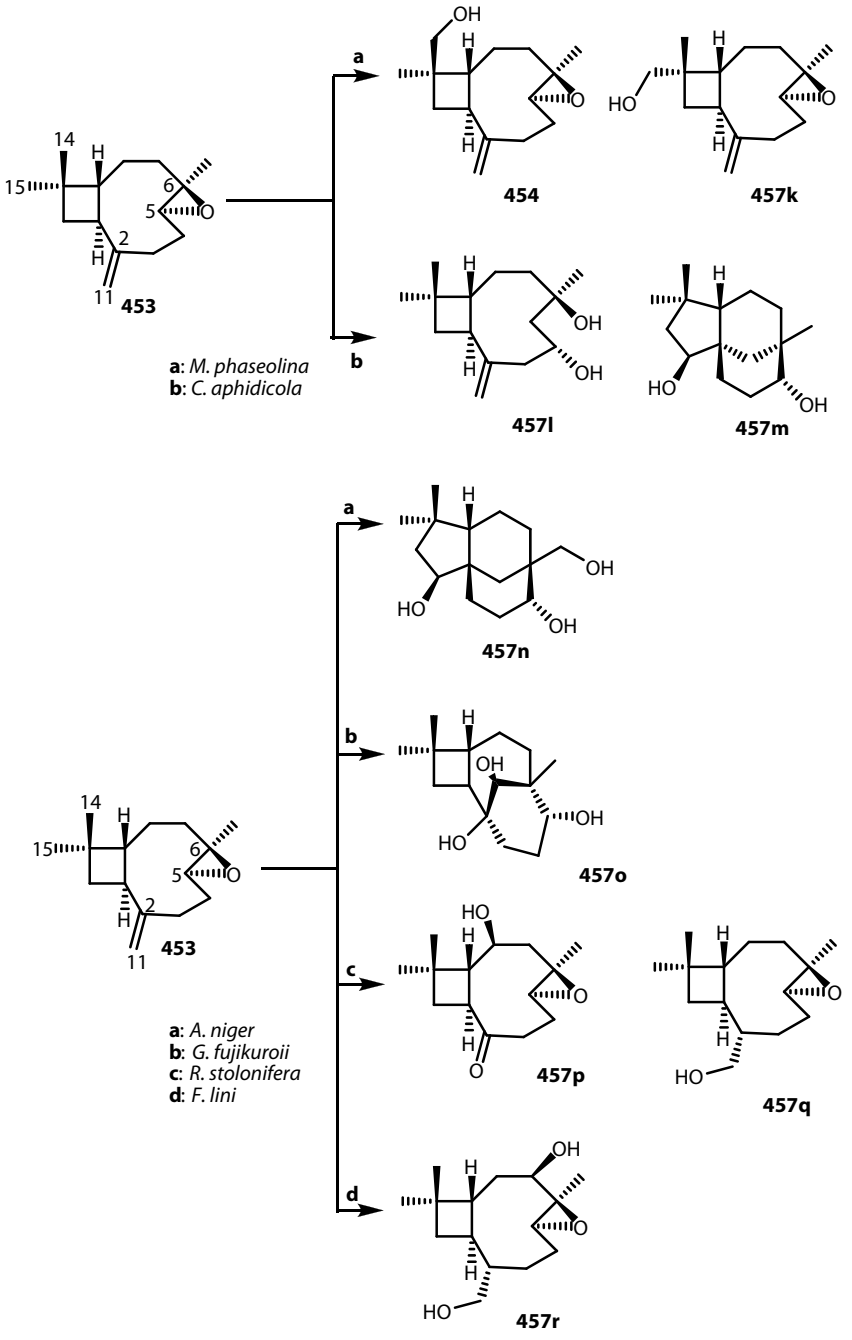


FIGURE 15.133 Biotransformation of (–)-β-caryophyllene epoxide (**453**) by various fungi.

Racemic *trans*-nerolidol (**469**) was also treated in the same fungus to afford ω–2 hydroxylated product (**471**) and C10,C11 hydroxylated compounds (**472**) as seen in racemic *cis*-nerolidol (**462**) (Miyazawa et al., 1996a) (Figure 15.137).

12-Hydroxy-*trans*-nerolidol (**472a**) is an important precursor in the synthesis of interesting flavor of α-sinensal. Hrdlicka et al. (2004) reported the biotransformation of *trans*-(**469**) and *cis*-nerolidol

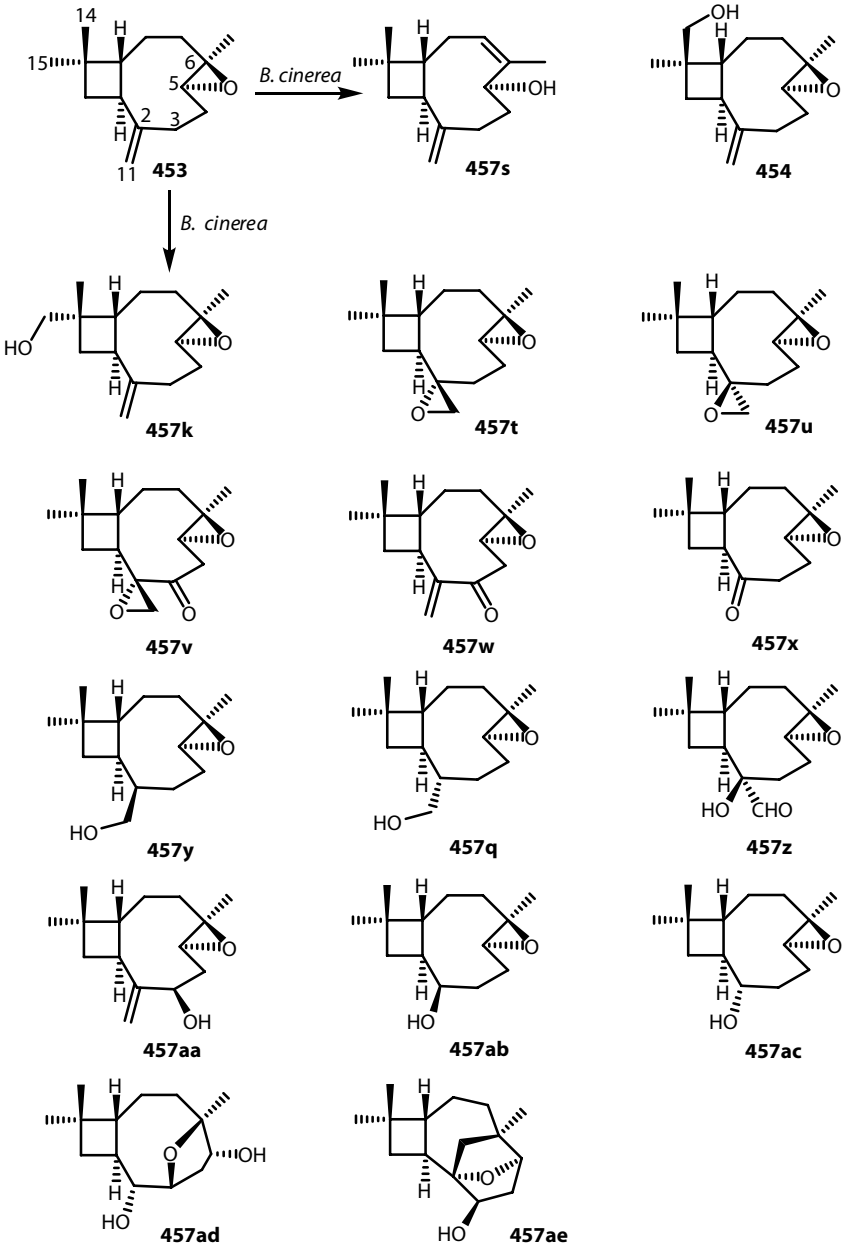


FIGURE 15.134 Biotransformation of (–)-β-caryophyllene epoxide (**453**) by *Botrytis cinerea*.

(**462**) and *cis-/trans*-mixture of nerolidol using repeated batch culture of *Aspergillus niger* grown in computer-controlled bioreactors. *Trans*-nerolidol (**469**) gave **472a** and **472** and *cis*-isomer (**462**) afforded **464a** and **464**. From a mixture of *cis*- and *trans*-nerolidol, 12-hydroxy-*trans*-neroridol **472a** (8%) was obtained in postexponential phase at high dissolved oxygen. At low dissolved oxygen condition, the mixture gave **472a** (7%) and **464a** (6%) (Figure 15.138).

From geranyl acetone (**470**) incubated with *Glomerella cingulata*, four products (**473**–**477**) were formed. It is noteworthy that the major compounds from both substrates (**469**, **470**) were ω–2

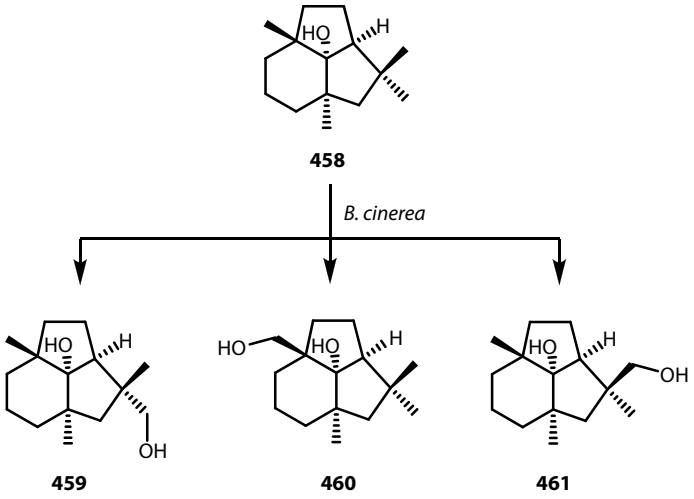


FIGURE 15.135 Biotransformation of isoprobotryan-9α-ol (**458**) by *Botrytis cinerea*.

hydroxylated products, but not C10,C11 dihydroxylated products as seen in *cis*-nerolidol (**462**) and nerylacetone (**463**) (Miyazawa et al., 1995c) (Figure 15.136).

The same fungus bioconverted (2*E*,6*E*)-farnesol (**478**) to four products, ω−2 hydroxylated product (**479**), which was further oxidized to give C10,C11 dihydroxylated compound (**480**) and 5-hydroxy derivative (**481**), followed by isomerization at C2,C3 double bond to afford a triol (**482**) (Miyazawa et al., 1996b) (Figure 15.140).

The same substrate was bioconverted by *Aspergillus niger* to afford two metabolites, 10,11-dihydroxy- (**480**) and 5,13-hydroxy derivative (**480a**) (Madyastha and Gururaja, 1993).

The same fungus also converted (2*Z*,6*Z*)-farnesol (**483**) to three hydroxylated products: 10,11-dihydroxy-(2*Z*,6*Z*)- (**484**), 10,11-dihydroxy (2*E*,6*Z*)-farnesol (**485**), and (5*Z*)-9,10-dihydroxy-6,10-dimethyl-5-undecen-2-one (**486**) (Nankai et al., 1996) (Figure 15.140).

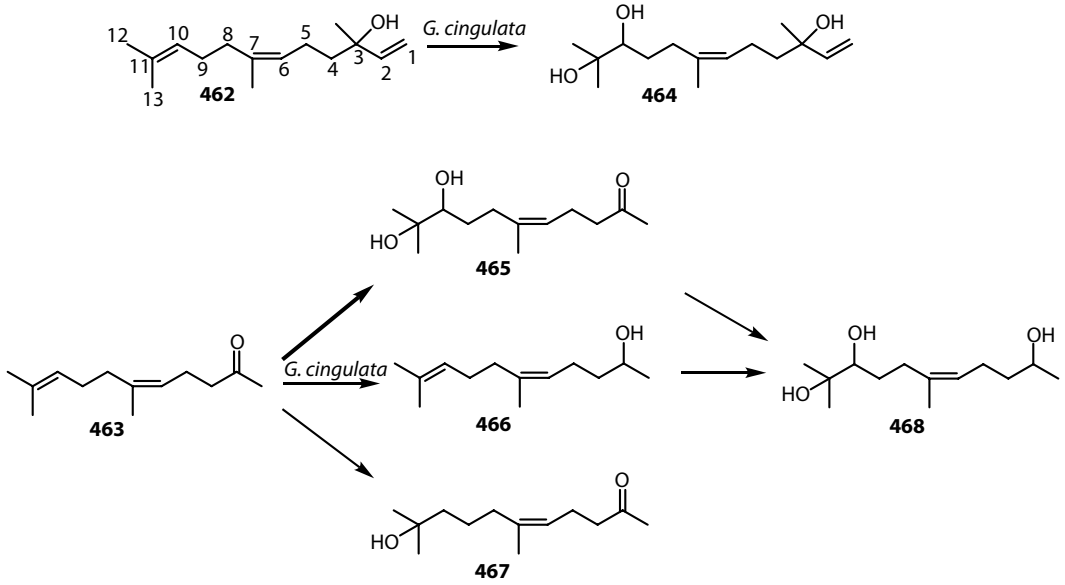


FIGURE 15.136 Biotransformation of *cis*-nerolidol (**462**) and *cis*-geranyl acetone (**463**) by *Glomerella cingulata*.

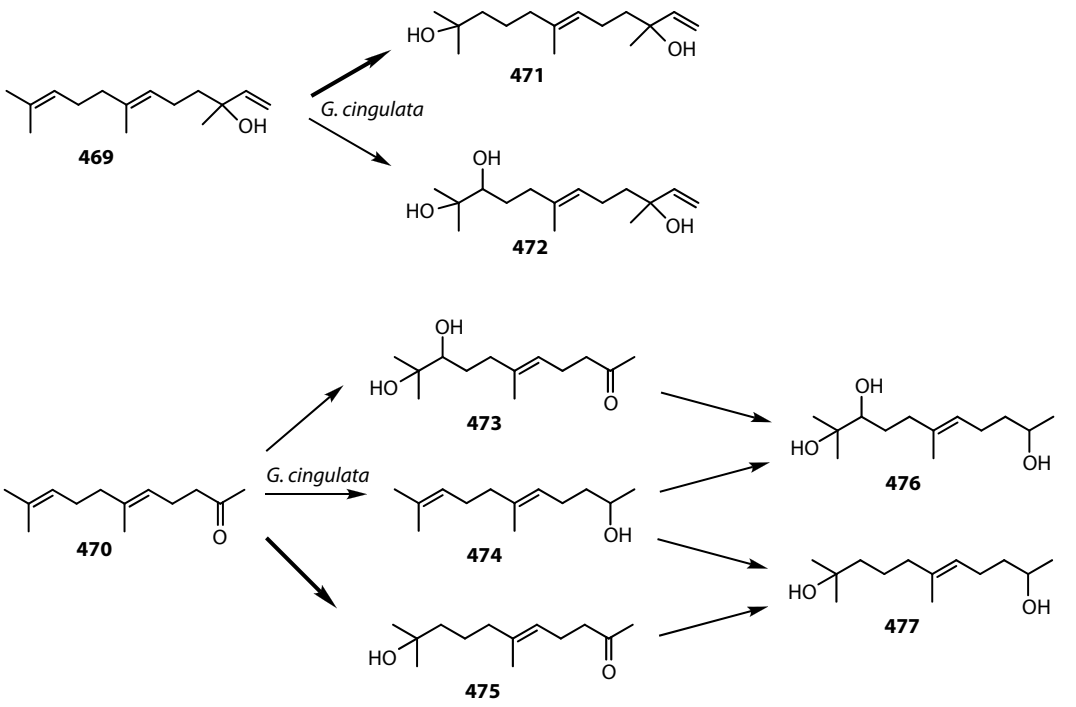


FIGURE 15.137 Biotransformation of *trans*-nerolidol (**469**) and *trans*-geranyl acetone (**470**) by *Glomerella cingulata*.

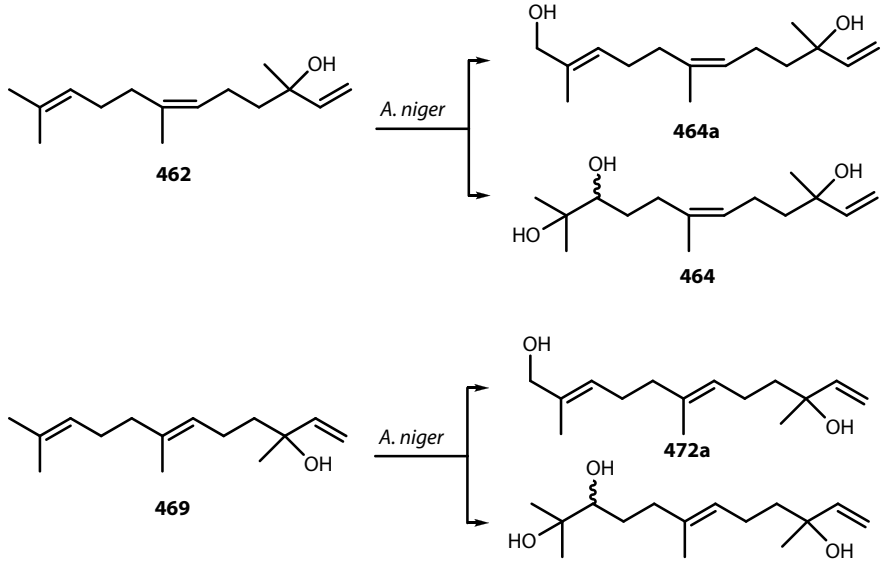


FIGURE 15.138 Biotransformation of *cis*- (**462**) and *trans*-nerolidol (**469**) by *Aspergillus niger*.

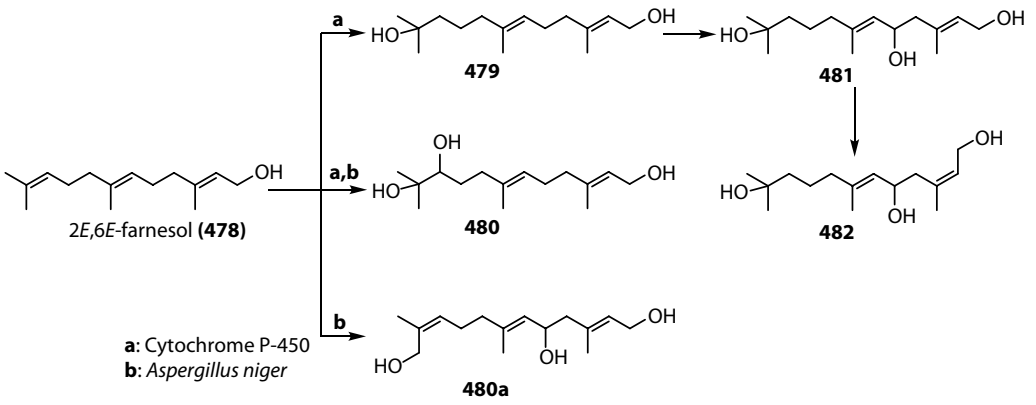


FIGURE 15.139 Biotransformation of *2E,6E*-farnesol (**478**) by Cytochrome P-450 and *Aspergillus niger*.

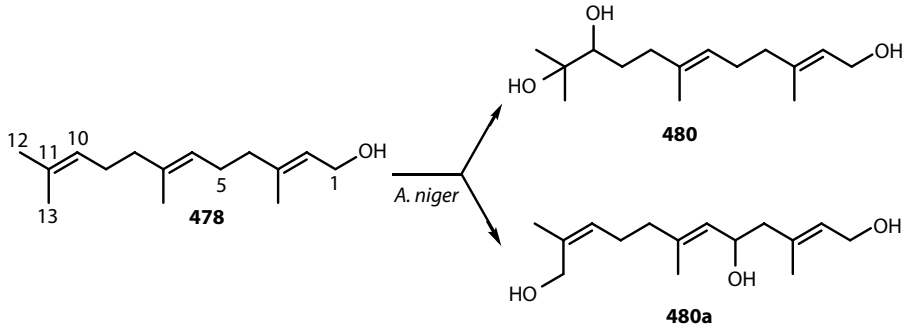


FIGURE 15.140 Biotransformation of *2E,6E*-farnesol (**478**) by *Aspergillus niger*.

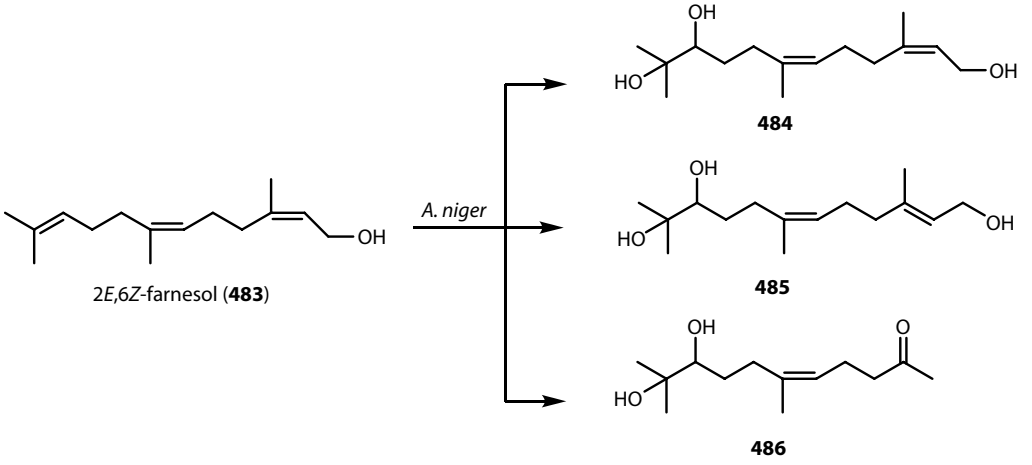


FIGURE 15.141 Biotransformation of *2Z,6Z*-farnesol (**478**) by *Aspergillus niger*.

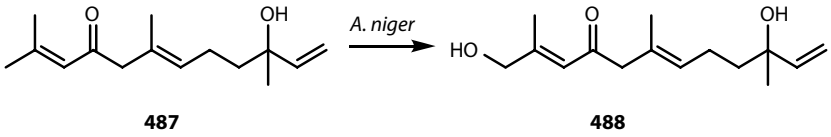


FIGURE 15.142 Biotransformation of 9-oxo-*trans*-nerolidol (**487**) by *Aspergillus niger*.

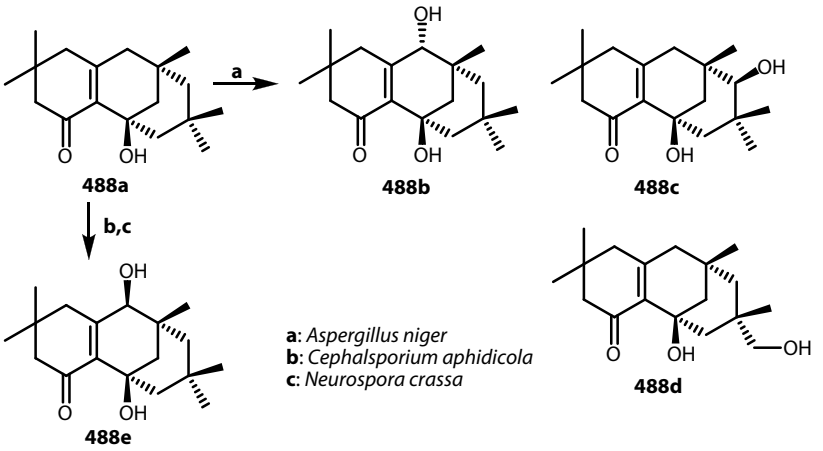


FIGURE 15.143 Biotransformation of diisophorone (**488a**) by *Aspergillus niger, Cephalosporium aphidicola,* and *Neurospora crassa.*

A linear sesquiterpene 9-oxonerolidol (**487**) was treated in *Aspergillus niger* to give ω−1 hydroxylated product (**488**) (Higuchi et al., 2001) (Figure 15.142).

Racemic diisophorone (**488a**) dissolved in ethanol was incubated with the Czapek–Dox medium of *Aspergillus niger* to afford 8α- (**488b**), 10β- (**488c**), and 17-hydroxydiisophorone (**488d**) (Kiran et al., 2004).

On the other hand, the same substrate was fed with *Nicotiana crassa* and *Cephalosporium aphidicola* to afford only 8β-hydroxydiisophorone (**488e**) in 20% and 10% yield, respectively (Kiran et al., 2005) (Figure 15.143).

From the metabolites of 5β,6β-dihydroxypresilphiperfolane 2β-angelate (**488f**) using the fungus *Mucor ramannianus*, 2,3-epoxyangeloyloxy derivative (**488g**) was obtained (Orabi, 2001) (Figure 15.144).

15.3 BIOTRANSFORMATION OF SESQUITERPENOIDS BY MAMMALS, INSECTS, AND CYTOCHROME P-450

15.3.1 Animals (Rabbits) and Dosing

Six male albino rabbits (2–3 kg) were starved for 2 days before experiment. Monoterpene were suspended in water (100 mL) containing polysorbate 80 (0.1 g) and were homogenized well. This solution (20 mL) was administered to each rabbit through a stomach tube followed by water (20 mL). This dose of sesquiterpenoids corresponds to 400–700 mg/kg. Rabbits were housed in stainless steal metabolism cages and were allowed rabbit food and water *ad libitum*. The urine was collected daily for 3 days after drug administration and stored at 0–5°C until the time of analysis. The urine was centrifuged to remove feces and hairs at 0°C and the supernatant was used for the experiments.

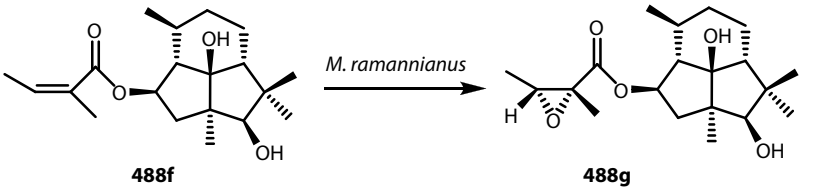


FIGURE 15.144 Biotransformation of 5β,6β-dihydroxypresilphiperfolane 2β-angelate (**488f**) by *Mucor ramannianus.*

The urine was adjusted to pH 4.6 with acetate buffer and incubated with β-glucuronidase-arylsufatase (3 mL/100 mL of fresh urine) at 37°C for 48 h, followed by continuous ether extraction for 48 h. The ether extracts were washed with 5% $NaHCO_3$ and 5% NaOH to remove the acidic and phenolic components, respectively. The ether extract was dried over $MgSO_4$, followed by evaporation of the solvent to give the neutral crude metabolites (Ishida et al., 1981).

15.3.2 Sesquiterpenoids

Wild rabbits (hair) and deer damage the young leaves of *Chamaecyparis obtusa*, one of the most important furniture and house-constructing tree in Japan. The essential oil of the leaves contains a large amount of (–)-longifolene (**489**). Longifolene (36 g) was administered to 18 of rabbits to obtain the metabolites (3.7 g) from which an aldehyde (**490**) (35.5%) was isolated as pure state. In the metabolism of terpenoids having an exomethylene group, glycol formation was often found, but in the case of longifolene such as a diol was not formed. Introduction of an aldehydes group in biotrans formation is very remarkable. Stereoselective hydroxylation of the gem dimethyl group on a seven-membered ring is first time (Ishida et al., 1982) (Figure 15.145).

(–)-β-Caryophyllene (**451**) is one of the ubiquitous sesquiterpene hydrocarbons in plant kingdom and the main component of beer hops and clove oil, and is being used as a culinary ingredient and as a cosmetic in soaps and fragrances. (–)-β-Caryophyllene also has cytotoxic against breast carcinoma cells and its epoxide is toxic to *Planaria* worms. It contains unique 1,1-dimethylcyclobutane skeleton. (–)-β-Caryophyllene (3 g) was treated in the same manner as described above to afford the crude metabolite (2.27 g) from which (10*S*)-14-hydroxycaryophyllene-5,6-oxide (**491**) (80%) and a diol (**492**) were obtained (Asakawa et al., 1981). Later, compound (**491**) was isolated from the Polish mushroom, *Lactarius camphorates* (Basidiomycetes) as a natural product (Daniewski et al., 1981). 14-Hyroxy-β-callyophyllene and 1-hydroxy-8-keto-β-caryophyllene have been found in Asteraceae and *Pseudomonas* species, respectively. In order to confirm that caryophyllene epoxide (**453**) is the intermediate of both metabolites, it was treated in the same manner as described above to give the same metabolites (**491**) and (**492**), of which **491** was predominant (Asakawa et al., 1981, 1986) (Figure 15.146).

The grapefruit aroma, (+)-nootkatone (**2**) was administered into rabbits to give 11,12-diol (**6**, **7**). The same metabolism has been found in that of biotransformation of nootkatone by microorganisms as mentioned in the previous paragraph. Compounds (**6**, **7**) were isolated from the urine of hypertensive subjects and named urodiolenone. The endogenous production of **6**, **7** seem to occur interdentally from the administrative manner of nootkatone or grapefruit. Synthetic racemic nootkatone epoxide (**14**) was incubated with rabbit-liver microsomes to give 11,12-diol (**6**, **7**) (Ishida, 2005). Thus, the role of the epoxide was clearly confirmed as an intermediate of nootkatone (**2**).

(+)-*ent*-Cyclocolorenone (**98**) and its enantiomer (**103**) were biotransformed by *Aspergillus* species to give cyclopropane-cleaved metabolites as described in the previous paragraph.

In order to compare the metabolites between mammals and microorganisms, the essential oil (2 g/rabbit) containing (–)-cyclocolorenone (**103**) obtained from *Solidago altissima* was administered in rabbits to obtain two metabolites; 9β-hydroxycyclocolorenone (**493**) and 10-hydroxycyclocolorenone (**494**) (Asakawa et al., 1986). 10-Hydroxyaromadendrane-type compounds are well

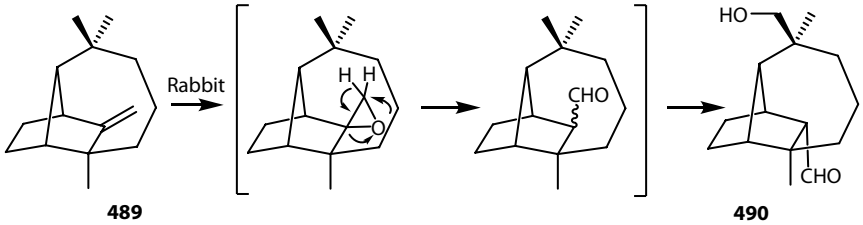


FIGURE 15.145 Biotransformation of longifolene (**489**) by rabbit.

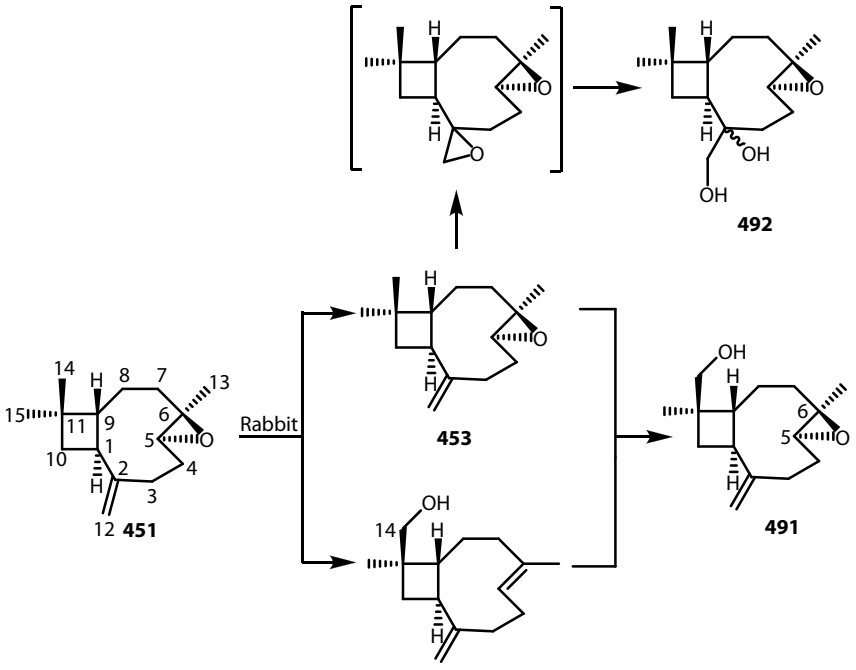


FIGURE 15.146 Biotransformation of (–)-β-caryophyllene (**451**) by rabbit.

known as the natural products. No oxygenated compound of cyclopropane ring was found in the metabolites of cyclocolorenone in rabbit (Figure 15.147).

From the metabolites of elemol (**495**) possessing the same partial structures of monoterpene hydrocarbon, myrcene, and nootkatone, one primary alcohol (**496**) was obtained from rabbit urine after the administration of **495** (Asakawa et al., 1986) (Figure 15.148).

Components of cedar wood such as cedrol (**414**) and cedrene shorten the sleeping time of mice. In order to search for a relationship between scent, olfaction, and detoxifying enzyme induction, (+)-cedrol (**414**) was administered to rabbits and dogs. From the metabolites from rabbits, two C3 hydroxylated products (**418** and **497**) and a diol (**415** or **416**), which might be formed after the hydrogenation of double bond. Dogs converted cedrol (**414**) into the different metabolite products,

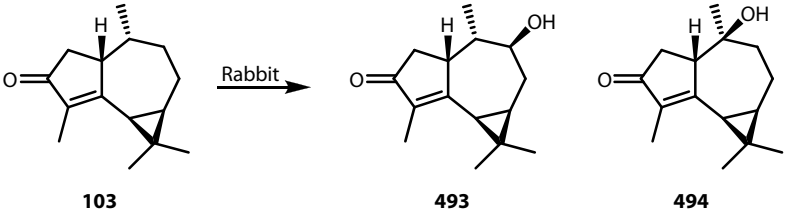


FIGURE 15.147 Biotransformation of (+)-*ent*-cyclocolorenone (**101**) by rabbit.

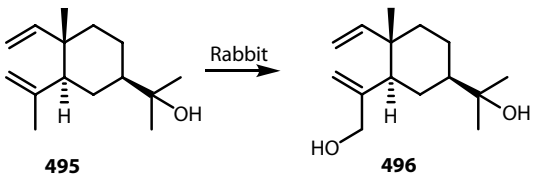


FIGURE 15.148 Biotransformation of elemol (**495**) by rabbit.

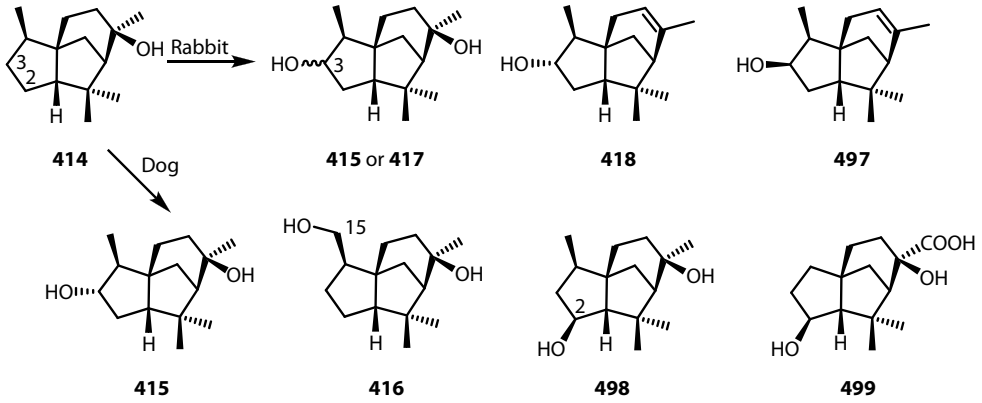


FIGURE 15.149 Biotransformation of cedrol (**414**) by rabbits or dogs.

C2 (**498**) and C2/C14 hydroxylated products (**499**), together with the same C3 (**415**) and C15 hydroxylated products (**416**) as those found in the metabolites of microorganisms and rabbits. The above species-specific metabolism is very remarkable (Bang and Ourisson, 1975).

The microorganisms, *Cephalosporium aphidicoda*, *Corynespora cassicoda*, *Botrytis cinerea*, and *Glomerella cingulata* also biotransformed cedrol to various C2, C3, C4, C6, and C15 hydroxylated products as shown in the previous paragraph. The microbial metabolism of cedrol resembles that of mammals (Figure 15.149).

Patchouli alcohol (**425**) with fungi static properties is one of the important essential oils in perfumery industry. Rabbits and dogs gave two oxidative products (**500**, **501**) and one norpatchoulen-1-one (**502**) possessing a characteristic odor. Plant pathogen, *Botrytis cinerea* causes many diseases for vegetables and flowers. This pathogen gave totally different five metabolites (**426**–**430**) from those found in the urine metabolites of mammals as described above (Bang et al., 1975) (Figure 15.150).

Sandalwood oil contains mainly α-santalol (**503**) and β-santalol. Rabbits converted α-santalol to three diastereomeric primary alcohols (**504**–**506**) and dogs did carboxylic acid (**507**) (Zundel, 1976) (Figure 15.151).

(2*E*,6*E*)-Farnesol (**478**) was treated in cockroach Cytochrome P-450 (CYP4C7) to form region- and diastereospecifically ω-hydroxylated at the C12 methyl group to the corresponding diol (**508**) with 10*E*-configuratation (Sutherland et al., 1998) (Figure 15.152).

Juvenile hormone III (**509**) was also treated in cockroach CYP4C7 to the corresponding 12-hydroxylated product (**510**) (Sutherland et al., 1998).

The African locust converted the same substrate (**509**) into a 7-hydroxy product (**511**) and a 13-hydroxylated product (**512**). It is noteworthy that the African locust and cockroach showed clear species specificity for introduction of oxygen function (Darrouzet et al., 1997).

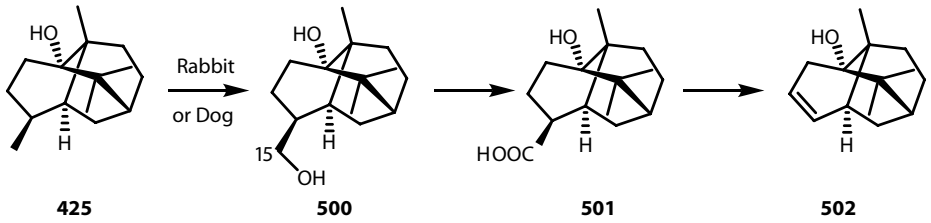


FIGURE 15.150 Biotransformation of patchouli alcohol (**425**) by rabbits or dogs.

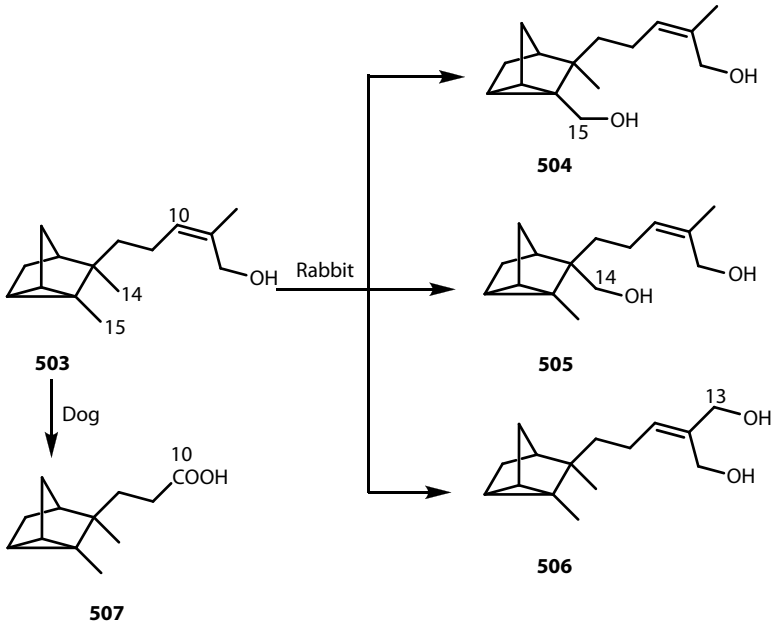


FIGURE 15.151 Biotransformation of santalol (**503**) by rabbits or dogs.

15.4 BIOTRANSFORMATION OF IONONES, DAMASCONES, AND ADAMANTANES

Racemic α-ionone (**513**) was converted to 4-hydroxy-α-ionone (**514**), which was further dehydrogenated to 4-oxo-α-ionone (**515**) by *Chlorella ellipsoidea* IAMC-27 and *Chlorella vulgaris* IAMC-209. α-Ionone (**513**) was reduced preferentially to α-ionol (**516**) by *Chlorella sorokiniana* and *Chlorella salina* (Noma et al. 1997a).

α-Ionol (**516**) was oxidized by *Chlorella pyrenoidosa* to afford 4-hydroxy-α-ionol (**524**). The same substrate was fed by the same microorganism and *Aspergillus niger* to furnish α-ionone (**513**) (Noma and Asakawa, 1998) (Figure 15.153).

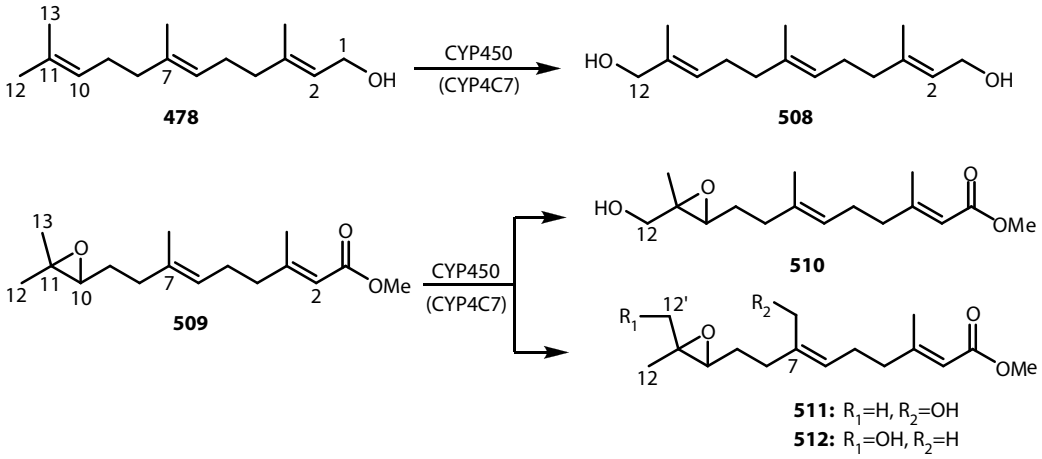


FIGURE 15.152 Biotransformation of *2E,6E*-farnesol (**478**) by cockroach Cytochrome P-450 and 10,11-epoxyfarnesic acid methyl ester (**509**) by African locust Cytochrome P-450.

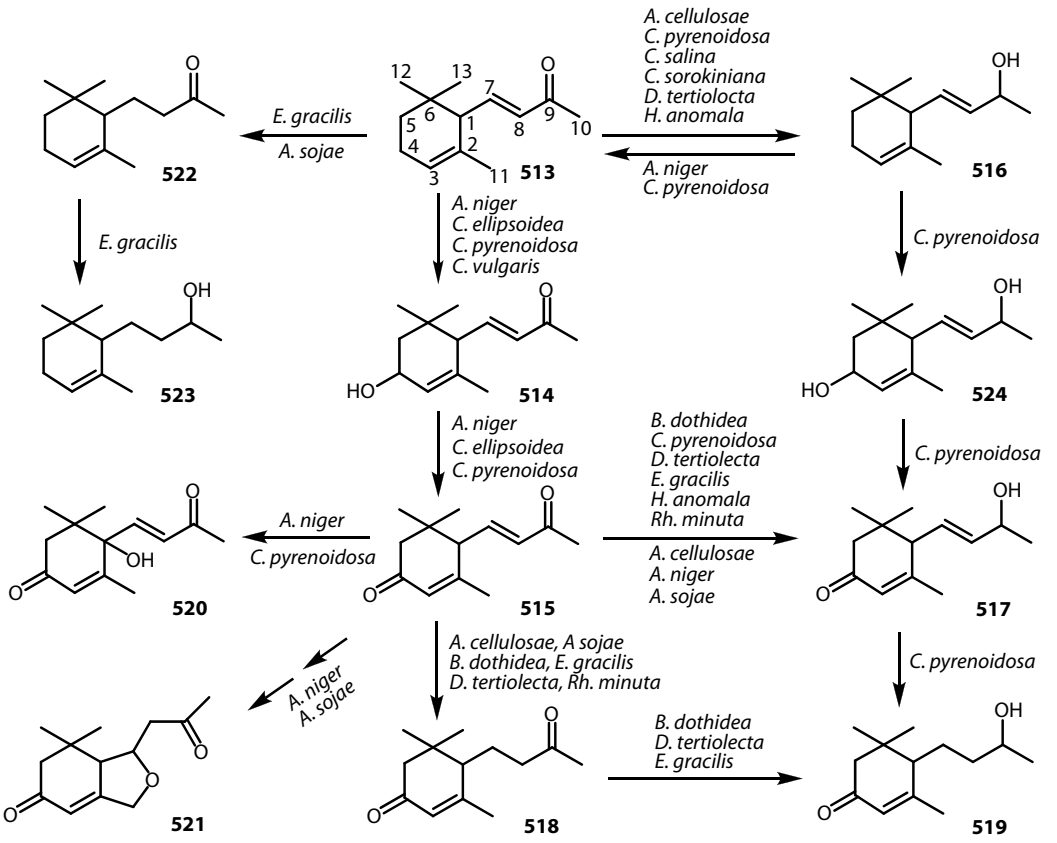


FIGURE 15.153 Biotransformation of α-ionone (**513**) by various microorganisms.

4-Oxo-α-ionone (**515**), which is one of the major product of α-ionone (**513**) by *Aspergillus Niger,* was transformed reductively by *Hansenula anomala, Rhodotorula minuta, Dunaliella tertiolecta, Euglena gracilis, Chlorella pyrenoidosa* C28 and other eight kinds of *Chlorella* species, *Botryosphaeria dothidea*, *Aspergillus cellulosae* IFO 4040 and *Aspergillus sojae* IFO 4389 to give 4-oxo-α-ionol (**517**), 4-oxo-7,8-dihydro-α-ionone (**518**), and 4-oxo-7,8-dihydro-α-ionol (**519**). Compound **515** was also oxidized by *Aspergillus niger* and *Aspergillus sojae* to give 1-hydroxy-4-oxo-α-ionone (**520**) and 7,11-oxido-4-oxo-7,8-dihydro-α-ionone (**521**). C7–C8 Double bond of α-ionone (**513**), 4-oxo α-ionone (**515**), and 4-oxo-α-ionol (**517**) were easily reduced to their corresponding dihydro products (**522**, **518**, **519**), respectively, by *Euglena, Aspergillus, Botrysphaeria*, and *Chlorella* species. The metabolite (**522**) was further reduced to **523** by *Euglena gracilis* (Noma et al., 1998).

Biotransformation of (+)-1*R*-α-ionone (**513a**), $[\alpha]_D$+386.5°; 99% ee and (–)-1*S*-α-ionone (**513a′**), $[\alpha]_D$–361.6°, 98% ee, which were obtained by optical resolution of racemic α-ionone (**513**), was fed by *Aspergillus niger* for 4 days in Czapek-peptone medium. From (**513a**), 4α-hydroxy-α-ionone (**514a**), 4β-hydroxy-α-ionone (**514b**), and 4-oxo-α-ionone (**515a**) were obtained, while from compound **513a′**, the enantiomers (**514a′**, **514b′**, **515a′**) of the metabolites from **513a** were obtained; however, the difference of their yields were observed. In case of **513a**, 4α-hydroxy-α-ionone (**514a**) was obtained as the major product, while **515a′** was predominantly obtained from **513a′**. This oxidation was inhibited by 1-aminobenzotriazole, thus CYP-450 is contributed to this oxidation process (Hashimoto et al., 2000) (Figure 15.154).

α-Damascone (**525**) was incubated with *Aspergillus niger* and *Aspergillus terreus*, in Czapek-peptone medium to give *cis*- (**525**) and *trans*-3-hydroxy-α-damascones (**527**) and 3-oxo-α-damscone

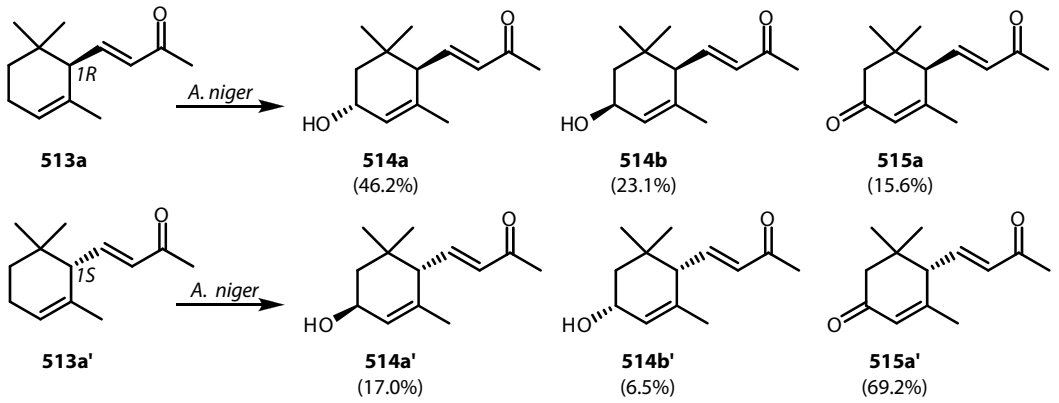


FIGURE 15.154 Biotransformation of (*1R*)-α-ionone (**513a**) and (*1S*)-α-ionone (**513a′**) by *Aspergillus niger.*

(**528**), while the latter *Aspergillus* species afforded 3-oxo-8,9-dihydro-α-damascone (**529**). The hydroxylation process of **525**–**527** was inhibited by CYP-450 inhibitor. *Hansenula anomala* reduced α-damascone (**525**) to α-damascol (**530**). *Cis*- (**526**) and *trans*-4-hydroxy-α-damascone (**527**) were fed by *Chlorella pyrenoidosa* in Noro medium to give 4-oxodamascone (**528**) (Noma et al., 2001a) (Figure 15.155).

β-Damascone (**531**) was also treated in *Aspergillus niger* to afford 5-hydroxy-β-damascone (**532**), 3-hydroxy-β-damascone (**533**), 5-oxo- (**534**), 3-oxo-β-damscone (**535**), and 3-oxo-1, 9-dihydroxy-1,2-dihydro-β-damascone (**536**) as the minor components. In case of *Aspergillus terreus*, 3-hydroxy-8,9-dihydro-β-damascone (**537**) was also obtained (Figure 15.156).

Adamantane derivatives have been used as many medicinal drugs. In order to obtain the drugs, adamantanes were incubated by many microorganisms, such as *Aspergillus niger*, *Aspergillus awamori, Aspergillus cellulosae, Aspergillus fumigatus, Aspergillus sojae, Aspergillus terreus,*

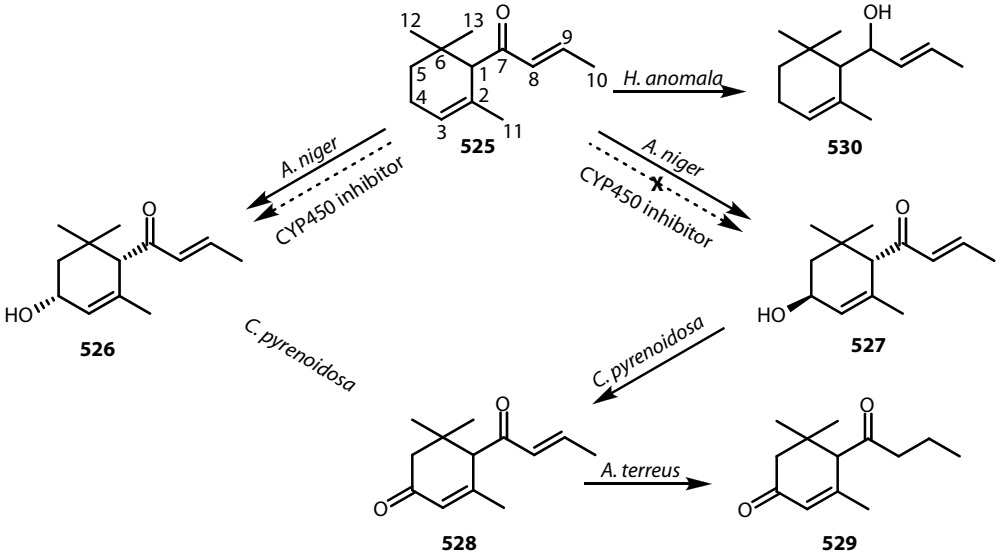


FIGURE 15.155 Biotransformation of α-damascone (**525**) by various microorganisms.

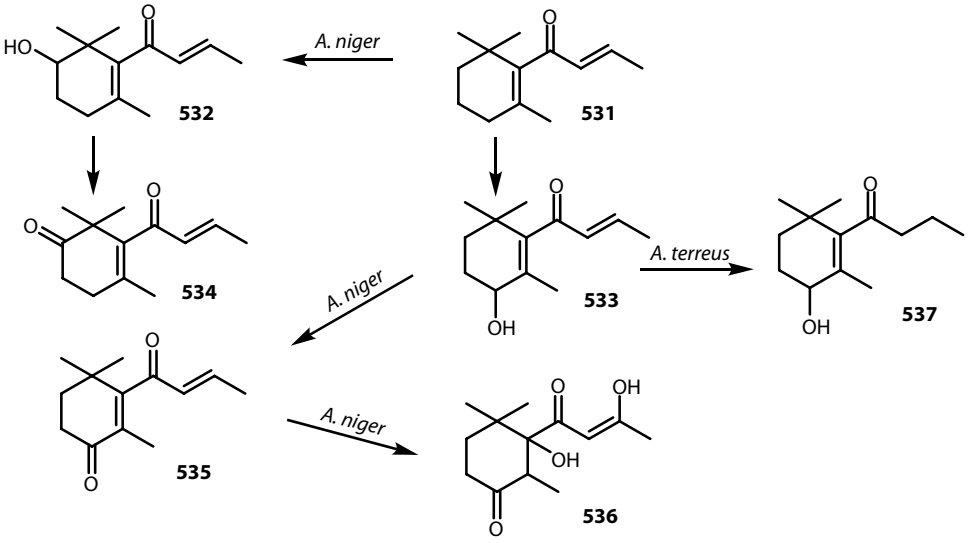


FIGURE 15.156 Biotransformation of β-damascone (**531**) by *Aspergillus niger* and *Aspergillus terreus.*

Botryospherica dothidea, Chlorella pyrenoidosa IMCC-28*, Chlorella sorokiriana, Fusarium culmurorum, Euglena glacilis*, and *Hansenula anomala* (Figure 15.157).

Adamantane (**538**) was incubated with *Aspergillus niger, Aspergillus Cellulosea,* and *Botryosphaeria dothidea* in Czapek-peptone medium. The same substrate was also treated in *Chlorella pyrenoidosa* in Noro medium. Compound **538** was converted into both 1-hydroxy- (**539**) and 9α-hydroxyadamantane (**540**) by all four microorganisms, followed by oxidation oxidized to give 1,9α-dihydroxyadamantanol (**541**) by *Aspergillus Niger,* which was further oxidized to 1-hydroxyadamantane-9-one (**542**), which was reduced to afford 1,9β-hydroxyadamantane (**544**). *Aspergillus niger* gave the metabolite (**541**) as the major product in 80% yield. *Aspergillus cellulosae* converted **538** to **539** and **540** in the ratio of 81:19. *Chlorella pyrenoidosa* gave **539**, **540** and adamantane-9-one (**543**) in the ratio 74:16:10. 4α-Adamantanol (**540**) was directly converted by *Chlorella pyrenoidosa*, *Aspergillus niger*, and *Aspergillus cellulosae* to afford **543**, which was also reduced to 9α-adamantanol (**540**) by *Aspergillus niger.* The biotransformation of adamantane, however, did not occur by the microorganisms: *Hansenula anomala, Chlorella sorokiriana, Dunaliella tertiolecta*, and *Euglena glacilis* (Noma et al., 1999).

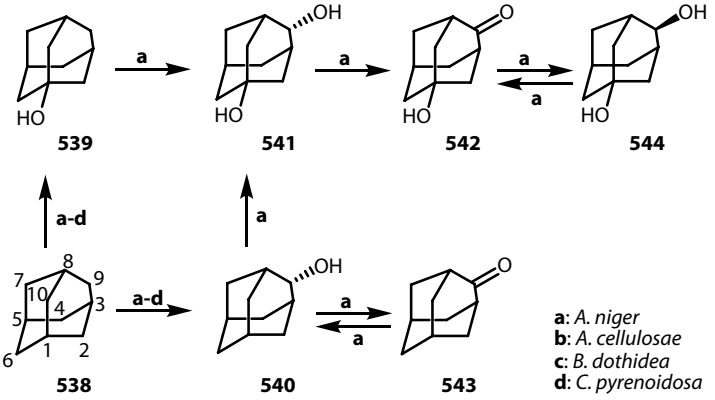


FIGURE 15.157 Biotransformation of adamantane (**538**) by various microorganisms.

Adamantanes (**538**–**543**, **542**) were also incubated with various fungi including with *Fusarium culmurorum*. 1-Hydroxyadamantane-9-one (**542**) was reduced stereoselectively to **541** by *Aspergillus niger, Aspergillus cellulosae*, *Botryosphaeria dothidea*, and *Fusarium culmurorum*. On the contrary, *Fusarium culmurorum* reduced **541**–**542**. *Aspergillus cellulosae* and *Botryosphaeria dothidea* bioconverted **542** to 1,9β-hydroxyadamantane (**544**) stereoselectively. Adamantane-9-one (**543**) was treated by *Aspergillus niger* to give nonsteroselectively **545**–**547** that were further converted into diketone (**548**, **549**) and a diol (**550**). It is noteworthy that oxidation and reduction reactions were observed between ketoalcohol (**547**) and diols (**551**, **552**). The same phenomenon was also seen between **546** and **553**. The latter diol was also oxidized by *Aspergillus niger* to furnish diketone (**549**) (Noma et al., 2001b, 2003). Direct hydroxylation at C3 of 1-hydroxyadamantane- 9-one (**542**) was seen in the incubation of **539** with *Aspergillus niger.*

4-Adamantanone (**543**) showed promotion effect of cell division of the fungus, while 1-adamantanol (**539**) and adamantane-9-one (**543**) inhibited germination of lettuce seed. 1-Hydroxyadamantane-9-one (**542**) inhibited the elongation of root of lettuce while and adamantane-1,4-diol (**544**) and adamantane itself (**538**) promoted root elongation (Noma et al., 1999, 2001b) (Figure 15.158).

Stereoselective reduction of racemic bicycle[3.3.1]nonane-2,6-dione (**555a**, **555a′**) was carried out by *Aspergillus awamori, Aspergillus fumigatus, Aspergillus cellulosae, Aspergillus sojae, Aspergillus terreus, Aspergillus niger, Botryosphaeria dothidea*, and *Fusarium culmorum* in Czapek-peptone, *Hansenula anomala* in yeast, *Euglena glacilis* in Hunter, and *Dunaliella tertiolecta* in Noro medium, respectively. All microorganisms reduced **555** and **555a′** to give

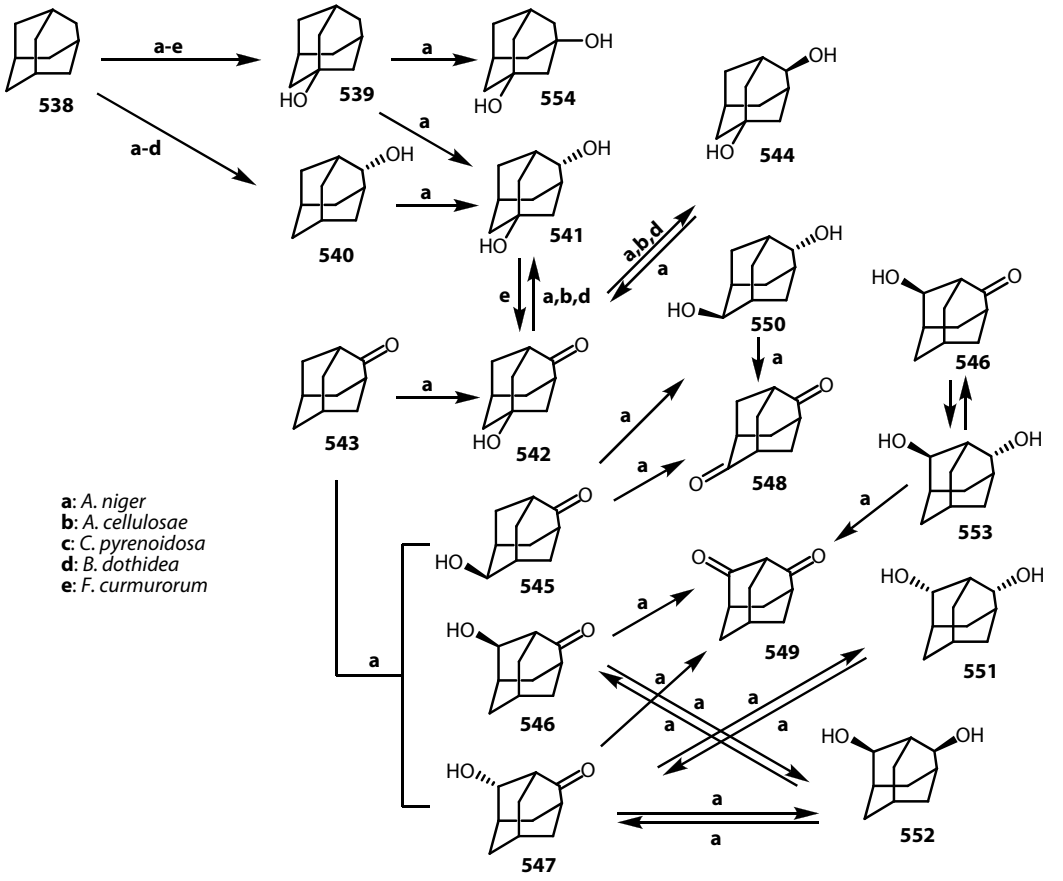


FIGURE 15.158 Biotransformation of adamantane (**538**) and adamantane-9-one by various microorganisms.

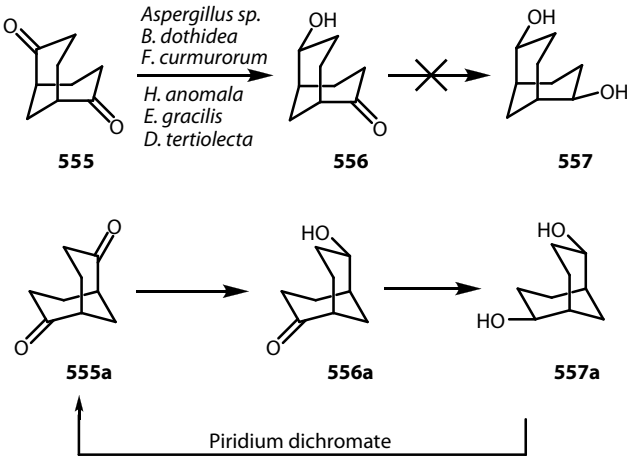


FIGURE 15.159 Biotransformation of bicyclo[3.3.1]nonane-2,6-dione (**555a**, **555a′**) by various microorganisms.

corresponding monoalcohol (**556**, **556a**) and optical-active (–)-diol (**557a**) ($[\alpha]_D$ –71.8° in the case of *Aspergillus terreus*), which was formed by enantioselective reduction of racemic monool, namely **556** and **556a** (Noma et al., 2003) (Figure 15.159).

15.5 BIOTRANSFORMATION OF AROMATIC COMPOUNDS

Essential oils contain aromatic compounds, such as *p*-cymene, carvacrol, thymol, vanillin, cinnamaldehyde, eugenol, chavicol, safrole, and asarone (**558**), among others.

Takahashi (1994) reported that simple aromatic compounds, propylbenzene, hexylbenene, decylbenzene, *o*- and *p*-hydroxypropiophenones, *p*-methoxypropiophenone, 4-hexylresorcinol, and methyl 4-hexylresorcionol were incubated with *Aspergillus niger.* From hexyl- and decylbenzenes, ω1-hydroxylated products were obtained, whereas from propylbenzene, ω2 hydroxylated metabolites were obtained (Takahashi, 1994).

Asarone (**558**) and dihydroeugenol (**562**) were not biotransformed by *Aspergillus niger.* However, dihydroasarone (**559**) and methyldihyroeugenol (**563**) were biotransformed by the same fungus to produce a small amount of 2-hydroxy (**560**, **561**) and 2-oxo derivatives (**564**, **565**), respectively. The chirality at C2 was determined to be *R* and *S* mixtures (1:2) by the modified Mosher method (Takahashi, 1994) (Figure 15.160).

Chlorella species are excellent microalgae as oxidation bioreactors as mentioned earlier. Treatment of monoterpene aldehydes and related aldehydes were reduced to the corresponding primary alcohols, indicating that these green algae possess reductase.

A microalgae, *Euglena gracillis* Z. also contains reductase. The following aromatic aldehydes were treated in this organism. Benzaldehyde, 2-cyanobenzaldehyde, *o*-, *m*-, and *p*-anisaldehyde, salicylaldehyde, *o*-, *m*-, and *p*-tolualdehyde, *o*-chlorobenzaldehyde, *p*-hydroxybenzaldehyde, *o*-nitro-, *m*-, and *p*-nitrobenaldeyde, 3-cyanobenzaldehyde, vanillin, isovanillin, *o*-vanillin, nicotine aldehyde, 3-phenylpropionaldehyde, ethyl vanillin. Veratraldehyde, 3-nitrosalicylaldehde, penylacetaldehyde, and 2-phenylproanaldehyde gave their corresponding primary alcohols. 2-Cyanobenzaldehyde gave its corresponding alcohol with phthalate. *m*- and *p*-Chlorobenzaldehyde gave its corresponding alcohols and *m*- and *p*-chlorobenzoic acids. *o*-Phthaldehyde and *p*-phthalate and iso- and terephthaldehydes gave their corresponding monoalohols and dialcohols. When cinnamaldehyde and α-methyl cinnamaldehyde were incubated in *Euglena gracilis*, cinnamyl alcohol and 3-phenylpropanol, and 2-methylcinnamyl alcohol, and 2-methyo-3-phenylpropanol were obtained

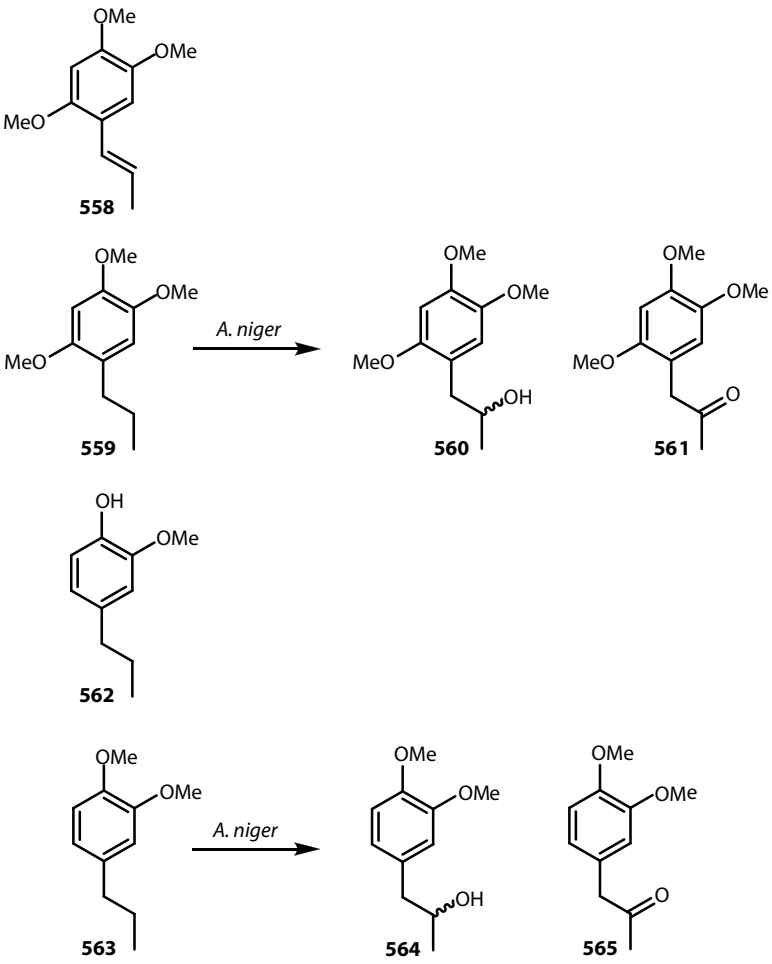


FIGURE 15.160 Biotransformation of dihydroasarone (**559**) and methyldihydro eugenol (**563**) by *Aspergillus niger*.

in good yield. *Euglena gracilis* could convert acetophenone to 2-phenylethanol; however, its enantio-excess is very poor (10%) (Takahashi, 1994).

Raspberry ketone (**566**) and zingerone (**574**) are the major components of raspberry (*Rubus idaeus*) and ginger (*Zingeber officinale*) and these are used as food additive and spice. Two substrates were incubated with the *Phytolacca americana* cultured cells for 3 days to produce two secondary alcohols (**567**, **568**) as well as five glucosides (**569**–**572**) from **566**, and a secondary alcohol (**576**) and four glycoside products (**575**, **577**–**579**) from **574**. In the case of raspberry ketone, phenolic hydroxyl group was preferably glycosylated after the reduction of carbonyl group of the substrate occurred. It is interesting to note that one more hydroxyl group was introduced into the benzene ring to give **568**, which were further glycosylated one of the phenolic hydroxyl groups and no glycocide of the secondary alcohol at C2 were obtained (Figure 15.161).

On the other hand, zingerone (**574**) was converted into **576**, followed by glycosylation to give both glucosides (**577**, **578**) of phenolic and secondary hydroxyl groups and a diglucoside (**579**) of both phenolic and secondary hydroxyl group in the molecule. It is the first report on the introduction of individual glucose residues onto both phenolic and secondary hydroxyl groups by cultured plant cells (Shimoda et al., 2007) (Figure 15.162).

Thymol (**580**), carvacrol (**583**), and eugenol (**586**) were glucosylated by glycosyl transferase of cell-cultured *Eucalyptus perriniana* to each glucoside (**581**, 3%; **584**, 5%; **587**, 7%) and gentiobioside

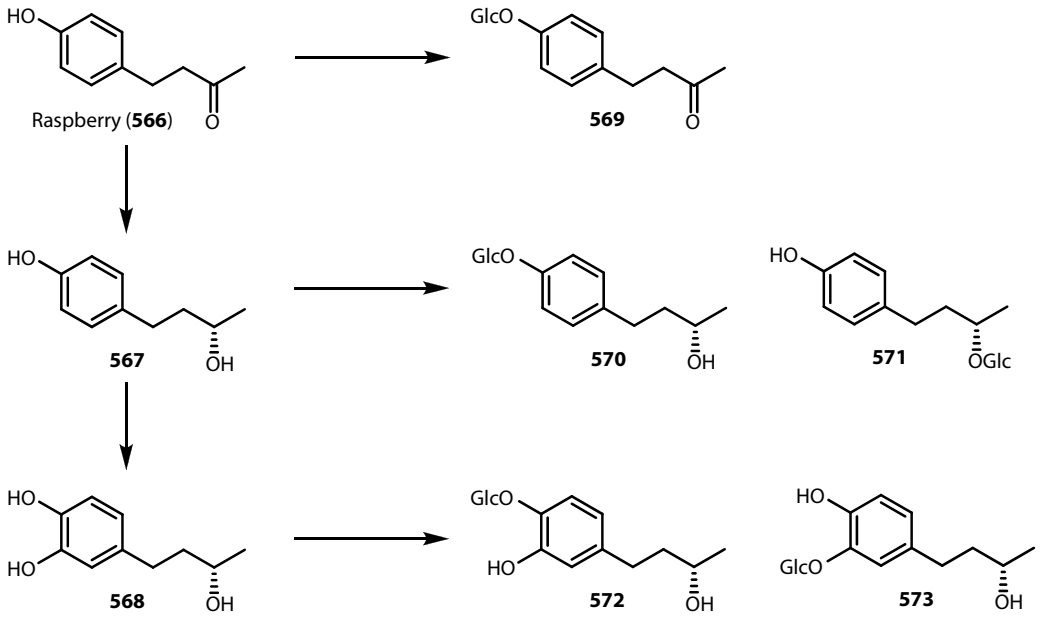


FIGURE 15.161 Biotransformation of raspberry ketone (**566**) by *Phytolacca americana* cells.

(**582**, 87%; **585**, 56%; **588**, 58%). The yield of thymol glycosides was 1.5 times higher than that of carvacrol and 4 times higher than that of eugenol. Such glycosylation is useful to obtain higher water-soluble products from natural and commercially available secondary metabolites for food additives and cosmetic fields (Shimoda et al., 2006) (Figure 15.163).

Hinokitiol (**589**), which is easily obtained from cell suspension cultures of *Thujopsis dolabrata* and possesses potent antimicrobial activity, was incubated with cultured cells of *Eucalyptus perriniana* for 7 days to give its monoglucosides (**590**, **591**, 32%) and gentiobiosides (**592**, **593**) (Furuya et al., 1997, Hamada et al., 1998) (Figure 15.164).

(–)-Nopol benzyl ether (**594**) was smoothly biotransformed by *Aspergillus niger, Aspergillus cellulosae, Aspergillus sojae, Aspergillus Usami*, and *Penicillium* species in Czapek-peptone medium to give (–)-4-oxonopol-2′,4′-dihydroxybenzyl ether (**595**, 23% in the case of *Aspergillus niger*), which demonstrated antioxidant activity (ID_{50} 30.23 μ/M), together with a small amount of nopol (6.3% in *Aspergillus niger*). This is very rare direct introduction of oxygen function on the phenyl ring (Noma and Asakawa, 2006) (Figure 15.165).

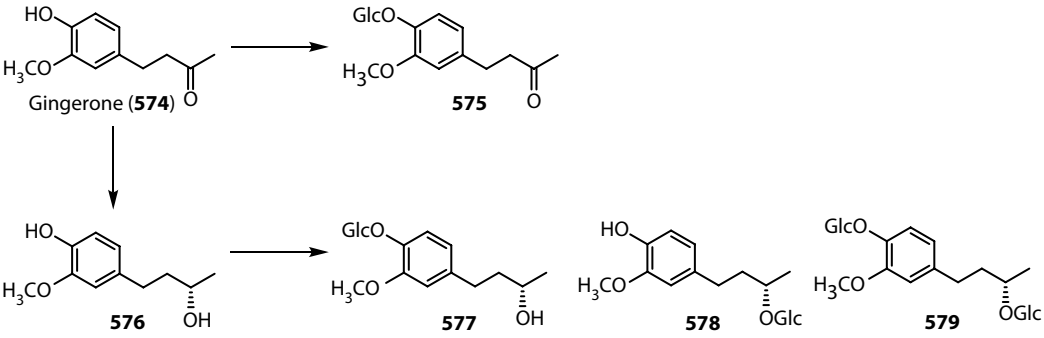


FIGURE 15.162 Biotransformation of zingerone (**574**) by *Phytolacca americana* cells.

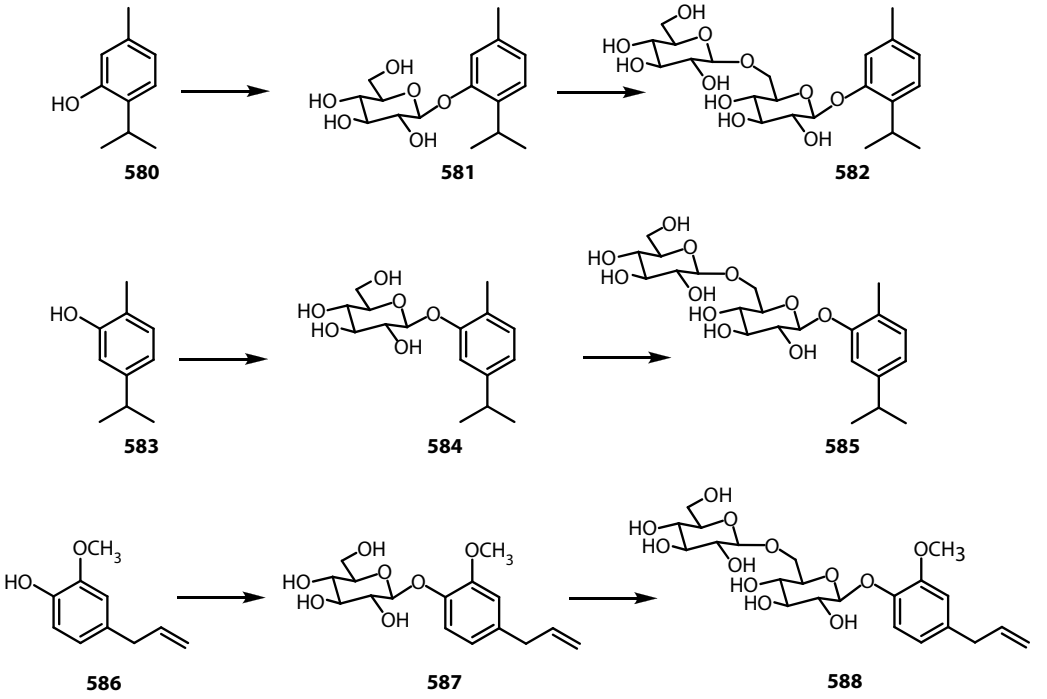


FIGURE 15.163 Biotransformation of thymol (**580**), carvacrol (**583**), and eugenol (**586**) by *Eucalyptus perriniana* cells.

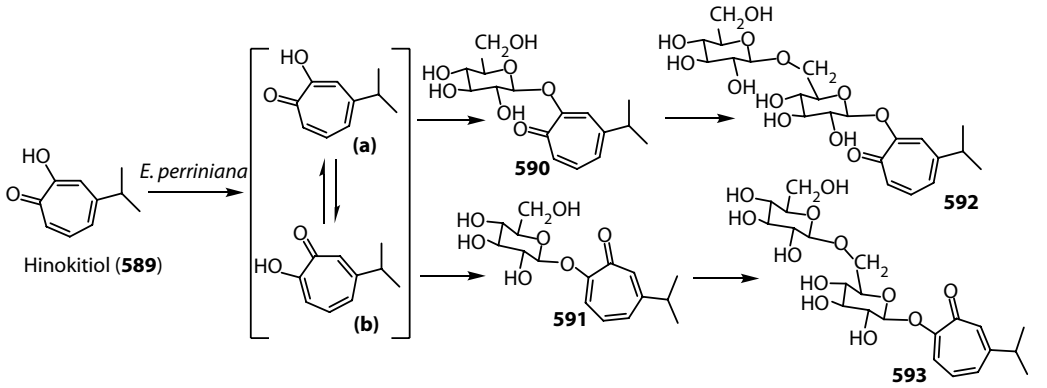


FIGURE 15.164 Biotransformation of hinokitiol (**589**) by *Eucalyptus perriniana* cells.

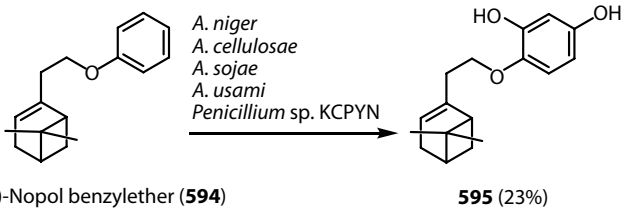


FIGURE 15.165 Biotransformation of nopol benzylether (**531**) by *Aspergillus* and *Penicillium* species.

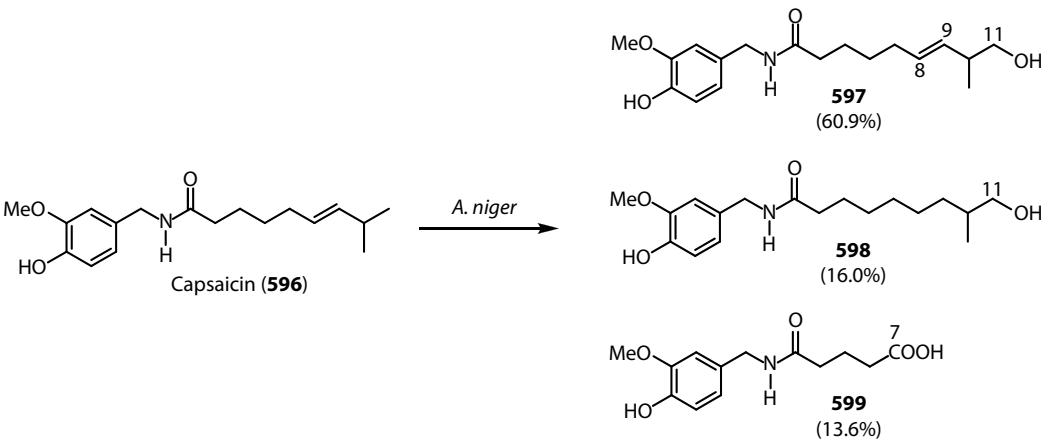


FIGURE 15.166 Biotransformation of capsaicin (**596**) by *Aspergillus niger.*

Capsicum annuum contains capsaicin (**596**) and its homologues having an alkylvanillylamides possess various interesting biological activities such as anti-inflammation, antioxidant, saliva, and stomach juice inducing activity, analgesic, antigenotoxic, antimutagenic, anticarcinogenic, antirheunatoid arthritis, diabetic neuropathy, and used as food additives. On the other hand, because of potent pungency and irritation on skin and mucous membrane, it has not yet been permitted as medicinal drug. In order to reduce this typical pungency and application of nonpungent capsaicin metabolites to the crude drug, capsaicin (**596**) (600 mg) including 30% of dihydrocapsaicin (**600**) was incubated in Czapek-peptone medium including *Aspergillus niger* for 7 days to give three metabolites, ω1-hydroxylated capsaicin (**597**, 60.9%), 8,9-dihydro-ω1-hydroxycapsaicin (**598**, 16%), and a carboxylic acid (**599**, 13.6%). All of the metabolites do not show pungency (Figure 15.166).

Dihydrocapsaicin (**600**) was also treated in the same manner as described above to afford ω1-hydroxydihyrocpsaicin (**598**, 80.9%) in high yield and the carboxylic acid (**599**, 5.0%). Capsaicin itself showed carbachol-induced contraction of 60% in the bronchus at a concentration of 1 μmol/L. 11-Hydroxycapsicin (**85**) retained this activity of 60% at a concentration of 30 μmol/L. Dihydrocapsicin (**600**) showed the same activity of contraction in the bronchus, at the same concentration as that used in capsaicin. However, the activity of contraction in the bronchus of 11-hydroxy derivative (**598**) showed weaker (50% at 30 μmol/L) than that of the substrate. Since both metabolites (**597** and **598**) are tasteless, these products might be valuable for the crude drug although the contraction in the bronchus is weak. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity test of capsaicin and dihydrocapsaicin derivatives was carried out. 11-Hydroxycapsaicin (**597**), 11-dihydrocapsaicin (**598**), and capsaicin (**596**) showed higher activity than (±)-α-tocopherol and 11-dihydroxycapsaicin (**598**) displayed the strong scavenging activity (IC_{50} 50 μmol/L) (Hashimoto, Asakawa, unpublished results) (Figure 15.167).

Shimoda et al. (2007a) reported the bioconversion of capsaicin (**596**) and 8-nordihydrocapsaicin (**601**) by the cultured cell of *Catharathus roseus* to give more water-soluble capsaicin derivatives. From capsaicin, three glycosides, capsaicin 4-*O*-β-D-glucopyranoside (**602**), which was one of the capsaicinoids in the fruit of *Capsicum* and showed 1/100 weaker pungency than capsaicin, 4-*O*-(6-*O*-β-D-xylopyranosyl)-β-D-glucoside (**603**) and 4-*O*-(6-*O*-α-L-arbinosyl)-β-D-glucopyranoside (**604**) were obtained. 8-Nor-dihydrocapsaicin (**601**) was also incubated with the same cultured cell to afford the similar products (**605**–**607**) all of which reduced their pungency and enhanced water solubility. Since many synthetic capsaicin glycosides possess remarkable pharmacological activity, such as decrease of liver and serum lipids, the present products will be used for valuable prodrugs (Figure 15.168).

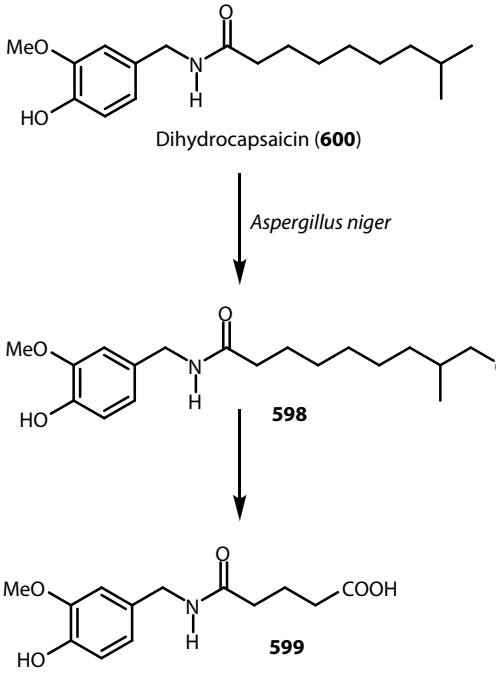


FIGURE 15.167 Biotransformation of dihydrocapsaicin (**600**) by *Aspergillus niger.*

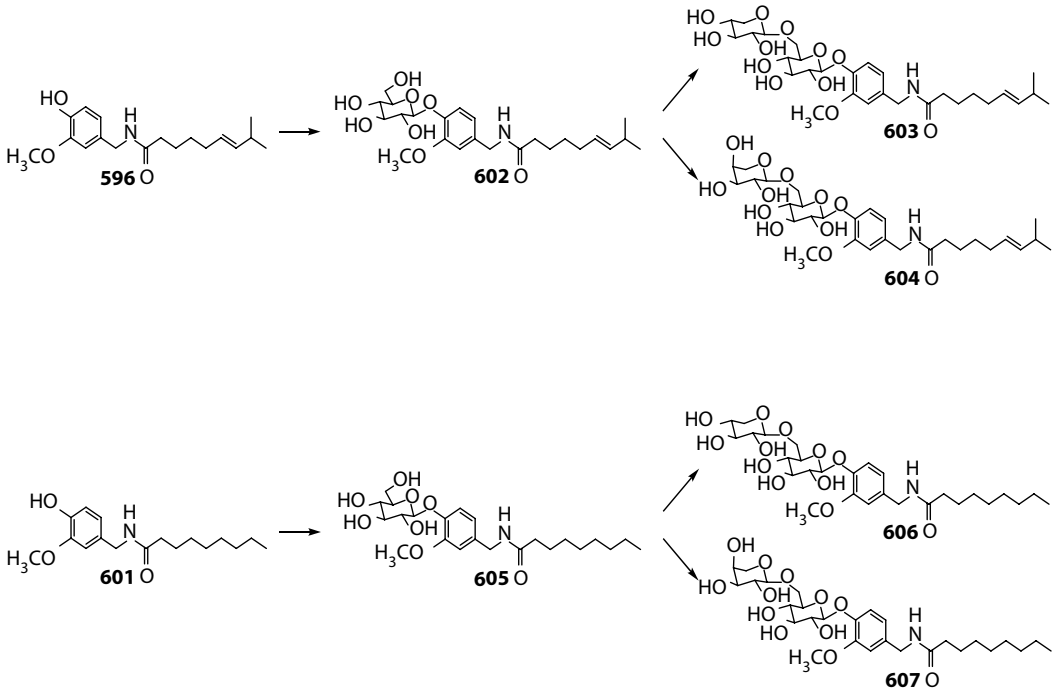


FIGURE 15.168 Biotransformation of capsaicin (**596**) and 8-nor-dihydrocapsaicin (**601**) by *Catharanthus roseus* cells.

Zingiber officinale contains various sesquiterpenoids and pungent aromatic compounds such as 6-shogaol (**608**) and 6-gingerol (**613**) and their pungent compounds that possess cardio tonic and sedative activity. 6-Shogaol (**608**) was incubated with *Aspergillus niger* in Czapek-peptone medium for 2 days to afford ω1-hydroxy-6-shagaol (**609**, 9.9%), which was further converted to 8-hydroxy derivative (**610**, 16.1%), a γ-lactone (**611**, 22.4%), and 3-methoxy-4-hydroxyphenylacetic acid (**612**, 48.5%) (Figure 15.169).

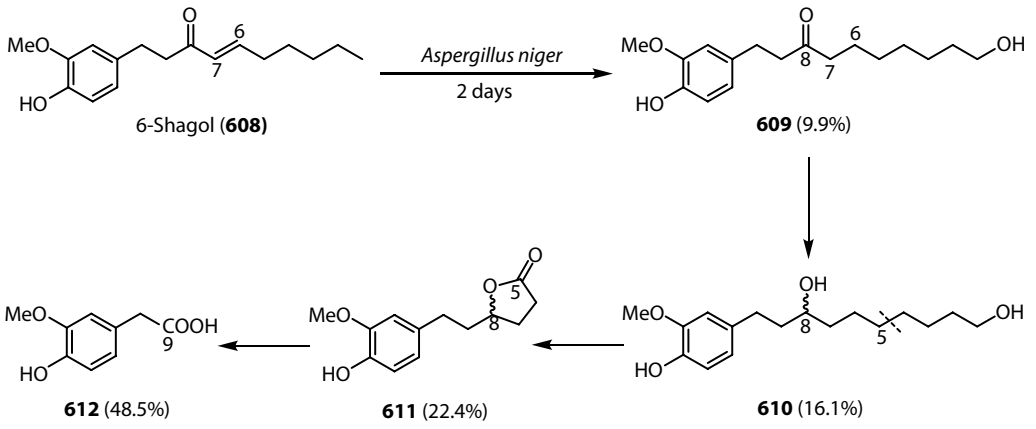


FIGURE 15.169 Biotransformation of 6-shogaol (**608**) by *Aspergillus niger*.

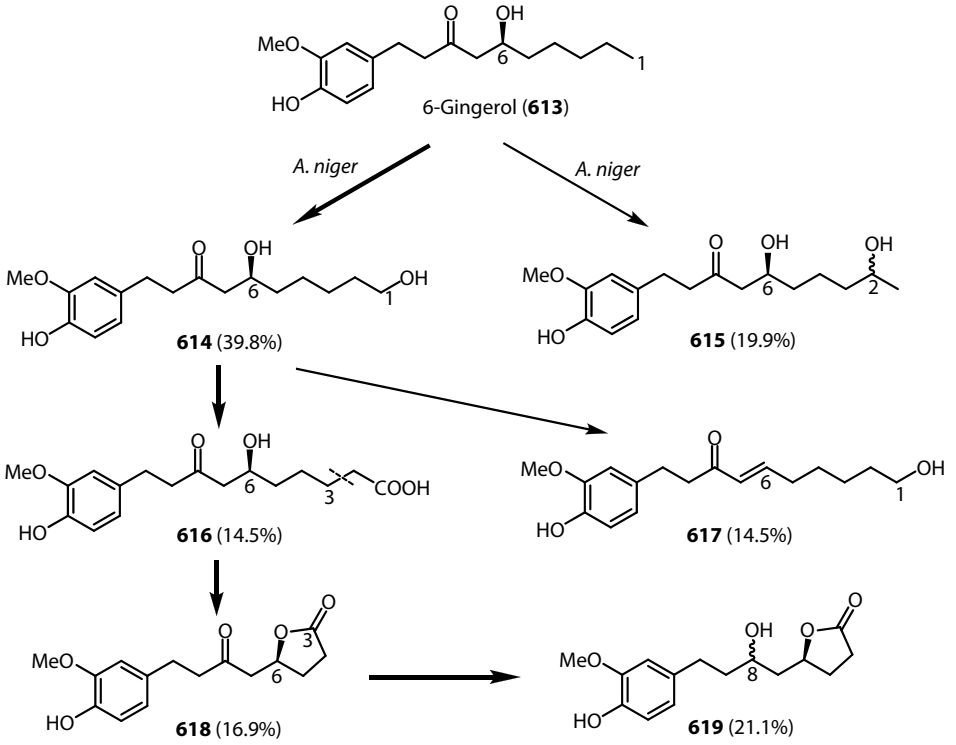


FIGURE 15.170 Biotransformation of 6-gingerol (**613**) by *Aspergillus niger*.

6-Gingerol (**613**) (1 g) was treated in the same condition as mentioned above to yield six metabolites, ω1-hydroxy-6-gingerol (**614**, 39.8%), its carboxylic derivative (**616**, 14.5%), a γ-lactone (**618**) (16.9%) that might be formed from (**616**), its 8-hydoxy-γ-lactone (**619**, 12.1%), ω2-hydroxy-6-gingerol (**615**, 19.9%), and 6-deoxy-gingerol (**617**, 14.5%) (Takahashi et al., 1993).

The metabolic pathway of 6-gingerol (**613**) resembles that of 6-shagaol (**608**). That of 6-shogaol and dihydrocapsaicin (**600**) is also similar since both substrates gave carboxylic acids as the final metabolites (Takahashi et al., 1993) (Figure 15.170).

In conclusion, a number of sesquiterpenoids were biotransformed by various fungi and mammals to afford many metabolites, several of which showed antimicrobial and antifungal, antiobesity, cytotoxic, neurotrophic, and enzyme inhibitory activity. Microorganisms introduce oxygen atom at allylic position to give secondary hydroxyl and keto groups. Double bond is also oxidized to give epoxide, followed by hydrolysis to afford a diol. These reactions precede stereo- and regiospecifically. Even at nonactivated carbon atom, oxidation reaction occurs to give primary alcohol. Some fungi like *Aspergillus niger* cleave the cyclopropane ring with a 1,1-dimethyl group. It is noteworthy that *Aspergillus niger* and *Aspergillus cellulosae* produce the totally different metabolites from the same substrates. Some fungi occurs reduction of carbonyl group, oxidation of aryl methyl group, phenyl coupling, and cyclization of a 10-membered ring sesquiterpenoids to give C6/C6- and C5/C7-cyclic or spiro compounds. Cytochrome P-450 is responsible for the introduction of oxygen function into the substrates.

The present methods are very useful for the production of medicinal and agricultural drugs as well as fragrant components from commercially available cheap, natural, and unnatural terpenoids or a large amount of terpenoids from higher medicinal plants and spore-forming plants like liverworts and fungi.

The methodology discussed in this chapter is a very simple one-step reaction in water, nonhazard, and very cheap, and it gives many valuable metabolites possessing different properties from those of the substrates.

REFERENCES

Abraham, W.-R., P. Washausen, and K. Kieslich, 1987. Microbial hydroxylation of cedrol and cedrene. *Z. Naturforsch.*, 42C: 414–419.

Abraham, W.R., L. Ernst, and B. Stumpf, 1990. Biotransformation of caryophyllene by *Diplodia gossypina*. *Phytochemisrty*, 29: 115–120.

Abraham, W.-R., K. Kieslich, B. Stumpf, and L. Ernst, 1992. Microbial oxidation of tricyclic sesquiterpenoids containing a dimethylcyclopropane ring. *Phytochemistry*, 31: 3749–3755.

Aleu, J., J.R. Hanson, R. Hernandez-Galan, and I.G. Collado, 1999. Biotransformation of the fungistatic sesquiterpenoid patchoulol by *Botrytis cinerea*. *J. Nat. Prod.*, 62: 437–440.

Aleu, J., R. Hernandez-Galan, J.R. Hanson, P.B. Hitchcock, and I.G. Collado, 1999a. Biotransformation of the fungistatic sesquiterpenoid ginsenol by *Botrytis cinerea*. *J. Chem. Soc., Perkin Trans*., 1: 727–730.

Aleu, J., R. Hernandez-Galan, and I.G. Collad, 2002. Biotransformation of the fungistatic sesquiterpenoid isobotryan-9α-ol by *Botrytis cinerea*. *J. Mol. Catal. B*, 16: 249–253.

Amate, A., A. Garcia-Granados, A. Martinez, et al., 1991. Biotransformation of 6α-eudesmanolides functionalized at C-3 with *Curvularia lunata* and *Rhizopus nigricans* cultures. *Tetrahedron*, 47: 5811–5818.

Aranda, G., M.S. Kortbi, J.-Y. Lallemand, et al., 1991. Microbial transformation of diterpenes: Hydroxylation of sclareol, manool and derivatives by *Mucor plumbeus*. *Tetrahedron*, 47: 8339–8350.

Aranda G., I. Facon, J.-Y. Lallemand, and M. Leclaire 1992. Microbiological hydroxylation in the drimane series. *Tetrahedron Lett.*, 33, 7845–7848.

Arantes, S.F., J.R. Hanson, and P.B. Hitchcok, 1999. The hydroxylation of the sesquiterpenoid valerianol by *Mucor plumbeu*s. *Phytochemistry*, 52: 1063–1067.

Asakawa, Y., 1982. Chemical constituents of the Hepaticae. In: *Progress in the Chemistry of Organic Natural Products*, W. Herz, H. Grisebach, and G.W. Kirby, eds, Vol. 42, pp. 1–285. Vienna: Springer.

Asakawa, Y., 1995. Chemical constituents of the bryophytes. In: *Progress in the Chemistry of Organic Natural Products*; W. Herz, G.W. Kirby, R.E. Moore, W. Steglich, and Ch. Tamm, eds, Vol. 65, pp. 1–618. Vienna: Springer.

Asakawa, Y., 1990. Terpenoids and aromatic compounds with pharmaceutical activity from bryophytes. In: *Bryophytes: Their Chemistry and Chemical Taxonomy*, D.H. Zinsmeister and R. Mues, eds, pp. 369–410. Oxford: Clarendon Press.

Asakawa, Y., 1999. Phytochemistry of bryophytes. In: *Phytochemicals in Human Health Protection, Nutrition, and Plant Defense*, J. Romeo, ed., Vol. 33, pp. 319–342. New York: Kluwer Academic, Plenum Publishers.

Asakawa, Y., 2007. Biologically active compounds from bryophytes. *Pure Appl. Chem.*, 75: 557–580.

Asakawa, Y., 2008. Recent advances of biologically active substances from the Marchantiophyta. *Nat. Prod. Commun.*, 3: 77–92.

Asakawa, Y., Z. Taira, T. Takemoto, T. Ishida, M. Kido, and Y. Ichikawa, 1981. X-ray crystal structure analysis of 14-hydroxycaryophyllene oxide, a new metabolite of (−)-caryophyllene in rabbits. *J. Pharm. Sci.*, 70: 710–711.

Asakawa, Y., T. Ishida, M. Toyota, and T. Takemoto, 1986. Terpenoid biotransformation in mammals IV. Biotransformation of (+)-longifolene, (−)-caryophyllene, (−)-caryophyllene oxide, (−)-cyclocolorenone, (+)-nootkatone, (−)-elemol, (−)-abietic acid and (+)-dehydroabietic acid in rabbits. *Xenobiotica*, 6: 753–767.

Asakawa, Y., H. Takahashi, and M. Toyota, 1991. Biotransformation of germacrane-type sesquiterpenoids by *Aspergillus niger*. *Phytochemistry*, 30: 3993–3997.

Asakawa, Y., T. Hashimoto, Y. Mizuno, M. Tori, and Y. Fukuzawa, 1992. Cryptoporic acids A-G, drimane-type sesquiterpenoid ethers of isocitric acid from the fungus *Cryptoporus volvatus*. *Phytochemistry*, 31: 579–592.

Asakawa, Y. and A. Ludwiczuk, 2008. Bryophytes-Chemical diversity, bioactivity and chemosystematics. Part 1. Chemical diversity and bioactivity. *Med. Plants Poland World,* 14: 33–53.

Ata, A. and J.A. Nachtigall, 2004. Microbial transformation of α-santonin. *Z. Naturforsch.,* 59C: 209–214.

Atta-ur Rahman, M.I. Choudhary, A. Ata, et al., 1994. Microbial transformation of 7α-hydroxyfrullanolide. *J. Nat. Prod.*, 57: 1251–1255.

Atta-ur-Rahman, A. Farooq, and M.I. Choudhary, 1997. Microbial transformation of sclareolide. *J. Nat. Prod.*, 60: 1038–1040.

Atta-ur Rahman, M.I. Choudhary, F. Shaheen, A. Rauf, and A. Farooq, 1998. Microbial transformation of some bioactive natural products. *Nat. Prod. Lett.*, 12: 215–222.

Ayer, W.A. and P.A. Craw, 1989. Metabolites of fairy ring fungus, *Marasmius oreades.* Part 2. Norsesquiterpenes, further sesquiterpenes, and argocybin. *Can. J. Chem.*, 67: 1371–1380.

Bang, L. and G. Ourisson, 1975. Hydroxylation of cedrol by rabbits. *Tetrahedron Lett.*, 16: 1881–1884.

Bang, L., G. Ourisson, and P. Teisseire, 1975. Hydroxylation of patchoulol by rabbits. Hemisynthesis of nor-patchoulenol, the odour carrier of patchouli oil. *Tetrahedron Lett.*, 16: 2211–2214.

Barrero, A.F., J.E. Oltra, D.S. Raslan, and D.A. Sade, 1999. Microbial transformation of sesquiterpene lactones by the fungi *Cunninghamella echinulata* and *Rhizopus oryzae*. *J. Nat. Prod.*, 62: 726–729.

Bhutani, K.K. and R.N. Thakur, 1991. The microbiological transformation of parthenin by *Beauveria bassiana* and *Sporotrichum pulverulentum*. *Phytochemistry*, 30: 3599–3600.

Buchanan, G.O., L.A.D. Williams, and P.B. Reese, 2000. Biotransformation of cadinane sesquiterpenes by *Beauveria bassiana* ATCC 7159. *Phytochemistry*, 54: 39–45.

Choudhary, M.I., S.G. Musharraf, A. Sami, and Atta-ur-Rahman, 2004. Microbial transformation of sesquiterpenes, (−)-ambrox® and (+)-sclareolide. *Helv. Chim. Acta*, 87: 2685–2694.

Choudhary, M.I., S.G. Musharraf, S.A. Nawaz, et al., 2005. Microbial transformation of (−)-isolongifolol and butyrylcholinesterase inhibitory activity of transformed products. *Bioorg. Med. Chem*., 13: 1939–1944.

Choudhary, M.I., Z.A. Siddiqui, S.A. Nawaz, and Atta-ur-Rahman., 2006. Microbial transformation and butyrylcholinesterase inhibitory activity of (−)-caryophyllene oxide and its derivatives. *J. Nat. Prod.*, 69: 1429–1434.

Choudhary, M.I., W. Kausar, Z.A. Siddiqui, and Atta-ur-Rahman., 2006a. Microbial Metabolism of (+)-Cycloisolongifol-5β-ol. *Z. Naturforsch.*, 61B: 1035–1038.

Clark, A.M. and C.D. Hufford, 1979. Microbial transformation of the sesquiterpene lactone costunolide. *J. Chem. Soc. Perkin Trans.*, 1: 3022–3028.

Collins, D.O. and P.B. Reese, 2002. Biotransformation of cadina-4,10(15)-dien-3-one and 3α-hydroxycadina-4,10(15)-diene by *Curvularia lunata* ATCC 12017. *Phytochemistry*, 59: 489–492.

Collins, D.O., P.L.D. Ruddock, J. Chiverton, C. de Grasse, W.F. Reynolds, and P.B. Reese, 2002. Microbial transformation of cadina-4,10(15)-dien-3-one, aromadendr-1(10)-en-9-one and methyl ursolate by *Mucor plumbeus* ATCC 4740. *Phytochemistry*, 59: 479–488.

Collins, D.O., W.F. Reynold, and P.B. Reese, 2002. Aromadendrane transformations by *Curvularia lunata* ATCC 12017. *Phytochemistry*, 60: 475–481.

Daniewski, W.M., P.A. Grieco, J. Huffman, A. Rymkiewicz, and A. Wawrzun, 1981. Isolation of 12-hydroxycaryophyllene-4,5-oxide, a sesquitepene from *Lactarius camphorates*. *Phytochemistry*, 20: 2733–2734.

Darrouzet, E., B. Mauchamp, G.D. Prestwich, L. Kerhoas, I. Ujvary, and F. Couillaud 1997. Hydroxy juvenile hormones: new putative juvenile hormones biosynthesized by locust corpora allata *in vitro. Biochem. Biophys. Res. Commun.*, 240: 752–758.

Devi, J.R., 1979. Microbiological transformation of terpenes: Part XXVI. Microbial transformation of caryophyllene. *Ind. J. Biochem. Biophys*., 16: 76–79.

Dhavlikar, R.S. and G. Albroscheit, 1973. Microbiologische Umsetzung von Terpenen: Valencen. *Dragoco Rep.*, 12: 250–258.

Duran, R., E. Corrales, R. Hernandez-Galan, and G. Collado, 1999. Biotransformation of caryophyllene oxide by *Botrytis cinerea*. *J. Nat. Prod.*, 62: 41–44.

El Sayed, K.A., M. Yousaf, M.T. Hamann, M.A. Avery, M. Kelly, and P. Wipf, 2002. Microbial and chemical transformation studies of the bioactive marine sesquiterpenes (*S*)-(+)-curcuphenol and -curcudiol isolated from a deep reef collection of the Jamaican sponge *Didiscus oxeata*. *J. Nat. Prod.*, 65: 1547–1553.

Furusawa, M., Y. Noma, T. Hashimoto, and Y. Asakawa, 2003. Biotransformation of Citrus oil nootkatone, dihydronootkatone and dehydronootkatone. *Proc. 47th TEAC*: 142–144.

Furusawa, M., T. Hashimoto, Y. Noma, and Y. Asakawa, 2005. Biotransformation of Citrus aromatics nootkatone and valencene by microorganisms. *Chem. Pharm. Bull.*, 53: 1423–1429.

Furusawa, M., T. Hashimoto, Y. Noma, and Y. Asakawa 2005a. Highly efficient production of nootkatone, the grapefruit aroma from valencene, by biotransformation. *Chem. Pharm. Bull.*, 53: 1513–1514.

Furusawa, M., T. Hashimoto, Y. Noma, and Y. Asakawa, 2005b. The structure of new sesquiterpenoids from the liverwort *Reboulia hemisphaerica* and their biotransformation. *Proc. 49th TEAC*: 235–237.

Furusawa, M., 2006. *Microbial biotransformation of sesquiterpenoids from crude drugs and liverworts: Production of functional substances*. PhD thesis. Tokushima Bunri University, pp. 1–156.

Furusawa, M., T. Hashimoto, Y. Noma, and Y. Asakawa, 2006a. Biotransformation of aristolene- and 2,3-secoaromadendrane-type sesquiterpenoids having a 1,1-dimethylcyclopropane ring by *Chlorella fusca* var. *vacuolata, Mucor* species, and *Aspergillus niger*. *Chem. Pharm. Bull*., 54: 861–868.

Furusawa, M., T. Hashimoto, Y. Noma, and Y. Asakawa, 2006b. Isolation and structures of new cyclomyltaylane and ent-chamigrane-type sesquiterpenoids from the liverwort *Reboulia hemisphaerica. Chem. Pharm. Bull*., 54: 996–1003.

Furuya, T., Y. Asada, Y. Matsuura, S. Mizobata, and H. Hamada, 1997. Biotransformation of b-thujaplicin by cultured cells of *Eucalyptus perriniana. Phytochemistry*, 46: 1355–1358.

Galal, A.M., A.S. Ibrahim, J.S. Mossa, and F.S. El-Feraly, 1999. Microbial transformation of parthenolide. *Phytochemistry*, 51: 761–765.

Galal, A.M., 2001. Microbial transformation of pyrethrosin. *J. Nat. Prod.*, 64: 1098–1099.

Garcia-Granados, A., M.C. Gutierrez, F. Rivas, and J.M. Arias, 2001. Biotransformation of 4β-hydroxyeudesmane- 1,6-dione by *Gliocladium roseum* and *Exserohilum halodes*. *Phytochemistry*, 58: 891–895.

Hamada, H., F. Murakami, and T. Furuya, 1998. The production of hinokitiol glycoside. *Proc. 42nd TEAC*: 145–147.

Hanson, J.R. and H. Nasir, 1993. Biotransformation of the sesquiterpenoid, cedrol, by *Cephalosporium aphidicola*. *Phytochemistry*, 33: 835–837.

Hanson, J.R. and A. Truneh, 1996. The biotransformation of ambrox and sclareolide by *Cephalosporium aphidicola*. *Phytochemistry*, 42: 1021–1023.

Hanson, R.L., J.M. Wasylyk, V.B. Nanduri, D.L. Cazzulino, R.N. Patel, and L.J. Szarka, 1994. Site-specific enzymatic hydrolysis of tisanes at C-10 and C-13. *J. Biol. Chem*., 269: 22145–22149.

Harinantenaina, L., Y. Noma, and Y. Asakawa, 2005. *Penicillium sclerotiorum* catalyzes the conversion of herbertenediol into its dimers: mastigophorenes A and B. *Chem. Pharm. Bull.*, 53: 256–257.

Harinantenaina, L., D.N. Quang, T. Nishizawa, et al., 2007. Bioactive compounds from liverworts: Inhibition of lipopolysaccharide-induced inducible NOS mRNA in RAW 264.7 cells by herbertenoids and cuparenoids. *Phytomedicine*, 14: 486–491.

Hashimoto, T., S. Kato, M. Tanaka, S. Takaoka, and Y. Asakawa, 1998. Biotransformation of sesquiterpenoids by microorganisms (4): Biotransformation of hinesol by *Aspergillus niger*. *Proc. 42nd TEAC*: 127–129.

Hashimoto, T., K. Shiki, M. Tanaka, S. Takaoka, and Y. Asakawa, 1998a. Chemical conversion of labdane-type diterpenoid isolated from the liverwort *Porella perrottetiana* into (–)-ambrox. *Heterocycles*, 49: 315–325.

Hashimoto, T., Y. Noma, Y. Akamatsu, M. Tanaka, and Y. Asakawa, 1999. Biotransformation of sesquiterpenoids by microorganisms. (5): Biotransformation of dehydrocostuslactone. *Proc. 43rd TEAC*: 202–204.

Hashimoto, T., Y. Noma, Y. Matsumoto, Y. Akamatsu, M. Tanaka, and Y. Asakawa, 1999a. Biotransformation of sesquiterpenoids by microorganisms. (6): Biotransformation of α-, β- and γ-cyclocostunolides. *Proc. 43rd TEAC*: 205–207.

Hashimoto, T., Y. Noma, S. Kato, M. Tanaka, S. Takaoka, and Y. Asakawa, 1999b. Biotransformation of hinesol isolated from the crude drug *Atractylodes lancea* by *Aspergillus niger* and *Aspergillus cellulosae*. *Chem. Pharm. Bull*., 47: 716–717.

Hashimoto, T., Y. Noma, H. Matsumoto, Y. Tomita, M. Tanaka, and Y. Asakawa, 2000. Microbial biotransformation of optically active (+)-α-ionone and (−)-α-ionone. *Proc. 44th TEAC*: 154–156.

Hashimoto, T., Y. Noma, C. Murakami, M. Tanaka, and Y. Asakawa, 2000a. Microbial transformation of α-santonin derivatives and nootkatone. *Proc. 44th TEAC*: 157–159.

Hashimoto, T., Y. Noma, and Y. Asakawa, 2001. Biotransformation of terpenoids from the crude drugs and animal origin by microorganisms. *Heterocycles*, 54: 529–559.

Hashimoto, T., Y. Noma, C. Murakami, N. Nishimatsu, M. Tanaka, and Y. Asakawa, 2001a. Biotransformation of valencene and aristolene. *Proc. 45th TEAC*: 345–347.

Hashimoto, T., Y. Asakawa, Y. Noma, et al., 2003. Production method of nootkatone. *Jpn. Kokai Tokkyo Koho*, 250591A.

Hashimoto, T., Y. Asakawa, Y. Noma, et al., 2003a. Production method of nootkatone. *Jpn. Kokai Tokkyo Koho*, 70492A.

Hashimoto, T., Y. Noma, N. Nishimatsu, M. Sekita, M. Tanaka, and Y. Asakawa, 2003b. Biotransformation of antimalarial sesquiterpenoids by microorganisms. *Proc. 47th TEAC*: 136–138.

Hashimoto, T., Y. Noma, Y. Goto, S. Takaoka, M. Tanaka, and Y. Asakawa, 2003c. Biotransformation of sesquiterpenoids from the liverwort *Plagiochila* species. *Proc. 47th TEAC*: 139–141.

Hashimoto, T., Y. Noma, Y. Goto, M. Tanaka, S. Takaoka, and Y. Asakawa, 2004. Biotransformation of (−)-maalioxide by *Aspergillus niger* and *Aspergillus cellulosae*. *Heterocycles*, 62: 655–666.

Hashimoto, T., M. Sekita, M. Furusawa, Y. Noma, and Y. Asakawa, 2005. Biotransformation of sesquiterpene lactones, (−)-parthenolide and (−)-frullanolide by microorganisms. *Proc. 49th TEAC*: 387–389.

Hashimoto, T., Y. Noma, and Y. Asakawa, 2006. Biotransformation of cuparane- and herbertane-type sesquiterpenoids. *Proc. 50th TEAC*: 263–265.

Hashimoto, T. and Y. Asakawa, 2007. Biological activity of fragrant substances from Citrus and herbs, and production of functional substances using microbial biotransformation. In: *Development of Medicinal Foods*; M. Yoshikawa, ed., pp. 168–184. Tokyo: CMC Publisher.

Hashimoto, T., M. Fujiwara, K. Yoshikawa, A. Umeyama, M. Tanaka, and Y. Noma, 2007. Biotransformation of sclareolide and sclareol by microorganisms. *Proc. 51st TEAC*: 316–318.

Hayashi, K., H. Morikawa, H. Nozaki, and D. Takaoka, 1998. Biotransformation of globulol and epiglubulol by *Aspergillus niger* IFO 4407. *Proc. 42nd TEAC*: 136–138.

Hayashi, K., H. Morikawa, A. Matsuo, D. Takaoka, and H. Nozaki, 1999. Biotransformation of sesquiterpenoids by *Aspergillus niger* IFO 4407. *Proc. 43rd TEAC*: 208–210.

Haze, S., K. Sakai, and Y. Gozu, 2002. Effects of fragrance inhalation on sympathetic activity in normal adults. *Jpn. J. Pharmacol*., 90: 247–253.

Hegazy, M.-E.F., C. Kuwata, Y. Sato, et al., 2005. Research and development of asymmetric reaction using biocatalysts-biotransformation of enones by cultured cells of *Marchantia polymorpha. Proc. 49th TEAC*: 402–404.

Higuchi, H., R. Tsuji, K. Hayashi, D. Takaoka, A. Matsuo, and H. Nozaki, 2001. Biotransformation of sesquiterpenoids by *Aspergillus niger. Proc. 45th TEAC*: 354–355.

Hikino, H., T. Konno, T. Nagashima, T. Kohama, and T. Takemoto, 1971. Stereoselective epoxidation of germacrone by *Cunninghamella blakesleeana*. *Tetrahedron Lett.*, 12: 337–340.

Hrdlicka, P.J., A.B. Sorensen, B.R. Poulsen, G.J.G. Ruijter, J. Visser, and J.J. L. Iversen 2004. Characterization of nerolidol biotransformation based on indirect on-line estimation of biomass concentration and physiological state in bath cultures of *Aspergillus niger*. *Biotech. Prog.,* 20: 368–376.

Ishida, T., Y. Asakawa, T. Takemoto, and T. Aratani, 1981. Terpenoid biotransformation in mammals. III. Biotransformation of α-pinene, β-pinene, pinane, 3-carene, carane, myrcene and p-cymene in rabbits. *J. Pharm. Sci.,* 70: 406–415.

Ishida, T., Y. Asakawa, and T. Takemoto, 1982. Hydroxyisolongifolaldehyde: A new metabolite of (+)-longifolene in rabbits. *J. Pharm. Sci.,* 71: 965–966.

Ishida, T., 2005. Biotransformation of terpenoids by mammals, microorganisms, and plant-cultured cells. *Chem. Biodivers.*, 2: 569–590.

Itsuzaki, Y., K. Ishisaka, and M. Miyazawa, 2002. Biotransformation of (+)-cedrol by using human skin microbial flora *Staphylococcus epidermidis*. *Proc. 46th TEAC*: 101–102.

Jacobsson, U., V. Kumar, and S. Saminathan, 1995. Sesquiterpene lactones from *Michelia champaca*. *Phytochemistry*, 39: 839–843.

Kaspera, R., U. Krings, T. Nanzad, and R.G. Berger, 2005. Bioconversion of (+)-valencene in submerged cultures of the ascomycete *Chaetomium globosum*. *Appl. Microbiol. Biotechnol*., 67: 477–583.

Kiran, I., H.N. Yildirim, J.R. Hanson, and P.B. Hitchcock, 2004. The antifungal activity and biotransformation of diisophorone by the fungus *Aspergillus niger*. *J. Chem. Technol. Biotechnol.*, 79: 1366–1370.

Kiran, I., T. Akar, A. Gorgulu, and C. Kazaz, 2005. Biotransformation of racemic diisophorone by *Cephalosporium aphidicola* and *Neurospora crassa*. *Biotechnol. Lett.*, 27: 1007–1010.

Kumari, G.N.K., S. Masilamani, R. Ganesh, and S. Aravind, 2003. Microbial biotransformation of zaluzanin D. *Phytochemistry*, 62: 1101–1104.

Lahlou, E.L., Y. Noma, T. Hashimoto, and Y. Asakawa, 2000. Microbiotransformation of dehydropinguisenol by *Aspergillus* sp. *Phytochemistry*, 54: 455–460.

Lamare, V. and R. Furstoss, 1990. Bioconversion of sesquiterpenes. *Tetrahedron*, 46: 4109–4132.

Lee, I.-S., H.N. ElSohly, E.M. Coroom, and C.D. Hufford, 1989. Microbial metabolism studies of the antimalarial sesquiterpene artemisinin. *J. Nat. Prod.*, 52: 337–341.

Ma, X.C., M. Ye, L.J. Wu, and D.A. Guo, 2006. Microbial transformation of curdione by *Mucor spinosus*. *Enzyme Microb. Technol*., 38: 367–371.

Maatooq, G.T., 2002. Microbial metabolism of partheniol by *Mucor circinelloides*. *Phytochemistry*, 59: 39–44.

Maatooq, G.A., 2002a. Microbial transformation of a β- and γ-eudesmols mixture. *Z. Naturforsch.*, 57C: 654–659.

Maatooq, G.A., 2002b. Microbial conversion of partheniol by *Calonectria decora*. *Z. Naturforsch.*, 57C: 680–685.

Madyastha, K.M. and T.L. Gururaja, 1993. Utility of microbes in organic synthesis: Selective transformations of acyclic isoprenoids by *Aspergillus niger*. *Ind. J. Chem*., 32B: 609–614.

Matsui, H., Y. Minamino, and M. Miyazawa, 1999. Biotransformation of (+)-cedryl acetate by *Glomerella cingulata*, parasitic fungus. *Proc. 43rd TEAC*: 215–216.

Matsui, H. and M. Miyazawa, 2000. Biotransformation of pathouli acetate suing parasitic fungus *Glomerella cingulata* as a biocatalyst. *Proc. 44th TEAC*: 149–150.

Matsushima, A., M.-E.F. Hegazy, C. Kuwata, Y. Sato, M. Otsuka, and T. Hirata, 2004. Biotransformation of enones using plant cultured cells-the reduction of α-santonin. *Proc. 48th TEAC*: 396–398.

Meyer, P. and C. Neuberg 1915. Phytochemische reduktionen. XII. Die umwandlung von citronellal in citronelol. *Biochem. Z.*, 71: 174–179.

Mikami, Y., 1988. *Microbial conversion of terpenoids. Biotechnology and Genetic Engineering Reviews*, Vol. 6, pp. 271–320. Wimborne, UK: Intercept Ltd.

Miyazawa, M., T. Uemura, and H. Kameoka, 1994. Biotransformation of sesquiterpenoids, (−)-globulol and (+)-ledol by *Glomerella cingulata*. *Phytochemistry*, 37: 1027–1030.

Miyazawa, M., H. Nakai, and H. Kameoka, 1995. Biotransformation of (+)-cedrol by plant pathogenic fungus, *Glomerella cingulata*. *Phytochemistry*, 40: 69–72.

Miyazawa, M., T. Uemura, and H. Kameoka, 1995a. Biotransformation of sesquiterpenoids, (+)-aromadendrene and (−)-alloaromadendrene by *Glomerella cingulata*. *Phytochemistry*, 40: 793–796.

Miyazawa, M., H. Nankai, and H. Kameoka, 1995b. Biotransformation of (−)-α-bisabolol by plant pathogenic fungus, *Glomerella cingulata*. *Phytochemistry*, 39: 1077–1080.

Miyazawa, M., H. Nakai, and H. Kameoka, 1995c. Biotransformation of cyclic terpenoids, (±)-cis-nerolidol and nerylacetone, by plant pathogenic fungus, *Glomerella cingulata*. *Phytochemistry*, 40: 1133–1137.

Miyazawa, M., Y. Honjo, and H. Kameoka, 1996. Biotransformation of guaiol and bulnesol using plant pathogenic fungus *Glomerella cingulata* as a biocatalyst. *Proc. 40th TEAC*: 82–83.

Miyazawa, M., H. Nakai, and H. Kameoka, 1996a. Biotransformation of acyclic terpenoid (±)-*trans*-nerolidol and geranylacetone by *Glomerella cingulata. J. Agric. Food Chem.*, 44: 1543–1547.

Miyazawa, M., H. Nakai, and H. Kameoka, 1996b. Biotransformation of acyclic terpenoid (*2E,6E*)-farnesol by plant pathogenic fungus *Glomerella cingulata*. *Phytochemistry*, 43: 105–109.

Miyazawa, M., S. Akazawa, H. Sakai, and H. Kameoka, 1997. Biotransformation of (+)-γ-gurjunene using plant pathogenic fungus, *Glomerella cingulata* as a biocatalyst. *Proc. 41st TEAC*: 218–219.

Miyazawa, M., Y. Honjo, and H. Kameoka, 1997a. Biotransformation of the sesquiterpenoid β-selinene using the plant pathogenic fungus *Glomerella cingulata. Phytochemistry*, 44: 433–436.

Miyazawa, M., H. Matsui, and H. Kameoka, 1997b. Biotransformation of patchouli alcohol using plant parasitic fungus *Glomerella cingulata* as a biocatalyst. *Proc. 41st TEAC*: 220–221.

Miyazawa, M., Y. Honjo, and H. Kameoka, 1998. Biotransformation of the sesquiterpenoid (+)-γ-gurjunene using a plant pathogenic fungus *Glomerella cingulata* as a biocatalyst. *Phytochemistry*, 49: 1283–1285.

Miyazawa, M., H. Matsui, and H. Kameoka, 1998a. Biotransformation of unsaturated sesquiterpene alcohol using plant parasitic fungus, *Glomerella cingulata* as a biocatalyst. *Proc. 42nd TEAC*: 121–122.
Miyazawa, M. and A. Sugawara, 2006. Biotransformation of (2)-guaiol by *Euritium rubrum*. *Nat. Prod. Res.*, 20: 731–734.
Morikawa, H., K. Hayashi, K. Wakamatsu et al., 2000. Biotransformation of sesquiterpenoids by *Aspergillus niger* IFO 4407. *Proc. 44th TEAC*: 151–153.
Nankai, H., M. Miyazawa, and H. Kameoka, 1996. Biotransformation of (*Z,Z*)-farnesol using plant pathogenic fungus, *Glomerella cingulata* as a biocatalyst. *Proc. 40th TEAC*: 78–79.
Nishida, E. and Y. Kawai, 2007. Bioconversion of zerumbone and its derivatives. *Proc. 51st TEAC*: 387–389.
Noma, Y., T. Hashimoto, A. Kikkawa, and Y. Asakawa, 1996. Biotransformation of (−)-α-eudesmol by *Asp. niger* and *Asp. cellulosae* M-77. *Proc. 40th TEAC*: 95–97.
Noma, Y., T. Hashimoto, S. Kato, and Y. Asakawa, 1997. Biotransformation of (+)-β-eudesmol by *Aspergillus niger. Proc. 41st TEAC*: 224–226.
Noma, Y., K. Matsueda, I. Maruyama, and Y. Asakawa, 1997a. Biotransformation of terpenoids and related compounds by *Chlorella* species. *Proc. 41st TEAC*: 227–229.
Noma, Y. and Y. Asakawa, 1998. Microbiological transformation of 3-oxo-α-ionone. *Proc. 44th TEAC*: 133–135.
Noma, Y. and Y. Asakawa, 2006. Biotransformation of (−)-nopol benzyl ether. *Proc 50th TEAC*, 434–436.
Noma, Y., T. Hashimoto, Y. Akamatsu, S. Takaoka, and Y. Asakawa, 1999. Microbial transformation of adamantane (Part 1). *Proc. 43rd TEAC*: 199–201.
Noma, Y., M. Furusawa, C. Murakami, T. Hashimoto, and Y. Asakawa, 2001. Formation of nootkatol and nootkatone from valencene by soil microorganisms. *Proc. 45th TEAC*: 91–92.
Noma, Y., T. Hashimoto, and Y. Asakawa, 2001a. Microbiological transformation of damascone. *Proc. 45th TEAC*: 93–95.
Noma, Y., T. Hashimoto, and Y. Asakawa, 2001b. Microbial transformation of adamantane. *Proc. 45th TEAC*: 96–98.
Noma, Y., T. Hashimoto, S. Sawada, T. Kitayama, and Y. Asakawa, 2002. Microbial transformation of zerumbone. *Proc. 46th TEAC*: 313–315.
Noma, Y., Y. Takahashi, and Y. Asakawa, 2003. Stereoselective reduction of racemic bicycle[33.1]nonane-2,6-dione and 5-hydroxy-2-adamantanone by microorganisms. *Proc. 46th TEAC*: 118–120.
Nozaki, H., K. Asano, K. Hayashi, M. Tanaka, A. Masuo, and D. Takaoka, 1996. Biotransformation of shiromodiol diacetate and myli-4(15)-en-9-one by *Aspergillus niger* IFO 4407. *Proc. 40th TEAC*: 108–110.
Okuda, M., K. Sonohara, and H. Takikawa, 1994. Production of natural flavors by laccase catalysis. *Jpn. Kokai Tokkyo Koho*, 303967.
Orabi, K.Y., 2000. Microbial transformation of the eudesmane sesquiterpene plectranthone. *J. Nat. Prod.,* 63: 1709–1711.
Orabi, K.Y., 2001. Microbial epoxidation of the tricyclic sesquiterpene presilphiperfolane angelate ester. *Z. Naturforsch.*, 56C: 223–227.
Otsuka, S. and K. Tani, 1991. Catalytic asymmetric hydrogen migration of ally amines. *Synthesis*: 665–680.
Parshikov, I.A., K.M. Muraleedharan, and M.A. Avery, 2004. Transformation of artemisinin by Cunninghamella elegans. *Appl. Microbiol. Biotechnol*., 64: 782–786.
Sakamoto, S., N. Tsuchiya, M. Kuroyanagi, and A. Ueno, 1994. Biotransformation of germacrone by suspension cultured cells. *Phytochemistry*, 35: 1215–1219.
Sakata, K. and M. Miyazawa, 2006. Biotransformation of (+)-isolongifolen-9-one by *Glomerella cingulata* as a biocatalyst. *Proc. 50th TEAC*: 258–260.
Sakata, K., I. Horibe, and M. Miyazawa, 2007. Biotransformation of (+)-α-longipinene by microorganisms as a biocatalyst. *Proc. 51st TEAC*: 321–322.
Sakui, N., M. Kuroyanagi, M. Sato, and A. Ueno, 1988. Transformation of ten-membered sesquiterpenes by callus of *Curcuma*. *Proc. 32nd TEAC*: 322–324.
Sakui, N., M. Kuroyamagi, Y. Ishitobi, M. Sato, and A. Ueno, 1992. Biotransformation of sesquiterpenes by cultured cells of *Curcuma zedoaria*. *Phytochemistry*, 31: 143–147.
Salvador, J.A.R. and J.H. Clark, 2002. The allylic oxidation of unsaturated steroids by *tert*-butyl hydroperoxide using surface functionalized silica supported metal catalysts. *Green Chem.*, 4: 352–356.
Sekita, M., M. Furusawa, T. Hashimoto, Y. Noma, and Y. Asakawa, 2005. Biotransformation of pungent tasting polygidial from *Polygonum hydropiper* and related compounds by microorganisms. *Proc. 49th TEAC*: 380–381.
Sekita, M., T. Hashimoto, Y. Noma, and Y. Asakawa, 2006. Biotransformation of biologically active terpenoids, sacculatal and cinnamodial by microorganisms. *Proc. 50th TEAC*: 406–408.

Shimoda, K., Y. Kondo, T. Nishida, H. Hamada, N. Nakajima, and H. Hamada, 2006. Biotransformation of thymol, carvacrol, and eugenol by cultured cells of *Eucalyptus perriniana*. *Phytochemistry*, 67: 2256–2261.

Shimoda, K., T. Harada, H. Hamada, N. Nakajima, and H. Hamda, 2007. Biotransformation of raspberry ketone and zingerone by cultured cells of *Phytolacca americana*. *Phytochemistry*, 68: 487–492.

Shimoda, K., S. Kwon, A. Utsuki, et al., 2007a. Glycosylation of capsaicin and 8-norhydrocapsaicin by cultured cells of *Catharanthus roseus*. *Phytochemistry*, 68: 1391–1396.

Shoji, N., A. Umeyama, Y. Asakawa, T. Takeout, K. Nocoton, and Y. Ohizumi, 1984. Structure determination of nootkatol, a new sesquiterpene isolated form *Alpinia oxyphylla* Miquel possessing calcium antagonist activity. *J. Pharm. Sci.*, 73: 843–844.

Sowden, R.J., S. Yasmin, N.H. Rees, S.G. Bell, and L.-L. Wong, 2005. Biotransformation of the sesquiterpene (+)-valencene by cytochrome $P450_{cam}$ and $P450_{BM\text{-}3}$. *Org. Biomol. Chem*., 3: 57–64.

Sugawara, A. and M. Miyazawa, 2004. Biotransformation of guaiene using plant pathogenic fungus, *Eurotium rubrum* as a biocatalyst. *Proc. 48th TEAC*: 385–386.

Sutherland, T.D., G.C. Unnithan, J.F. Andersen, et al., 1998. A cytochrome P450 terpenoid hydroxylase linked to the suppression of insect juvenile hormone synthesis. *Proc. Natl. Acad. Sci. USA*, 95: 12884–12889.

Takahashi, H., 1994. *Biotransformation of terpenoids and aromatic compounds by some microorganisms*. PhD thesis. Tokushima Bunri University, pp. 1–115.

Takahashi, H., T. Hashimoto, Y. Noma, and Y. Asakawa, 1993. Biotransformation of 6-gingerol, and 6-shogaol by *Aspergillus niger*. *Phytochemistry*, 34: 1497–1500.

Takahashi, H., M. Toyota, and Y. Asakawa, 1993a. Drimane-type sesquiterpenoids from *Cryptoporus volvatu*s infected by *Paecilomyces varioti*. *Phytochemistry*, 33: 1055–1059.

Takahashi, T. and M. Miyazawa, 2005. Biotransformation of (+)-nootkatone by *Aspergillus wentii,* as biocatalyst. *Proc. 49th TEAC*: 393–394.

Takahashi, T. and M. Miyazawa, 2006. Biotransformation of sesquiterpenes which possess an eudesmane skeleton by microorganisms. *Proc. 50th TEAC*: 256–257.

Takahashi, T., I. Horibe, and M. Miyazawa, 2007. Biotransformation of β-selinene by *Aspergillus wentii. Proc. 51st TEAC*: 319–320.

Tani, K., T. Yamagata, S. Otsuka, et al., 1982. Cationic rhodium (I) complex-catalyzed asymmetric isomerization of allylamines to optically active enamines. *J. Chem. Soc. Chem. Commun.*: 600–601.

Tori, M., M. Sono, and Y. Asakawa, 1990. The reaction of three sesquiterpene ethers with *m*-chloroperbenzoic acid. *Bull. Chem. Soc. Jpn.*, 63: 1770–1776.

Venkateswarlu, Y., P. Ramesh, P.S. Reddy, and K. Jamil, 1999. Microbial transformation of $\Delta^{9(15)}$-africane. *Phytochemistry*, 52: 1275–1277.

Wang, Y., T.-K. Tan, G.K. Tan, J.D. Connolly, and L.J. Harrison, 2006. Microbial transformation of the sesquiterpenoid (−)-maalioxide by *Mucor plumbeus. Phytochemistry*, 67: 58–61.

Wilson, C.W. III and P. E. Saw, 1978. Quantitative composition of cold-pressed grapefruit oil. *J. Agric. Food Chem.*, 26: 1430–1432.

Yang, L., K. Fujii, J. Dai, J. Sakai, and M. Ando, 2003. Biotransformation of α-santonin and its C-6 epimer by fungus and plant cell cultures. *Proc. 47th TEAC*: 148–150.

Zhan, J., H. Guo, J. Dai, Y. Zhang, and D. Guo, 2002. Microbial transformation of artemisinin by *Cunninghamella echinulata* and *Aspergillus niger*. *Tetrahedron Lett.*, 43: 4519–4521.

Zundel, J.-L., 1976. PhD thesis. Universite Louis Pasteur. Strasbourg, France.

16 Industrial Uses of Essential Oils

W. S. Brud

CONTENTS

16.1 INTRODUCTION

The period when essential oils were used first on an industrial scale is difficult to identify. The nineteenth century is generally regarded as the commencement of the modern phase of industrial application of essential oils. However, the large-scale usage of essential oils dates back to ancient Egypt. In 1480 BC, Queen Hatshepsut of Egypt sent an expedition to the country of Punt (now Somalia) to collect fragrant plants, oils, and resins as ingredients for perfumes, medicaments, and flavors and for the mummification of bodies. Precious fragrances have been found in many Egyptian archeological excavations, as a symbol of wealth and social position.

If significant international trade of essential oil-based products is the criterion for industrial use, "Queen of Hungary Water" was the first alcoholic perfume in history. This fragrance, based on rosemary essential oil distillate, was created in the mid-fourteenth century for the Polish-born Queen Elisabeth of Hungary. Following a special presentation to King Charles V, The Wise of France in 1350, it became popular in all medieval European courts. The beginning of the eighteenth century saw the introduction of "Eau de Cologne," based on bergamot and other citrus oils, which remains widely used to this day. This fresh citrus fragrance was the creation of Jean Maria Farina, a descendant of Italian perfumers who came to France with Catherine de Medici and settled in Grasse in the sixteenth century. According to the city of Cologne archives, Jean Maria Farina and Karl Hieronymus Farina, in 1749, established factory (Fabriek) of this water, which sounds very "industrial." The "Kölnisch Wasser" became the first unisex fragrance rather than one simply for men, known and used all over Europe, and it has been repeated subsequently in innumerable countertypes as a fragrance for men.

16.2 THE HISTORY

The history of production of essential oils dates back to ca. 3500 BC when the oldest known water distillation equipment for essential oils was employed, and may be seen today in the Texila museum in Pakistan. Ancient India, China, and Egypt were the locations where essential oils were produced and widely used as medicaments, flavors, and fragrances. Perfumes came to Europe most probably from the East at the time of the crusades, and perfumery was accorded a professional status by the approval of a French guild of perfumers in Grasse by King Philippe August in 1190. For centuries, Grasse remained the center of world perfumery and was also the home of the first ever officially registered essential oils-producing company—Antoine Chiris—in 1768. (It is worth noting that not much later, in 1798, the first American essential oil company—Dodge and Olcott Inc.—was established in New York.)

About 150 years earlier, in 1620, an Englishman, named Yardley, obtained a concession from King Charles I to manufacture soap for the London area. Details of this event are sparse, other than the high fee paid by Yardley for this privilege. Importantly, however, Yardley's soap was perfumed with English lavender, which remains the Yardley trademark today, and it was probably the first case of use of an essential oil as a fragrance in large-scale soap production.

The use of essential oils as food ingredients has a history dating back to ancient times. There are many examples of the use of citrus and other squeezed (manually or mechanically expressed) oils for sweets and desserts in ancient Egypt, Greece, and the Roman Empire. Numerous references exist to flavored ice creams in the courts of the Roman Emperor Nero and of China. The reintroduction of recipes in Europe is attributed to Marco Polo on his return from traveling to China. In other stories, Catherine de Medici introduced ice creams in France, whereas Charles I of England served the first dessert in the form of frozen cream. Ice was used for freezing drinks and food in many civilizations and the Eastern practice of using spices and spice essential oils both as flavoring ingredients and as food conservation agents was adopted centuries ago in Europe.

Whatever may be regarded as the date of their industrial production, essential oils, together with a range of related products—pomades, tinctures, resins, absolutes, extracts, distillates, concretes, and so on—were the only ingredients of flavor and fragrance products until the late nineteenth century. At this stage, the growth in consumption of essential oils as odoriferous and flavoring materials stimulated the emergence of a great number of manufacturers in France, the United Kingdom, Germany, Switzerland, and the United States (Table 16.1).

The rapid development of the fragrance and flavor industry in the nineteenth century was generally based on essential oils and related natural products. In 1876, however, Haarman and Reimer started the first production of synthetic aroma chemicals—vanillin, then coumarin, anisaldehyde, heliotropin, and terpineol. Although aroma chemicals made a revolution in fragrances with top discoveries in the twentieth century, for many decades both flavors and fragrances were manufactured with constituents of natural origin, the majority of which were essential oils.

16.3 FRAGRANCES

The main reason for the expansion of the essential oils industry and the growing demand for products was the development of the food, soap, and cosmetics industries. Today's multinational companies, the main users of fragrances and flavors, have evolved directly from the developments during the mid-nineteenth century.

In 1806, William Colgate opened his first store for soaps, candles, and laundry starch on Dutch Street in New York. In 1864, B.J. Johnson in Milwaukee started the production of soap, which came to be known as Palmolive from 1898. In 1866, Colgate launched its first perfumed soaps and perfumes. In 1873, Colgate launched toothpaste in a glass jug on the market and in the tube first in 1896. In 1926, two soap manufacturers—Palmolive and Peet—merged to create Palmolive–Peet, which 2 years later merged with Colgate to establish the Colgate–Palmolive–Peet company (renamed as the Colgate–Palmolive Company in 1953).

TABLE 16.1
The First Industrial Manufacturers of Essential Oils, Flavors, and Fragrances

Company Name	Country	Established
Antoine Chiris	France (Grasse)	1768
Cavallier Freres	France (Grasse)	1784
Dodge & Olcott Inc.	USA (New York)	1798
Roure Bertrand Fils and Justin Dupont	France (Grasse)	1820
Schimmel & Co.	Germany (Leipzig)	1829
J. Mero-Boyveau	France (Grasse)	1832
Stafford Allen and Sons	United Kingdom (London)	1833
Robertet et Cie	**France (Grasse)**	**1850**
W.J. Bush	United Kingdom (London)	1851
Payan-Bertrand et Cie	**France (Grasse)**	**1854**
A. Boake Roberts	United Kingdom (London)	1865
Fritsche-Schimmel Co	USA (New York)	1871
V. Mane et Fils	**France (Grasse)**	**1871**
Haarman&Reimer	Germany (Holzminden)	1874
R.C. Treatt Co.	**United Kingdom (Bury)**	**1886**
N.V. Polak und Schwartz	Holland (Zaandam)	1889
Ogawa and Co.	**Japan (Osaka)**	**1893**
Firmenich and Cie	**Switzerland (Geneve)**	**1895**
Givaudan S.A.	**Switzerland (Geneve)**	**1895**
Maschmeijer Aromatics	Holland (Amsterdam)	1900

Note: Companies continuing to operate under their original name are printed in bold.

In October 1837, William Procter and James Gamble signed a formal partnership agreement to develop their production and marketing of soaps (Gamble) and candles (Procter). "Palm oil," "rosin," "toilet," and "shaving" soaps were listed in their advertisements. An "oleine" soap was described as having a violet odor. Only 22 years later, Procter & Gamble (P&G) sales reached 1 million dollars. In 1879, a fine but inexpensive "ivory" white toilet soap was offered to the market with all purpose applications as a toilet and laundry product. In 1890, P&G was selling more than 30 different soaps.

The story of a third player started in 1890 when William Hesket Lever created his concept of the Sunlight Soap, which revolutionized the idea of cleanliness and hygiene in Victorian Britain.

The very beginning of twentieth century marked the next big event when the young French chemist Eugene Schueller prepared his first hair color in 1907 and established what is now L'Oreal. These were the flagships in hundreds of emerging (and disappearing by fusions, takeovers, or bankruptcy) manufacturers of perfumes, cosmetics, toiletries, detergents, household chemicals, and related products, the majority of which were and are perfumed with essential oils.

16.4 FLAVORS

Over the same time period, another group of users of essential oils entered the markets. In 1790, the term "soda water" for carbon dioxide saturated water as a new drink appeared for the first time in the United States and in 1810, the first U.S. patent was issued for the manufacture of imitations of natural gaseous mineral waters. Only 9 years later the "soda fountain" was patented by Samuel Fahnestock. In 1833, carbonated lemonade flavored with lemon juice and citric acid was on sale in England. In 1835, the first bottled soda water appeared in the United States. It is, however, interesting that the first flavored sparkling drink—Ginger Ale—was created in Ireland in 1851. The milestones in flavored soft drinks

appeared 30 years later: 1881—the first cola-flavored drink in the United States; 1885—Dr Pepper was invented by Charles Aderton in Waco, Texas; 1886—Coca-Cola by Dr John S. Pemberton in Atlanta, Georgia; and in 1898—Pepsi-Cola, created by Caleb Bradham, known from 1893 as "Brad's Drink."

Dr Pepper was advertised as the king of beverages, free from caffeine (which was added to it later on), was flavored with black cherry artificial flavor, and was first sold in the Old Corner Drug Store owned by Wade Morrison. Its market success and position as one of the most popular U.S. soft drinks started by a presentation during the St Louis World's Fair, where some other important flavor-consuming products—ice cream cones, hot dog rolls, and hamburger buns—were also shown. All of them remain major users of natural flavors based on essential oils. Hundred years later after the merger with another famous lemon–lime drink 7UP in 1986, it finally became a part of Cadbury.

Dr. John Pemberton was a pharmacist and he mixed up a combination of lime, cinnamon, coca leaves, and cola to make the flavor for his famous drink, first as a remedy against headache (Pemberton French Wine Coca) and then reformulated according to the prohibition law and used it to add taste to soda water from his "soda fountain." The unique name and logo was created by his bookkeeper Frank Robinson and Coca-Cola was advertised as a delicious, exhilarating, refreshing and invigorating temperance drink. Interestingly, the first year of sales resulted in $20 loss, as the cost of the flavor syrup used for the drink was higher than the total sales of $50. In 1887, another pharmacist, Asa Candler, bought the idea and with aggressive marketing in 10 years introduced his drink all over the United States and Canada by selling syrup to other companies licensed to manufacture and retail the drink. Until 1905, Coca-Cola was known as a tonic drink and contained the extract of cocaine and cola nuts and with the flavoring of lime and sugar.

Like Pemberton, Caleb Bradham was a pharmacist and in his drugstore, he offered soda water from his "soda fountain." To promote sales, he flavored the soda with sugar, vanilla, pepsin, cola, and "rare oils"—obviously the essential oils of lemon and lime—and started selling it as a cure for dyspepsia, "Brad's Drink" than Pepsi-Cola.

The development of the soft drinks industry is of great importance because it is a major consumer of essential oils, especially those of citrus origin. It is enough to say that nowadays, according to their web pages, only Coca-Cola-produced beverages are consumed worldwide in a quantity exceeding 1 billion drinks per day. If we consider that the average content of the appropriate essential oil in the final drink is about 0.001–0.002%, and the standard drink is ca. 0.31 (300 g), we approach a daily consumption of essential oils by this company alone at the level of 3–6 tons per day, which gives an annual usage well over 2000 tons. Although all other brands of the food industry use substantial quantities of essential oils in ice creams, confectionary, bakery, and a variety of fast foods (where spice oils are used), these together use less oils than the beverage manufacturers.

There is one special range of products that can be situated between the food and cosmetic–toiletries industry sectors and it is a big consumer of essential oils, especially of all kinds of mint, eucalyptus, and some other herbal and fruity oils. These are oral care products, chewing gums, and all kinds of mouth refreshing confectioneries. As mentioned above, toothpastes appeared on the market in the late nineteenth century in the the United States. Chewing gums or the custom of chewing certain plant secretions were known to the ancient Greeks (e.g., mastic tree resin) and to ancient Mayans (e.g., sapodilla tree gum). Chewing gum, as we know it now, started in America around 1850 when John B. Curtis introduced flavored chewing gum, which was first patented in 1859 by William Semple. In 1892, William Wrigley used chewing gum as a free gift with sales of baking powder in his business in Chicago and very soon he realized that chewing gum has real potential. In 1893, Juicy Fruit gum came into market and was followed in the same year by Wrigley's Spearmint; today, both products are known and consumed worldwide and their names are global trademarks.

16.5 PRODUCTION AND CONSUMPTION

This brief and certainly incomplete look into the history of industrial usage of essential oils as flavor and fragrance ingredients shows that the real industrial scale of flavor and fragrance industry

developed in the second half of the nineteenth century together with transformation of "manufacture" into "industry."

There are no reliable data on the scale of consumption of essential oils in specific products. On the basis of different sources, it can be estimated that the world market for the flavors and fragrances has a value of 10–12 billion euro, being equally shared by each group of products. It is very difficult to estimate the usage of essential oils in each of the groups. More oils are used in flavors than in fragrances which today are mainly based on aroma chemicals, especially in large volume compounds used in detergents and household products. Table 16.2 presents estimated data on world consumption of major essential oils (each used over 500 tons per annum).

TABLE 16.2
Estimated World Consumption of the Major Essential Oils

Oil Name	Consumption (tons)	Approximate Value (€ million)[a]	Major Applications[b]
Orange	50,000	275	Soft drinks, sweets, fragrances
Cornmint (*Mentha arvensis*)[c]	25,000	265	Oral care, chewing gum, confectionery, fragrances, menthol crystals
Peppermint	4500	120	Oral care, chewing gum, confectionery, liquors, tobacco, fragrances
Eucalyptus (*Eucalyptus globulus*)	4000	22	Oral care, chewing gum, confectionery, pharmaceuticals, fragrances
Lemon	3500	21	Soft drinks, sweets, diary, fragrances, household chemicals
Citronella	3000	33	Perfumery, toiletries, household chemicals
Eucalyptus (*Eucalyptus citriodora*)	2100	10	Confectionery, oral care, chewing gum, pharmaceuticals, fragrances
Clove leaf	2000	24	Condiments, sweets, pharmaceuticals, tobacco, toiletries, household chemicals
Spearmint (*Mentha spicata*)	2000	46	Oral care, chewing gum, confectionery
Cedarwood (*Virginia*)	1500	22	Perfumery, toiletries, household chemicals
Lime	1500	66	Soft drinks, sweets, diary, fragrances
Lavandin	1000	15	Perfumery, cosmetics, toiletries
Litsea cubeba	1000	20	Citral for soft drinks, fragrances
Cedarwood (China)	800	11	Perfumery, toiletries, household chemicals
Camphor	700	3	Pharmaceuticals
Coriander	700	40	Condiments, pickles, processed food, fragrances
Grapefruit	700	9	Soft drinks, fragrances
Star anise	700	7	Liquors, sweets, bakery, household chemicals
Patchouli	600	69	Perfumery, cosmetics, toiletries
Basil	500	12	Condiments, processed food, perfumery, toiletries
Mandarine	500	30	Soft drinks, sweets, liquors, perfumery, toiletries

[a] Based on average prices offered in 2007.
[b] Almost all of the major oils are used in alternative medicine.
[c] Main source of natural menthol.

The following oils are used in quantities between 100 and 500 tons per annum: bergamot, cassia, cinnamon leaf, clary sage, dill, geranium, lemon petitgrain, lemongrass, petitgrain, pine, rosemary, tea tree, and vetivert. It must be emphasized that most of the figures given above on the production volume are probably underestimates because no reliable data are available on the domestic consumption of essential oils in major producing countries, such as China, India, and Indonesia. Therefore quantities presented in various sources are sometimes very different. For example, consumption of *Mentha arvensis* is given as 5000 and 25,000 tons per annum. The lower one probably relates to the direct usage of the oil, the higher includes the oil used for the production of menthol crystals. In Table 16.2, the highest available figures are presented. Considering the above and general figures for flavors and fragrances, it can be estimated that the total value of essential oils used worldwide is somewhere between 2 and 3 billion euro. Price fluctuations (e.g., the patchouli oil price jump in mid-2007) and many other unpredictable changes cause any estimation of essential oils consumption value to be very risky and disputable. The figures given in the table are based on average trade offers. Table 16.2 does not include turpentine, which is sometimes added into essential oils data. Being used mainly as a chemical solvent or a raw material in the aroma chemicals industry, it has no practical application as an essential oil, except in some household chemicals.

As noted earlier, the largest world consumer of essential oils is the flavor industry, especially for soft drinks. However, this is limited to a few essential oils, mainly citrus (orange, lemon, grapefruit, mandarin, lime), ginger, cinnamon, clove, and peppermint. Similar oils are used in confectionery, bakery, desserts, and dairy products, although the range of oils may be wider and include some fruity products and spices. The spicy oils are widely used in numerous salted chips, which are commonly consumed along with beverages and long drinks. Also, the alcoholic beverage industry is a substantial user of essential oils; for example, anis in numerous specialties of the Mediterranean region; herbal oils in liqueurs; ginger in ginger beer; peppermint in mint liquor; and in many other flavored alcohols.

Next in importance to beverages in the food sector are the sweet, dairy, confectionery, dessert (fresh and powdered), sweet bakery, and cream manufacturing sector, for which the main oils used are citrus, cinnamon, clove, ginger, and anis. Many other oils are used in an enormous range of very different products in this category.

The fast food and processed food industries are also substantial users of essential oils, although the main demand is for spicy and herbal flavors. Important oils here are coriander (especially popular in the United States), pepper, pimento, laurel, cardamom, ginger, basil, oregano, dill, and fennel, which are added to the spices with the aim of strengthening and standardizing the flavor.

The major users of essential oils are the big compounders—companies that emerged from the historical manufacturers of essential oils and fragrances and flavors and new ones established by various deals between old players in the market or, like International Flavors and Fragrances (IFF), were created by talented managers who left their parent companies and started on their own. Today's big 10 are listed in Table 16.3.

Out of the 20 companies listed in Table 16.1, seven were located in France but by 2007, out of 10 largest, only two are from France. Also, only four of today's big 10 are over a century old with two leaders—Givaudan and Firmenich—from Switzerland and Mane and Robertet from France.

The flavor and fragrance industry is the one where the majority of oils are introduced into appropriate flavor and fragrance compositions. Created by flavorists and perfumers, an elite of professionals in the industry, the compositions, complicated mixtures of natural and nature identical ingredients for flavoring, and natural and synthetic components for fragrances, are offered to end users. The latter are the manufacturers of millions of very different products from luxurious "haute couture" perfumes, and top-class-flavored liquors and chocolate pralines through cosmetics, household chemicals, sauces, condiments, cleaning products, air fresheners, and aroma marketing.

It is important to emphasize that a very wide range of essential oils are used in alternative or "natural" medicine with aromatherapy—treatment of many ailments with the use of essential oils as bioactive ingredients—being the leading outlet for the oils and products in which they are applied as major active components. The ideas of aromatherapy from a niche area dominated by lovers of

TABLE 16.3
Leading Producers of Flavors and Fragrances

Position	Company Name (Headquarters)	Sales in Million (€)[a]
1	Givaudan S.A. (Vernier, Switzerland)	2550
2	Firmenich S.A. (Geneve, Switzerland)	1620
3	International Flavors and Fragrances (New York, USA)	1500
4	Symrise AG (Holzminden, Germany)	1160
5	Takasago International Corporation (Tokyo, Japan)	680
6	Sensient Technologies Flavors&Fragrances (Milwaukee, USA)	400
7	T. Hasegawa Co. Ltd (Tokyo, Japan)	280
8	Mane S.A. (Le Bar-sur-Loup, France)	260
9	Frutarom Industries Ltd (Haifa, Israel)	220
10	Robertet S.A. (Grasse, France)	210

[a] Estimated data based on web pages of the companies, various reports, and journals.

nature and some kind of magic, although based on very old and clinically proved experience, came into mass production appearing as an advertising "hit" in many products including global ranges. Examples include Colgate–Palmolive liquid soaps, a variety of shampoos, body lotions, creams, and so on by many other producers, and fabric softeners emphasizing the benefits to users' mood and condition from the odors of essential oils (and other fragrant ingredients) remaining on fabrics. Aromatherapy and "natural" products, where essential oils are emphasized as "the natural" ingredients, are a very fast developing segment of the industry and this is a return to what was a common practice in ancient and medieval times.

16.6 CHANGING TRENDS

Until the second half of the nineteenth century, formulas of perfumes and flavors (although much less data are available on flavoring products in history) were based on essential oils and some other naturals (musk, civet, amber, resins, pomades, tinctures, extracts, etc.). Now, some 150 years later, old formulations are being taken out of historical books and are advertised as the "back to nature" trend. Perfumery handbooks published until the early twentieth century listed essential oils, and none or only one or two aroma chemicals (or isolates from essential oils). A very good illustration of the changes that affected the formulation of perfumes in the twentieth century is a comparison of rose fragrance as recorded in perfumery handbooks. Dr Heinrich Hirzel in his *Die Toiletten Chemie* (1892, p. 384) gave the following formula for high-quality white rose perfume:

400 g of rose extract
200 g of violet extract
150 g of acacia extract
100 g of jasmine extract
120 g of iris infusion
25 g of musk tincture
5 g of rose oil
10 drops of patchouli oil.

Felix Cola's milestone work *Le Livre de Parfumeur* (1931, p. 192) recorded a white rose formula containing only 1% of rose oil, 2% of rose absolute, 7.5% other oils, and aroma chemicals.

Rose Blanche	
Rose oil	10 g
Rose absolute	20 g
Patchouli oil	25 g
Bergamot oil	50 g
Linalool	60 g
Benzyl acetate	7 g
Phenylethyl acetate	75 g
Citronellol	185 g
Geraniol	200 g
Phenylethyl alcohol	300 g

In the mid-twentieth century, perfumers were educated to consider chemicals as the most convenient, stable, and useful ingredients for fragrance compositions. Several rose fragrance formulas with less than 2% rose oil or absolute can be found in F.V. Wells and M. Billot's *Perfumery Technology*, (1975), and rose fragrance without any natural rose product is nothing curious in a contemporary perfumers' notebook. However, looking through descriptions of new fragrances launched in the last few years, one can observe a very strong tendency to emphasize the presence of natural ingredients—oils, resinoids, and absolutes—in the fragrant mixture. The "back to nature" trend creates another area for essential oils usage in many products.

A very fast growing group of cosmetics and related products today are the so-called organic products. These are based on plant ingredients obtained from wild harvesting or from "organic cultivation" and which are free of pesticides, herbicides, synthetic fertilizers, and other chemicals widely used in agriculture. According to different sources, sales of "organic" products in 2007 will reach 4–5 billion U.S. dollars. The same "organic raw materials" are becoming more and more popular in the food industry, which in consequence will increase the consumption of "organic flavors" based on "organic essential oils." "Organic" certificates, available in many countries (in principle for agricultural products, although they are institutions that also certify cosmetics and related products), are product passports to a higher price level and selective shops or departments in supermarkets. The importance of that segment of essential oils consumption can be illustrated by comparison of the average prices for standard essential oils as listed in Table 16.4 and the same oils claimed as "organic."

The consumption of essential oils in perfumed products varies according to the product (Table 16.5): from a very high level in perfumes (due to the high concentration of fragrance compounds in perfumes and the high content of natural ingredients in perfume fragrances) and in a wide range of "natural" cosmetics and toiletries to relatively low levels in detergents and household chemicals, in which fragrances are based on readily available low-priced aroma chemicals. However, it must be emphasized that although the concentration of essential oils in detergents and related products is low, the large volume sales of these consumer products result in substantial consumption of the oils.

Average values given for fragrance dosage in products and for the content of oils in fragrances are based on literature data and private communications from the manufacturers. It should be noted that in many cases the actual figures for individual products can be significantly different. "Eau Savage" from Dior is a very good example: analytical data indicate a content of essential oils (mainly bergamot) of over 70%. Toothpastes are exceptional in that the content of essential oils in the flavor is in some cases nearly 100% (mainly peppermint, spearmint cooled with natural menthol).

While the average dosage of fragrances in the final product can be very high, flavors in food products are used in very low dosages, well below 1%. The high consumption of essential oils by this sector results from the large volume of sales of flavored foods. Average dosages of flavors and the content of essential oils in the flavors are given in Table 16.6.

As in the case of fragrances, the average figures given in Table 16.6 vary in practice in individual cases, both in the flavor content in the product and much more in the essential oils

TABLE 16.4
Prices of Selected Standard and "Organic" Essential Oils

Oil Name	Standard Quality (€/kg)[a]	Organic Quality (€/kg)[a]
Orange	5.50	35
Cornmint (*M. arvensis*)	10.50	50
Peppermint	27.00	100
Eucalyptus (*E. globulus*)	5.50	26
Lemon	6.00	30
Citronella	11.00	23
Eucalyptus (*E. citriodora*)	5.00	34
Clove leaf	12.00	60
Spearmint (*M. spicata*)	23.00	40
Cedarwood (*Virginia*)	15.00	58
Lime	44.00	92
Lavandin	15.00	36
Litsea cubeba	20.00	44
Cedarwood (China)	14.00	53
Camphor	4.50	24
Coriander	57.00	143
Grapefruit	13.00	170
Patchouli	115.00	250

[a] Average prices based on commercial offers in 2007.

TABLE 16.5
Average Dosage of Fragrances in Consumer Products and Content of Essential Oils in Fragrance Compounds

Position	Product	Average Dosage of Fragrance Compound in Product (%)	Average Content of Essential Oils in Fragrance (%)
1	Perfumes	10.0–25.0	5–30[a]
2	Toilet waters	3.0–8.0	5–50[a]
3	Skin care cosmetics	0.1–0.6	0–10
4	Deodorants (inclusive deoparfum)	0.5–5.0	0–10
5	Shampoos	0.3–2.0	0–5
6	Body cleansing products (liquid soaps)	0.5–3.0	0–5
7	Bath preparations	0.5–6.0	0–10
8	Soaps	0.5–3.0	0–5
9	Toothpastes	0.5–2.5	10–50[b]
10	Air fresheners	0.5–30.0	0–20
11	Washing powders and liquids	0.1–0.5	0–5
12	Fabric softeners	0.1–0.5	0–10
13	Home care chemicals	0.5–5.0	0–5
14	Technical products	0.1–0.5	0–5
15	Aromatherapy and organic products	0.1–0.5	100

[a] Traditional perfumery products contained more natural oils than modern ones.
[b] Mainly mint oils.

TABLE 16.6
Average Content of Flavors in Food Products and of Essential Oils in Flavors

Position	Food Products	Flavor Dosage in Food Product (%)	Essential Oils Content in Flavor (%)
1	Alcoholic beverages	0.05–0.15	3–100
2	Soft drinks	0.10–0.15	2–5
3	Sweets (confectionery, chocolate, etc.)	0.15–0.25	1–100
4	Bakery (cakes, biscuits, etc.)	0.10–0.25	1–50
5	Ice creams	0.10–0.30	2–100
6	Diary products, desserts	0.05–0.25	1–50
7	Meat and fish products (also canned)	0.10–0.25	10–20
8	Sauces, ketchup, condiments	0.10–0.50	2–10
9	Food concentrates	0.10–0.50	1–25
10	Snacks	0.10–0.15	2–20

percentage in the flavor. Again "natural" or "organic" products contain only essential oils, since it is unacceptable to include any synthetic aroma chemicals or so-called nature identical food flavors. It should be noted that a substantial number of flavorings are oleoresins: products that are a combination of essential oils and other plant-derived ingredients, which are especially common in hot spices (pepper, chili, pimento, etc.) containing organoleptically important pungent components that do not distill in steam. This group of oleoresin products must be included in the total consumption of essential oils.

For many years after World War II, aroma chemicals were considered the future for fragrance chemistry and there was strong, if unsuccessful, pressure by the manufacturers to get approval for the wide introduction of synthetics (especially those regarded as "nature identical") in food flavors. The very fast development of production and usage of aroma chemicals caused increasing concern over safety issues for the human health and for the environment. One by one certain products were found harmful either for human health (e.g., nitro musks) or for nature. This resulted in wide research on the safety of the chemicals and the development of new safe synthetics. Concurrently, the attention of perfumers and producers turned in the direction of essential oils, which as derived from natural sources and known and used for centuries were generally considered safe. According to recent research, however, this belief is not entirely true and some, fortunately very few, oils and other fragrance products obtained from plants have been found dangerous, and their use has been banned or restricted. However, these are exceptional cases and the majority of essential oils are found safe both for use on the human body as cosmetics and related products as well as for consumption as food ingredients.

It is important to appreciate that the market for "natural," "organic," and "ecological" products both in body care and food industries has changed from a niche area to a boom in recent years with the growth exceeding 30% per annum. The estimated value of sales for "organic" cosmetics and toiletries is 600–800 million euro in Europe, the United States, and Japan and will grow steadily together with organic foods. This creates a very sound future for the essential oils industry, which as such or as isolates derived from the oils will be widely used for fragrance compounds in cosmetic and related products as well as for flavors.

Furthermore, the modernization of agricultural techniques and the growth of plantation areas result in better economical factors for the production of essential oil-bearing plants, creating workplaces in developing countries of Southeast Asia, Africa, and South America as well as further development of modern farms in the United States and Europe (Mediterranean area, Balkans). Despite some regulatory restrictions (EU, REACH, FDA, etc.), essential oils are and will have an

important and growing share in the fragrance and flavor industry. The same will be true for the usage of essential oils and other products of medicinal plants in pharmaceutical products. It is well known that the big pharmaceutical companies invest substantial resources in studies of folk and traditional medicine as well as in research on biologically active constituents of plant origin. Both of these areas cover applications of essential oils. The same is observed in cosmetic and toiletries using essential oils as active healing ingredients.

16.7 CONCLUSIONS

It can be concluded that the industrial use of essential oils is a very promising area and that regular growth shall be observed in future. Much research work will be undertaken both on the safety of existing products and on development of new oil-bearing plants that are used locally in different regions of the world both as healing agents and as food flavorings. Both directions are equally important. Global exchange of tastes and customs shall not lead to unification by Coca-Cola or McDonalds. With all the positive aspects of these products, there are many local specialties that can become world property, like basil-oregano-flavored pizza, curry dishes, spicy kebab, or the universal and always fashionable Eau de Cologne. With the growth of the usage of the commonly known essential oils, new ones coming from exotic flowers of the Amazon jungle or from Indian Ayurveda books can add new benefits to the flavor and fragrance industry.

ACKNOWLEDGMENTS

The author is most grateful to K.D. Protzen of Paul Kaders GmbH and Dr C. Green for their help and assistance in preparation of this chapter.

REFERENCES

Cola, F., 1931. *Le Livre du Parfumeur*. Paris: Casterman.
Hirzel, H., 1892. *Die Toiletten Chemie*. Leipzig, Germany: J.J. Weber Verlag.

FURTHER READING

Dorland, W.F. and J.A. Rogers Jr., 1977. *The Fragrance and Flavor Industry*. New Jersey: V.E. Dorland.
Lawrence, B.M., 2000, *Essential Oils 1995–2000*. Wheaton, IL: Allured Publishing.
Lawrence, B.M., 2004, *Essential Oils 2001–2004*. Wheaton, IL: Allured Publishing.
Lawrence, B.M., 2007, *Essential Oils 2005–2007*. Wheaton, IL: Allured Publishing.
Wells, F.V. and M. Billot, 1981. *Perfumery Technology*. London: E. Horwood Ltd.

WEB SITES

American Beverage Association: http://www.ameribev.org
The Coca-Cola Company: http://www.thecoca-colacompany.com/heritage/ourheritage.html
Colgate-Palmolive: http://www.colgate.com/app/Colgate/US/Corp/History/1806.cvsp
Pepsi Cola History: http://www.solarnavigator.net/sponsorship/pepsi_cola.htm
Procter & Gamble: http://www.pg.com/company/who_we_are/ourhistory.shtml
Unilever: http://www.unilever.com/aboutus

17 Encapsulation and Other Programmed Release Techniques for Essential Oils and Volatile Terpenes

Jan Karlsen

CONTENTS

17.1 INTRODUCTION

In order to widen the applications of volatiles (essential oils), it is necessary to lower the volatility of the compounds to obtain a longer shelf life of products made with these compounds. By lowering the volatility, one can also imagine a possibility to better test the biological effects of these compounds. The encapsulation processes are means by which a liquid essential oil is enclosed in a carrier matrix to provide a dry, free-flowing powder. However, for the prolonged effect of volatile compounds many other techniques are used, where methods are copied from other fields of research when one wants to control the release of active ingredients.

To lower the volatility, one needs to encapsulate the volatiles into a polymer matrix, utilize complex formation, use the covalent bonding to a matrix—to mention a few techniques. We therefore need to formulate the volatiles and take many of these techniques from areas where controlled release formulations have already been in use for many years. The area with the maximum number of applications of controlled release formulations is the field of drug delivery. In this area of research, there exists a large number of publications as well as a large number of patents where one can find inspiration for the formulation of programmed release of volatiles (Deasy, 1984).

So far in the area of volatile terpenes/essential oils, we have seen a large number of investigations that focused on plant selection, volatiles isolation techniques, separation of the volatiles isolated, identification of isolated compounds, and the biochemical formation of terpenes. The formulation

of volatiles into products has been seen as an area of industrial research. This has naturally led to a large number of patents but very few scientific publications on the formulation of essential oils and lower terpenes.

The idea of this chapter is to give an introduction to the area of making a controlled release product of volatiles and, in particular, of essential oils and their constituents.

17.2 CONTROLLED RELEASE OF VOLATILES

The main interest of volatiles encapsulation is the possibility to extend the biological effect of the compounds. For essential oils, we want to prolong the activity by lowering the evaporation of the volatile compounds. During the last 10 years, there are not many publications on this topic in the scientific literature but there are quite a number of patents that describe the various ways of prolonging the effect of volatiles (Porzio, 2008; Sair and Sair, 1980a; Sair, 1980b; Zasypkin and Porzio, 2004; Fulger and Popplewell, 1997, 1998; McIver, 2005). One reason for this fact is that the prolonging of the effect of volatile compounds is regarded as so close to practical applications and therefore the area of industrial research where the results will be bonded in patent applications. However, there are signs that this idea is changing. In order to lower the volatility, thus prolonging the effect of essential oils and terpenes, we have to look into another area of scientific research. In the field of drug delivery, many techniques have been studied for the controlled delivery of active molecules.

The reasons for controlled release (encapsulation of volatiles) may be the following:

Changing the impact of fragrance and flavors
Adding fragrance to textiles
Stabilizing specific compounds
Tailoring the fragrance to the intended use of a product
Lowering the volatility and thereby prolonging the shelf life of a fragrance product.

The slow or controlled release of volatiles is achieved by:

Encapsulation
Solution/dispersion in a polymer matrix
Complex formation
Covalent bonding to another molecule or matrix.

For essential oils and lower terpenes, the following techniques can be utilized depending on the volatiles and the intended use of the final product:

Microcapsule production
Microparticle production
Melt extrusion
Melt injection
Complex formation
Liposomes
Micelles
Covalent bonding to a matrix
Combination of nanocapsules into larger microcapsules.

Since the making of one of the above-mentioned type of products and techniques will influence the activity toward the human biological membranes in one way or the other. Therefore, the relevant sizes of biological units are listed in Table 17.1 and the average sizes of units produced in consumer products, where volatile compounds are involved are listed in Table 17.2.

TABLE 17.1
Size Diameters of Biological Entities

Human blood cells	7000–8000 nm
Bacteria	800–2000 nm
Human cell nucleus	1000 nm
Nanoparticles that can cross biomembranes	60 nm
Virus	17–300 nm
Hemoglobin molecule	3.3 nm
Nanoparticle that can cross blood–brain barrier	4 nm
DNA helix	3 nm
Water molecule	0.3 nm

TABLE 17.2
Average Size of Formulation Units in nm (Sizes below 150 nm may be Invisible to the Naked Eye)

Solutions	0.1
Micellar solutions	0.5
Macromolecular solutions	0.5
Microemulsions	5–20
Liposomes SUV	20–150
Nanospheres	100–500
Nanocapsules	100–500
Liposomes LUV	200–500
Liposomes MLV	200–1000
Microcapsules	5000–30,000
Simple emulsions	500–5000
Multiple emulsions	10,000–100,000

Abbreviations: SUV is small unilamellar vesicles, LUV is large unilamellar vesicles, and MUV is multilamellar vesicles.

Since the introduction of the encapsulation of volatiles (essential oils/lower terpenes), the number of applications has multiplied. Encapsulation of volatiles gives us a more predictable and long-lasting effect of the products. The areas of applications are large and the industry of essential oils and terpenes foresee many prospects for microencapsulated products.

Application markets of encapsulated essential oils and terpenes are:

Medicine
Food, household items, and personal care
Biotechnology
Pharmaceuticals
Electronics
Photography
Chemical industry
Textile industry
Cosmetics.

It is therefore easy to understand that the encapsulation procedures will open up a much larger market for essential oil/terpene products. Experience from all the areas mentioned above can be applied to the study of volatile compounds in products.

In the area of essential oils and lower terpenes, simple encapsulation procedures from the area of drug delivery are applied. The essential oils or single active constituents are mixed with a hydrophilic polymer and spray-dried using a commercial spray-drier. Depending on whether we have an emulsion or a solution of the volatile fraction in the polymer, we obtain monolithic particles or a normal microcapsule.

The most usual polymers used for encapsulation are:

Oligosaccharides from α-amylase
Modified starches from maize, cassava, rice, and potato
Acacia gum
Gum arabic
Alginate
Chitosan.

Many different emulsifiers are used to solubilize the essential oils totally or partly, prior to the encapsulation procedure. This can result in a monolithic particle or a usual capsule, where the essential oil is surrounded by a hydrophilic coating. When the mixture of an essential oil and a hydrophilic polymer is achieved, the application of a spray-drying procedure of the resulting mixture will result in the formation of microcapsules. The techniques for achieving an encapsulated product in high efficiency will depend on many technical parameters and can be found in the patent literature. Normally a mixture of essential oil:hydrophilic polymer (4:1) can be used, but this will also depend on the type of equipment used. The reader is advised to refer to the parameters given for the polymer used in the experiment. To achieve the encapsulated product, a mixture of low pressure and temperature is used in the spray-drying equipment and a loss of essential oil/volatiles is inevitable. However, a recovery of more than 70% can be achieved by carefully monitoring the production conditions.

17.3 USE OF HYDROPHILIC POLYMERS

In product development, one tends to use cheap derivatives of starches or other low-grade quality polymers. Early studies with protein-based polymers such as gelatine, gelatine derivatives, soy proteins, and milk-derived proteins gave reasonable technical quality of the products. However, even if these materials show stable emulsification properties with essential oils, they have some unwanted side effects in products. We have seen that a more careful control of the polymer used can result in real high-tech products, where the predictability of the release of the volatiles can be assured like a programmed release of drug molecules in drug delivery devices. The polymer quality to be used will, of course, depend on the intention of the final product. In the cosmetic industry, where one is looking for an essential oil product, free-flowing and dry, to mix with a semisolid or a solid matrix, the use of simple starch derivatives will be very good. For other applications, where the release of the volatiles needs to be controlled or predicted more accurately, it is recommended that a more thorough selection of a well-characterized polymer is done. One example of a very good and controllable polymer is alginate. This polymer is available in many qualities and can be tailored to any controlled release product. The chemistry of alginate is briefly discussed below as this discussion will allow the reader to decide whether to opt for an alginate of technical quality or, if a high-tech product is the aim, to choose a better characterized hydrocolloid.

17.4 ALGINATE

Alginates are naturally occurring polycarbohydrates consisting of the monomers α-L-glucuronic acid (G) and β-D-mannuronic acid (M). The relative amounts of these two building blocks will influence the total chemistry of the polymer. The linear polymer is water soluble due to its polarity. Today the alginate can be produced by the bacteria that allow us to control the composition of the monomers (G/M ratio). The chemical composition of the alginate is dependent on the origin of the raw material. The marine species display seasonal differences in the composition and different parts of the plant produce different alginates. Alginates may undergo epimerization to obtain the preferred chemical composition. This composition (G/M ratio) will determine the diffusion rate through the swollen alginate gel, which surrounds the encapsulated essential oils (Elias, 1997; Amsden, 1998a, 1998b; Ogston, 1973). An important structure parameter is also the distribution of the carboxyl groups along the polymer chain. The molecular weight of the polymer is equally important, and molecular weights between 12,000 and 250,000 are readily available in the market. The alginate polymer can form a swollen gel by hydrophobic interaction or by cross-linking with divalent ions like calcium. The G/M ratio determines the swelling rate and therefore also the release of encapsulated compounds. The diffusion of different substances has been studied and references can be made to essential oil encapsulation. The size of alginate capsules can also vary from 100 μm or more down to the nanometer range depending on the production procedure chosen (Draget et al., 1994, 1997; Donati et al., 2005; Tønnesen and Karlsen, 2002; Shilpa, 2003).

17.5 STABILIZATION OF ESSENTIAL OIL CONSTITUENTS

The encapsulation of essential oils in a hydrophilic polymer may stabilize the constituents of the oil but a better technique for this purpose will often be to use cyclodextrins in the encapsulation process. The use of cyclodextrins will lead to a complexation of the single compounds, which will again stabilize the complexed molecule. Complex formation with cyclodextrins is often used in drug delivery to promote solubility of lipophilic compounds; however, in the case of volatiles containing compounds that may oxidize, the complex formation will definitely prolong the shelf life of the finished product. A good review of the flavor encapsulation advantages is given by Risch and Reineccius (1988). The most important aspect of essential oil encapsulation in a hydrophilic polymer is that the volatility is lowered. Lowering the volatility will result in longer shelf life of products and a better stability of the finished product in this respect.

17.6 CONTROLLED RELEASE OF VOLATILES FROM NONVOLATILE PRECURSORS

The limited effect of volatiles for olfactive perception has led to the development of encapsulated volatiles and also to the development of covalent-bonded fragrance molecules to matrices. In this way, molecules release their fragrance components by the cleavage of the covalent bond. Mild reaction conditions met in practical life initiated by light, pH, hydrolysis, temperature, oxygen, and enzymes may release the flavors. The production of "profragrances" is a very active field for the industry and has led to numerous patents. The plants producing essential oils have invented means by which the volatiles are produced, stored, and released into the atmosphere at predestined times related to the environmental factors. The making of a profragrance involves mimicking these natural procedures into flavor products. However, we are simplifying the process by using only one parameter in this release process, that is, the splitting of a covalent bond. In theory, the making of a long-lasting biological impact and the breakdown of a constituent are contradictory reactions. However, in practice the use of a covalent bond and thereafter the control of the splitting of this bond by parameters such as light, humidity, temperature, and so on can be built into suitable flavor

and fragrance products. Naturally, this technique using covalent bonding is only applicable to single essential oil constituents but constitutes a follow-up of essential oil encapsulation (Herrmann, 2004, 2007; Powell, 2006).

17.7 CYCLODEXTRIN COMPLEXATION OF VOLATILES

Cyclodextrin molecules are modified carbohydrates that have been used for many years to modify the solubility properties of drug molecules by complexation. The cyclodextrin can also be applied to volatiles to protect them against the environmental hazards and thus prolong the shelf life of these compounds. Cyclodextrin complexation will also modify the volatility of the essential oils and prolong the bioactivity. The cyclodextrins will give a molecular encapsulation by the complexation reaction with volatile molecules. The complexation of the volatiles with cyclodextrin may improve the heat stability, improve the stability toward oxygen, and improve the stability against light (Szente, 1988). A significant lowering of the volatility has been observed for the complexation with essential oils (Risch and Reineccius, 1988). The complexation of essential oils by the use of cyclodextrins will also result in increased heat stability. This is in contrast with the stability of volatiles that have been adsorbed on a polymer matrix. The use of cyclodextrins can protect the volatiles against

Loss of volatiles upon storage of a finished product
Light-induced instability
Heat decomposition
Production of free-flowing "dry" powders
Oxidation.

17.8 CONCLUDING REMARKS

The encapsulation/complexation of essential oils, volatiles, or single oil constituents will result in a remarkable lowering of the volatility, stabilize the constituents, improve the shelf life of finished products, and prolong the biological activity. The control of these parameters will depend on the nature of the volatiles to be encapsulated. Most of the literature on the encapsulation of volatiles is found in the patent literature. Techniques described in the literature allow the user of essential oils to choose the polymer matrix in which to encapsulate an essential oil according to the use of the finished product. The effect of controlled delivery of flavor and fragrance molecules opens up large areas of applications, which previously were limited due to the volatility of the essential oils and their constituents. The encapsulation or lowering the volatility of compounds like essential oil ingredients will allow for more relevant studies of the biological effects of volatile compounds.

REFERENCES

Amsden, B., 1998a. Solute diffusion within hydrogels. *Macromolecules*, 31: 8382–8395.
Amsden, B., 1998b. Solute diffusion in hydrogels. *Polym. Gels Networks*, 6: 13–43.
Deasy, P.B., 1984. *Microencapsulation and Related Drug Processes*. New York: Marcel Dekker.
Donati, I., S. Holtan, Y.A. Morch, M. Borgogna, M. Dentini, and G. Skjåk-Bræk, 2005. New hypothesis on the role of alternating sequences in calcium-alginate gels. *Biomacromolecules*, 6: 1031–1040.
Draget, K.I., G. Skjåk-Bræk, B.E. Christensen, O. Gåserød, and O. Smidsrød, 1997. Swelling and partial solubization of alginic acid beads in acids. *Carbohydr. Polym*., 29: 209–215.
Draget, K.I., G. Skjåk-Bræk, and O. Smidsrød, 1994. Alginic acid gels; the effect of alginate chemical composition and molecular weight. *Carbohydr. Polym.*, 25: 31–38.
Elias, H.-G., 1997. *An Introduction to Polymer Science*. Weinheim, Germany: VCH.
Fulger, C. and M. Popplewell, 1997. Flavour encapsulation. U.S. Patent 5.601.845.
Fulger, C. and M. Popplewell, 1998. Flavour encapsulation. U.S. Patent 5.792.505.
Herrmann, A., 2004. Photochemical fragrance delivery systems based on the norrish type II reaction—a review. *Spectrum*, 17: 10–13 and 19.

Herrmann, A., 2007. Controlled release of volatiles under mild reaction conditions: From nature to everyday products. *Angew. Chem. Int. Ed.*, 46: 5836–5863.

McIver, B., 2005. Encapsulation of flavour and/or fragrance composition. U.S. Patent 6.932.982.

Ogston, A.G., B.N. Preston, and J.D. Wells, 1973. On the transport of compact particles through solutions of chain polymers. *Proc. R. Soc. (London) A*, 333: 297–309.

Porzio, M., 2008. Melt extrusion and melt injection. *Perfumer Flavorist*, 33: 48–53.

Powell, K., J. Benkhoff, W. Fischer, and K. Fritsche, 2006. Secret sensations: Novel functionalities triggered by light—Part II: Photolatent fragrances. *Eur. Coat. J.*, 9: 40–49.

Risch, S.J. and G.A. Reineccius, 1988. *Flavor Encapsulation*. ACS Symposium Series 370, American Chemical Society, Washington.

Sair, L., 1980b. Food supplement concentrate in a dense glass house extrudate. U.S. Patent 4.232.047.

Sair, L. and R. Sair, 1980a. Encapsulation of active agents as microdispersions in homogeneous natural polymers. U.S. Patent 4.230.687.

Shilpa, A., S.S. Agarwal, and A.R. Ray, 2003. Controlled delivery of drugs from alginate matrix. *Macromol. Sci. Polym. Rev.*, C, 43: 187–221.

Szente, L. and J. Szejtli, 1988. Stabilization of flavors by cyclodextrins. In: *Flavor Encapsulation*, S.J. Risch (ed.), pp. 148–157. ACS Symposium Series 370, Washington DC: American Chemical Society.

Tønnesen, H.H. and J. Karlsen, 2002. Alginate in drug delivery systems. *Drug Dev. Ind. Pharm.*, 28: 621–630.

Zasypkin, D. and M. Porzio, 2004. Glass encapsulation of flavours with chemically modified starch blends. *J. Microencapsulation*, 21: 385–397.

18 Aroma-Vital Cuisine
Healthy and Delightful Consumption by the Use of Essential Oils

Maria M. Kettenring and Lara-M. Vucemilovic-Geeganage

CONTENTS

> Your nourishment ought to be your remedies and your medicaments shall be your food.
>
> **—Hippocrates**

Certainly, the value of our nutrition, in terms of nutritional physiology, is not only conditioned by its nutrient and calorie contents. Moreover, also health-conscious and constitutional eating habits require an adequate preparation of meals as well as an appropriate form of presentation. Early sophisticated civilizations and their health doctrines, like that of the Traditional Chinese Medicine (TCM), Ayurveda in Southeast Asia, and for instance the medical schools during the ancient Greek period examined individuals and their reaction on life circumstances, habits, nutrition, and substances, to contribute to a long-lasting health. To support a person's balance the aim was to develop a conscious way of using the senses and a balanced sensory perception.

Thus fragrances are a kind of soul food, as the information of scents can be perceived in every section of our self, physical, energetic as well as intellectual, from a holistic point of view. Adding spice with essential oils according to the Aroma-Vital cuisine combines sensuality with sanative potential.

People across continents and cultures have experimented with the healing virtues of "nature's bouquet" or just simply tried to enhance the flavor and vitality of their meals. The ancient Egyptian civilization reverted to an elaborated dinner ceremony by using the efficacy of essential oils to get

the participants in the mood for the meal. Before the food was served, heated chalices with scented fats, enriched with a variety of herbs and spices were provided, not only to spread pleasant smells, rather as a kind of odorous aperitif to activate ones saliva to prepare for digestion. Meals that have been enriched with essential oils or expressed oils, rebound to a conscious awareness of consuming food, are well-nigh comparable, like going on a culinary expedition. This fare is perceived as a composition of tastes, which is not only tastefully ingenious, but also might be able to raise the food's virtue.

In this regard the entropy rather than the potency of the condiment is significant. The abundance of nuances, the art of adding flavor on the cusp of being noticeable, becomes more important than giving aroma officiously. The scents hovering above the meals, almost like a slight breeze, compound the food's own natural flavor in a subtle manner. "Less is more" is the economic approach which in this context is indicative.

The sensation of satiety is taking place early on. Due to this desire to savor to the fullest, the taste is excited and leads to longer chewing. This in turn activates α-amylase (amylolytic enzyme, already working in the oral cavity). Conditionally on the high bioavailability, especially of the monoterpenes, which are significant and available in the paring of citrus fruits and some kind of herbs, in a sense the Aroma-Vital cuisine shows aspects of the salutary genesis (Salutogenese). The savoriness of the food, pleasant smell, and appetizing appearance plays a prominent role here, at last the appetite regulates between physiological needs and pleasure and thus variety and vitally enhanced meals are in demand.

18.1 BASIC PRINCIPLES OF THE AROMA-VITAL CUISINE

18.1.1 The Heart of Culinary Arts is Based on Exquisite Ingredients and an Accomplished Rounding

Natural aromas, from blossoms, herbs, seeds, and spices, extracted in artificial pure essential oils, delicately accompany the elaborate cuisine. They are not supposed to supersede fresh herbs, rather complementing them. If, however, herbs are not available, natural essences are delightfully suited to add nuances. They are giving impetus to and are flexible assistants for preparing last-minute menus. One should use this rich source to compile a first-aid assortment of condiments or even a mobile spice rack.

18.1.2 Quality Criteria and Specifics that have to be Adhered to, while Handling Essential Oils for Food Preparation

The regional legal regulations of the food chemical codex or the local food legislation might differ and if one is going to use essential oils professionally, one has to be firm with them, but still there are certain basics that deserve attention and lead to a safe and healthy way of practicing this subtle culinary art.

For cooking, solely 100% pure essential oils from controlled organic cultivation should be used. Oils that are not available of controlled organic origin, particularly those that are cold-pressed, a residue check should be guaranteed by the manufacturer to ensure that the product does not contain harmful amounts of pesticides. The label should not only contain name, contents, and quantity but also

- Latin definition
- Country of origin
- Description of used plant parts
- Used method of extraction

- Date of expiry
- If the oil has been thinned, the exact ratio of mixture
- If solvents have been used, they should be mentioned.

For the Aroma-Vital cuisine, the only acceptable solvent would be alcohol. As the oil is used in very small and thinned concentrations it would not be harmful to children. Less qualitative oils from industrial origin sometimes might even contain other substances. It should be indicated that natural flavorings used in food production should be pure and free of animal by-products such as gelatin or glycerin, which has been obtained by saponification of animal fat.

18.1.3 Storage

Essential oils are very sensible to the disposure of light, air and temperature; therefore they should be stored adequately. In this way, long-lasting essential oils keep their aroma as well as their ingredients and might even develop their bouquet. Foods or processed foods with essential oils may not be stored in tin boxes. Very important: essential oils should be kept away from children.

18.1.4 Quantity

The internal use of essential oils has to be practiced carefully. This subtle art is an amazing tool, but swallowed in too huge amounts, they are bad for one's health. One should never add the pure concentrate of essential oils to foods; it should not be forgotten that 1 drop is often comparable to a huge amount of plant material. Therefore, they ought to be always thinned and the dilution should be used teaspoon by teaspoon.

18.1.5 Emulsifiers and Forms of Administering

Essential oils are not water soluble; therefore, emulsifiers are necessary to spread their aroma, they are for example

1. Basic oils, special oils, or macerated oils
2. Butter, milk, curd, egg yolk, and mayonnaise
3. Alcohol and vinegar
4. Syrups, molasses, honeys, treacles, and sugars
5. Salt
6. Tofu, soy sauce and tamarind sauce
7. Avocado, lemon juice, and coconut
8. Sesame seeds, sunflower seeds, almonds, and walnuts.

On the basis of these emulsifiers and a mixture of essential oils, a variety of "culinary assistants" can be conjured up: spiced oils, spiced butter or mayonnaises, spiced alcohols, spiced syrups, spiced sauces, or even spiced salts. These blends can be prepared in advance and stored to use them for everyday meals. Another nice variation is the use of hydrolates (a partial extract of plant material extracted by distillation) such as rose water, for food preparation.

18.1.6 To Add Spice with Natural Aromas in a Balanced Way

To know how food and essential oils interact is a great help to create a harmonic assembly of foods, which is nourishing us from a holistic point of view. In this manner, the sun-pervaded seed oils of anise, bay, dill, fennel, or caraway might be able to aerate the earthy corm- and root-vegetable. Salads can be enhanced and prepared to be more digestive by adding pure natural essential oils such as thyme, rosemary, and clementine to the marinade, or another rather Asian variation would be to add ginger, pepper, and lemon grass.

18.1.7 Essential Oils are able to Lift Our Spirits as well

A condiment ensemble of orange, vanilla extract, cacao extract, and rose for example, is able to support soul foods such as milk rice, milk shakes, and desserts in their attitude to supply security and confidence.

18.2 A SMALL CULINARY TRIP: AROMA-VITAL CUISINE RECIPES AND INTRODUCTION

TABLE 18.1
Basic Spice Rack of Essential Oils: How to Prepare Essential Oil Mixtures and Essential Oil Seasonings

Basic Essential Oils	Mixtures	Emulsifier Seasonings		Recipes Example
		EURO ASIA		
Lime (*Citrus aurantiifolia*)	5 drops	1. Oil	50 mL sesame oil	Asian style
		2. Dairy prod.	50 mL mayonnaise	Eggs
Coriander seed (*coriandrum sativum*)	1 drop	3. Vinegar	50 mL rice vinegar	Sushi
		4. Sweetener	50 mL agave syrup	Chutney
Ginger (*Zingiber officinalis*)	2 drops	5. Salt	50 mg sea-salt	Spice
		6. Tofu and co	50 mL soy sauce	Marinated fried tofu
Lemongras (*Cymbopogon citratus*)	1 drop	7. Vegetables and fruits	50 mL coconut milk	Rice and curry
		8. Nuts and seeds	50 mg sesame seeds	Spice
Green pepper (*Piper nigrum*)	1 drop			
		O SOLE MIO		
		1.	50 mL olive oil	Pasta
Thyme linalool (*Thymus vulgaris*)	1 drop	2.	50 mL egg yolk	Omelette
		3.	50 mL balmy vinegar	Salad
Rosemary cineole (*Rosmarinus officinalis*)	1/2 drop	4.	50 mL honey	Cuisine Provencal
		5.	50 mg sea-salt	Spice
Clementine (*Citrus deliciosa*)	5 drops	6.	50 mg tofu	Grilled tofu
		7.	50 mg avocado	Guacamole
		8.		Pesto
		CAPRI		
Orange (*Citrus sinensis*)	5 drops	1.	50 mL hazelnut oil	Desserts
		2.	50 mL buttermilk	Drink
Lemon (*Citrus limon*)	3 drops	3.	50 mL cider vinegar	Salad
		4.	100 mL maple syrup	Desserts
		5.	50 mg sea-salt	Spice
		6.	50 mL apple vinegar	Fruit salad
		7.	50 mg avocado	Sauce
		8.	50 mg walnuts	Cakes
		BERGAMOT-GRAND MANIER		
Grapefruit (*Citrus paradisi*)	5 drops	1.	50 mL walnut oil	Salad
		2.	50 mg butter	Cake

continued

TABLE 18.1 (continued)
Basic Spice Rack of Essential Oils: How to Prepare Essential Oil Mixtures and Essential Oil Seasonings

Basic Essential Oils	Mixtures	Emulsifier Seasonings		Recipes Example
Orange (*Citrus sinensis*)	5 drops	3.	1 L white vine	Beverage
		4.	50 mg raw sugar	Sweets
Limon (*Citrus limon*)	2 drops	5.	50 mg sea-salt	Spice
		6.	50 mL tamarind sauce	Thai cuisine
Bergamot (*Citrus bergamia*)	2 drops	7.	50 mL lemon juice	Drink
		8.	50 mg pumpkin seeds	Soup
		MAGIC ORANGE		
Orange (*Citrus sinensis*)	5 drops	1.	50 mL almond oil	Sweets
		2.	50 mg goat cheese	Oriental
Vanillaextract (*Vanilla planifolia*)	3 drops	3.	50 mL raspberry vinegar or balsamic vinegar	Fruit salad
		4.	100 mL honey/treacle	Sweets
Kakaoextract (*Theobroma cacao*)	3 drops	5.	—	
		6.	50 mL seitan tofu	Oriental
Rose (*Rosa damascena*)	1/2 drop	7.	50 mg bananas	Desserts
		8.	50 mg almonds	Spice
		CLARY SAGE AND BERGAMOT		
Clary sage (*Salvia sclarea*)	2 drops			Spice
Bergamot (*Citrus bergamia*)	5 drops	5.	50 g sea-salt	
		PEPPERMINT		
Peppermint (*Mentha piperita*)	Rather less—2 drops per 100 mL/mg	4.	100 mL maple syrup	Drink
		LAVENDER		
Lavender (*Lavandula officinalis*)	Rather less—2 drops per 100 mL/mg	4.	100 mL honey	Cuisine Provencal

MENU

Basics

Crispy Coconut Flakes (Flexible Asian Spice Variation)
Gomasio (Sesame Sea-Salt Spice)
Honey Provencal

Beverages

Aroma Shake with Herbs
Earl Grey at His Best
Lara's Jamu
Rose-Cider
Syrup Mint-Orange

Entrees

a. Soups:
   Peppermint Heaven
   Perky Pumpkin Soup
b. Salads:
   Melon-Plum Purple Radish Salad
   Salad with Goat Cheese and Ricotta

Appetizer and Finger Food

Crudities—Flavored Crispy Raw Vegetables
Maria's Dip
Tapenade
Tofu Aromanaise
Vegetable Skewer

Main Course

Celery—Lemon Grass Patties
Chèvre Chaude-Goat Cheese "Provence" with Pineapple
Crispy Wild Rice-Chapatis
Mango–Dates–Orange Chutney
Prawns Bergamot

Dessert, Cakes, and Baked Goods

Apple Cake Rose
Chocolate Fruits and Leaves
Homemade Fresh Berry Jelly
Rose Semifreddo
Sweet Florentines
(Chocolate should not be heated up more than 40°. Essential oils are best at 40° as well.)

AROMA-VITAL CUISINE RECIPES

Basics

Crispy Coconut Flakes (Flexible Asian Spice Variation)

Nice with Asian flavored dishes or sweet baked goods.
Ingredients:

- 50 g dried coconut flakes
- 10 drops EURO ASIA intermixture (spicy variation) or 10 drops MAGIC ORANGE intermixture (sweet variation)
- 1 preserving jar.

Preparation: Roast the coconut flakes in a frying pan. Lightly scatter the chosen essential oils into the empty jar. Spread the oil well, then fill in the roasted coconut rasps and shake it well.

Gomasio (A Sesame Sea-Salt Spice)

Gomasio is a secret of the Middle Eastern cuisine, which completes your spice rack and gives a subtle salty flavor to the dish. Nice to combine with soy sauce, fresh thyme leaves, or cumin.

Ingredients:

- 50 g sesame seeds
- 1 teaspoon EURO ASIA seasoning salt no. 5
- 1 preserving jar.

Preparation: Roast the sesame seeds in a frying pan, then mix the seeds with the salt in a mortar. Crush them lightly with a pestle to release the flavor. Fill into a preserving jar and shake it well. If necessary add a few more drops of EURO ASIA intermixture.

Honey Provencal

A great basic for the cuisine Provencal.
Ingredients:

- 100 ml acacia honey
- 5 drops O SOLE MIO intermixture
- 2 drops LAVENDER pure essential oil
- 1 drop EURO ASIA intermixture
- 1 drop CLARY SAGE AND BERGAMOT intermixture.

Preparation: Emulsify the ingredients well. Use the honey to brush grilled vegetables, tofu, goat and sheep cheese, or to season gratins, to add a fabulous distinctly French flavor to a simple dish.

Beverages

Aroma Shake with Herbs

This green fruity flavored cleansing juice certainly is a great rejuvenator.
Ingredients:

- 500 mL organic buttermilk
- 100 mL organic soy milk
- 5 tablespoons sprouts (alfalfa, adzuki bean sprouts, and cress)
- 100 mL carrot juice
- 3 drops CAPRI intermixture
- 2 drops EURO ASIA intermixture
- 1 tablespoon maple syrup
- 1 tablespoon parsley finely chopped.

Preparation: Pour the buttermilk and soy milk in a blender and process for a few minutes until combined. Add the carrot juice, then emulsify the essential oils with the maple syrup and stir it into the mixture. Fill into iced tall glasses and serve chilled. A decorative idea is to dive the top of the glasses into lemon juice and then into the finely chopped parsley, before filling in the shake.

Earl Grey at His Best

Ingredients:

- 1 preserving jar (100 g capacity)
- 100 g Darjeeling tea “first flush”
- 10 drops BERGAMOT basic essential oil.

Preparation: Lightly scatter the "BERGAMOT" basic essential oil into the empty jar. Add the tea, close the jar and shake it well. Repeat the procedure to shake the jug for the next 5–10 days; then this incredible sort of flavored tea will be ready to serve.

Lara's Jamu

Jamu is a kind of herbal tonic from Southeast Asia. Every country and family has their own recipes. This one is a tasty booster for the immune system.
Ingredients:

- The rind of two limes in thin shreds
- Juice of two limes
- 2 tablespoons freshly grated ginger
- 1 handful fresh or dried nettle
- 50 ml maple treacle
- 2 teaspoons curcuma powder
- 500 ml water
- 750 ml of sparkling water (optional)
- 5 drops EURO ASIA intermixture
- 2 drops PEPPERMINT basic essential oil
- 3 drops CAPRI intermixture.

Courtesy of Subash J. Geeganage.

Preparation: Boil the mixture of lime, ginger, and nettle with 500 ml water for 10 min; then let it cool down a bit to be able to sieve it later into decorative chalices. Mix the curcuma powder with fresh lime juice and the EURO ASIA basic essential oil and stir it into the herbal mixture. Now the maple treacle mixed with PEPPERMINT basic essential oil will be stirred in as a sweetener. Serve hot or chilled with sparkling water, fresh mint sprigs, and sliced lime.

Rose-Cider

Refreshing and inspiring.
Ingredients:

- 1 L cider
- 1/2 drop rose basic essential oil or 1 tablespoon organic rose water.

Preparation: Stir in the rose oil or rose water. Serve cold.

Syrup Mint-Orange

A refreshing hot summer drink.
Ingredients:

- 50 mL PEPPERMINT seasoning syrup no. 4
- 5 drops CAPRI intermixture.

Preparation: Simply mix the ingredients and you have a refreshing basic syrup, which can be used for drinks, baked goods, to pour it into soda water, tea juices, or even into ice cubes. To serve, garnish the drinks with some fresh peppermint leaves.

Entrees

Soups

Peppermint Heaven
Ingredients:

- 500 mL vegetable stock
- Fresh peppermint leaves for decoration
- 2–3 drops PEPPERMINT basic essential oil
- 1 drop BERGAMOT basic essential oil
- 150 mL cream
- O SOLE MIO salt no. 5 or regular salt to season to taste.

Preparation: Whip the cream; then add the basic essential oils to it. Meanwhile boil the vegetable stock; then stir in the cream. Ladle into soup bowls to serve and garnish each with a little bit whipped cream and fresh mint leaves.

Perky Pumpkin Soup
Warm and spicy—the perfect autumn dinner.
Ingredients:

- 2 drops CAPRI intermixture
- 1 large onion, finely chopped
- 2 carrots, sliced finely
- 1 tablespoon pumpkin seed-oil or butter
- 500 g peeled pumpkin, finely chopped into cubes
- 200 mL vegetable stock
- 50 mL cream
- 1 teaspoon curry powder
- 1 tablespoon EURO ASIA seasoning oil no. 1
- Fresh coriander to garnish
- 1 tablespoon CAPRI seasoning salt no. 5
- A little bit sherry.

Preparation: Heat the pumpkin seed oil in a saucepan. Add the onion and carrots and cook over moderate heat until it softens. Stir in the pumpkin pieces and cook until the pumpkin is soft. Process the mixture in a blender and pour it to the pan. Stir in the vegetable stock and cream and season with the essential oils, salt, and sherry. Ladle into warm soup bowls and garnish each with some fresh coriander leaves.

Salads

Melon-Plum Purple Radish Salad

A refreshing hot summer party dish.
Ingredients:

- 1 mid-size watermelon or 2 Galia melons
- 1 handful radishes rinsed and chopped
- 1 bell pepper rinsed and sliced
- 3 pears rinsed and chopped
- Juice of 1 lemon
- 1 tablespoon CAPRI or O SOLE MIO seasoning oil no. 1
- 250 g sour cream
- 150 g curd
- Salt
- Freshly ground black pepper
- Some fresh summer herbs like thyme, cress or lemon balm.

Courtesy of Ulla Mayer-Raichle.

Preparation: Half the melon in a zigzag manner, separate the halves, remove the seeds from the melon halves, and use a melon baller to scoop out even-sized balls. Place the half of the melon balls, radishes, bell pepper, and pears in a large salad bowl and marinade the salad with lemon juice. Then store the melon halves and the salad in the fridge for at least half an hour. Meanwhile mix the seasoning oil of your choice with sour cream and curd and season with salt and pepper. Stir the mixture into the salad carefully and fill the salad into the melon halves. Garnish them with herbs and some of the extra melon balls.

Salad with Goat Cheese and Ricotta

A refreshing companion for spicy foods.
Ingredients:

- 1 red bell pepper rinsed, sliced
- 1 green bell pepper rinsed, sliced
- 1 scallion, chopped
- 1 head salad greens (Aragula, Sorrel, Dandelion, etc.), rinsed, dried, and chopped.

For the salad dressing:

- 1 drop O SOLE MIO intermixture
- 3 drops CAPRI intermixture
- 4 tablespoons dark olive oil
- Juice of 1 lemon
- Sea-salt
- 100 g goat cheese or ricotta, chopped
- 1/2 handful fresh eatable spring blossoms (daisies, primroses, etc.), rinsed
- 2 handfuls fresh herbs of your choice (coriander, parsley, basil, etc.), rinsed
- Roasted sesame.

Preparation: Emulsify the essential oil intermixtures with the olive oil; add the lemon juice and season with salt. Place the dressing in a large bowl, marinade the cheese, and add the salad leaves, bell peppers, and scallion. Mix well and garnish with the herbs and blossoms and the roasted sesame.

Appetizer and Finger Food

Crudities—Flavored Crispy Raw Vegetables

Simple and delicious.
Ingredients:

- 750 g vegetables well rinsed and cut into crudities (radishes, scallions, chicory, carrots, etc.)
- Juice of 1 lemon
- 5 drops CAPRI intermixture.

Preparation: Emulsify the CAPRI intermixture into the lemon juice, fill it into a spray flacon, and spread it on top of the sliced vegetables. Serve with dip and breadsticks or baguette.

Maria's Dip

Ingredients:

- 3 drops CAPRI intermixture
- 1 tablespoon creme fraiche
- 1/2 teaspoon salt
- 250 g sour cream.

Preparation: Emulsify the CAPRI essential oil intermixture into the creme fraiche. Stir in the salt and sour cream until combined. Ready to serve with bread, toast, and for example, the flavored crudities.

Tapenade

An Italian secret simple to make and perfect for dipping or seasoning.
Ingredients:
For the olives:

- 200 g pitted green or black olives, rinsed and halved
- 100 mL dark olive oil
- 1 handful fresh rosemary
- 10 drops O SOLE MIO intermixture.

For the tapenade:

- 60 g capers
- 1 crushed garlic clove
- Freshly ground black pepper.

Preparation: Marinate the olives in a mixture of olive oil, rosemary, and O SOLE MIO intermixture for at least 1 h. Place the olives, capers, and garlic in a food processor or blender and process until combined. Gradually add the flavored marinade and blend to a coarse paste; season with pepper. Keep stored in the fridge for up to 1 week.

Tofu Aromanaise

Served with the veggie skewers—a truly impressive dinner party dish.
Ingredients:

- 200 g organic pure tofu or smoked tofu
- 3 tablespoons sunflower oil
- 2 tablespoons EURO ASIA seasoning oil no. 1
- EURO ASIA seasoning salt no. 5
- A few chives.

Preparation: Put the tofu in a blender and process it until the tofu is smooth. Transfer the creamy tofu to a bowl and stir in the sunflower oil very slowly, then add the EURO ASIA seasoning oil, and season with EURO ASIA salt. Garnish the top with chopped chives. Serve cold.

Veggie Skewers

A tasty idea for your next barbecue.
Ingredients:

- 20 skewers
- 1000 g fresh young vegetables

(tomatoes, fennel, eggplants, carrots, bell peppers, scallions, etc.).
For the marinade:

- 5 tablespoons dark olive oil
- 3 tablespoons either O SOLE MIO or EURO ASIA seasoning oil no. 1
- Freshly grounded pepper
- 1 handful fresh chopped herbs (basil, thyme, parsley, etc.) or dried herbs.

Courtesy of Ulla Mayer-Raichle.

Preparation: Prepare the vegetables and cut them into cubes. Mix all the marinade ingredients in a shallow dish and add the vegetable cubes. Spoon the marinade over the vegetables and leave to marinate in the fridge for at least 1 h. Then thread the cubes onto skewers. Brush with the marinade and broil or grill until golden, turning occasionally. Serve with baguette, tofu aromannaise tapenade, or any other dip.

Main Course

Celery Lemon Grass Patties

Delicious, little, and flexible to combine.
Ingredients:

- 1–2 large celery
- 250 mL liquid vegetable stock
- 1 organic free range egg
- 4 lemon slices
- 1 pinch of BERGAMOT CLARY SAGE no. 5.

Asian variation:

- 3 tablespoons coconut flakes
- 2 tablespoons EURO ASIA seasoning no. 1
- Coconut oil or roasted sesame oil to fry.

Mediterranean variation:

- 2 tablespoons O SOLE MIO seasoning no. 1
- 3 tablespoons sesame seeds
- Soy oil to fry.

Preparation: Blanche the washed and sliced celery roots in the vegetable stock. Choose your favorite cookie cutter, like heart or star, and cut them out of the blanched celery. Whisk the egg and stir in the essential oil variation of your choice. Marinate the celery stars and hearts, then coat them with coconut flakes or sesame seeds and fry them until they have a delicious golden brown color. To serve, top them with a small amount of the essential oil seasoning. They are great to accompany salads, baked potatoes with sour cream, and other vegetarian dishes or if you prefer, beef creations.

Chévre Chaude-Goat Cheese "Provence" with Pineapple

Ingredients:

- 4 slices of fresh pineapple
- 1 tablespoon sunflower oil or butter or ghee
- 1 teaspoon "O SOLE MIO honey" no. 4
- 1 tablespoon CAPRI honey no. 4
- 2 tablespoons honey PROVENCAL (basics)
- 2–3 small goat or sheep cheese
- A little bit fresh or dried thyme to garnish
- Sour cream
- Salad or Parma ham (optional).

Courtesy of Ulla Mayer-Raichle.

Preparation: Halve the pineapple slices and fry them on both sides. Lower the heat and top them with CAPRI honey. Preheat the oven to 180°C. Halve the cheese and place them on top of each of the two pineapple slices. Drop a little bit of honey PROVENCAL on each portion and bake it shortly until the cheese starts to caramelize. Serve immediately with the rest of the aromatized honeys dispersed on the surface, fresh herbs above, the sour cream on top, and with Parma ham or fresh salad aside.

Crispy Wild Rice-Chapatis

Ingredients:

- 200 g wild rice
- 400–500 mL warm water
- 1 laurel leaf
- 1 small onion or 3 scallions, finely chopped
- 1 teaspoon EURO ASIA seasoning oil no. 1
- 1 tablespoon EURO ASIA seasoning soy sauce no. 6
- 2 organic or free range eggs
- Curry powder
- Lemon juice as you like
- Around 2 tablespoons oil or ghee to fry.

Preparation: Steam the wild rice briefly, then fill it up with the rest of the warm water, and add the laurel leave. Cook it for another 15–20 min, then turn the heat down and stir in the EURO ASIA seasoning oil no. 1. Cover it, leave it and let it chill until firm. Then stir all ingredients into the wild rice. Divide the mixture into walnut-sized balls; then flatten them slightly. Heat the oil or ghee in a pan and fry the chapatis until golden brown on each side. Drain on paper towels and serve at once. These crispy wild rice-chapatis taste delicious with steamed vegetables and dips or even salads. They are ideal as a snack or a nice idea for the next picnic.

Mango–Dates–Orange Chutney

A spice dip-trip to Asia.
Ingredients:
For 1000 g you need

- 250 g organic well-scrubbed oranges (e.g., sweet and juicy sorts like Valencia)
- 250 g onions

- 250 g sliced mangoes
- 350 mL acacia honey
- If this is not available choose any other treacle or honeys, which is neutral in taste and of organic origin
- 50 mL maple syrup
- 2 teaspoons CAPRI essential oil seasoning salt no. 5
- A little bit of chile powder or 1 fresh chile pepper
- 350 mL cider vinegar
- 250 mg chopped dates
- 50 mL of either EURO ASIA
- or MAGIC ORANGE essential oil seasoning vinegar no. 3
- 2 tablespoons CAPRI essential oil seasoning syrup no. 4
- 5 drops pure EURO ASIA condiment intermixture.

Preparation: Remove long, thin shreds of orange rind, using a grater (zester). Scrape it firmly along the surface of the fruit. Remove the white layer of the oranges; then slice the oranges and remove the pits. Finely chop the onions. Peel the mangoes and cut them into small chunks. Mix honey, syrup, chile powder, and vinegar with 1 teaspoon of the CAPRI salt no. 5 and boil it in a huge saucepan until the honey melts, stir it well. Add mangoes, onions, dates, oranges, and the half of the shredded orange rind. Then lower the heat and let it simmer for 1 h, until the mixture has formed a thick mass. Stir in the rest of the shredded orange rind and the chosen essential oil vinegar no. 3. Then emulsify the pure EURO ASIA condiment intermixture into the CAPRI syrup no. 4 and stir it in the chutney. Use the rest of the CAPRI salt no. 5 to add spice. Fill the mixture into sterilized warm preserving jars, store them cold and dark. Nice to serve with the Chévre chaude or the crispy wild rice-chapatis and veggie skewers.

Prawns Bergamot

Ingredients:

- 500 g large prawns

Marinade:

- 5 drops pure CAPRI essential oil intermixture
- 1 small onion
- 1/2 crushed garlic clove
- 1 handful flat leaf parsley
- 3 scallions
- Juice of a lemon
- 2 drops pure BERGAMOT essential oil
- 1/2 teaspoon fennel seed
- 6 tablespoons olive oil
- Salt and fresh pepper
- 3 tablespoons BERGAMOT–GRAND MANIER vine no. 3.

Preparation: Prepare and wash the prawns as usual. Slice the onions and garlic, chop the parsley finely and cut the scallions into quarters. Take a teaspoon of lemon juice and emulsify the essential oils in it and mix in the rest of the ingredients. Let the prawns soak in the marinade and keep it in the fridge for 1 h. Then separate the prawns from the marinade; filter the marinade and keep the parts separately. Fry the prawns inside of the liquid parts of the marinade, then add the rest.

Stir it well for another minute, season with salt, pepper, and BERGAMOT vine and let it simmer slowly. Nice to serve with baguette or the crispy wild rice chapatis and vegetables like green asparagus tips.

Dessert, Cakes, and Baked Goods

Apple Cake Rose

This classic combination is an apples favorite destiny. Suited even for diabetics.
Ingredients:

- 250 g spelt flour
- 120 g finely sliced cold butter
- 1 organic or free range egg
- 1 tablespoon CAPRI essential oil seasoning no. 1
- Salt
- 50–100 mL warm water
- 1000 g sweet ripe apples
- Juice of a half lemon
- 1 tablespoon organic rose water.

For the topping:

- 250 ml cream
- 1 egg yolk of an organic or free range egg
- 5–7 drops MAGIC ORANGE pure seasoning intermixture
- 1 tablespoon organic rose water
- 50 g sliced almonds to garnish the top of the cake.

Preparation: Sift the flour, butter, egg, warm water, and the CAPRI seasoning no. 1 into a large mixing bowl. Mix everything together until combined; then store the cake mixture in the fridge for a half hour. In the meanwhile, peel and core the apples, slice them into wedges, and slice the wedges thinly. Combine lemon juice with rose water and splash it over the apples. For the topping, beat the egg yolk with the cream and the pure essential oil intermixture MAGIC ORANGE. Then pour the cake mixture into the prepared pan, smooth the surface, then make a shallow hollow in a ring around the edge of the mixture. Arrange the apple slices on top of the cake mixture. Pour the topping carefully above the apple slices and garnish the sliced almonds above. Cover the cake with aluminum foil. Bake for 30–40 min, until firm and the mixture comes away from the side of the pan. Lower the heat, remove the foil, and bake it for another 5 min. Serve warm.

Chocolate Fruits and Leaves

A delicious way to consume your favorite fruits, dried fruits, nuts, or even leaves like rose leaves.
Ingredients:

- 250 g organic chocolate couverture (bitter chocolate)
- 5 drops MAGIC ORANGE or BERGAMOT–GRAND MANIER or CAPRI intermixture – or 2–3 drops PEPPERMINT, LAVENDER, or GINGER pure basic essential oil, depending on your taste—spicy, mint, or fruity.

Courtesy of Ulla Mayer-Raichle.

Preparation: Warm up the chocolate couverture until you have a creamy consistency. Stir in your choice of basic essential oils or intermixture. Dive in the fruits, and let them dry. Serve chilled.

Homemade Fresh Berry Jelly

Ingredients:

- 500 gm mixed berries
- (blue berries; rasp berries; red, white, and black currant; black berries; strawberries, cranberries, cherries)
- 100 mL water
- 1 tablespoon agar or 2 tablespoons kuzu or sago (binding agent)
- 1–2 tablespoons cold water
- 12 drops MAGIC ORANGE intermixture
- 3 tablespoons maple syrup.

Preparation: Take the clean fruits and boil them in the water. Stir the binding agent into the small amount of cold water, then add it to the warm fruits and let them boil for another 3–5 min before you lower the heat, then leave the mixture to cool. Emulsify the essential oils intermixture with the maple honey; then stir it into the jelly. Serve cool with fresh berries or a spoonful of whipped cream with mint leaves.

Rose Semifreddo

Romantic and delicate aromatic dessert.
Ingredients:

- 150 g creme fraiche
- 75 g low fat quark
- 100 mL acacia honey
- 1 tablespoon rose water
- Rose leaves from 2 roses (organic farming)
- 2 tablespoons cognac

- Nonalcoholic alternative—1 drop pure MAGIC ORANGE intermixture in 2 tablespoons maple syrup
- 150 mL whipped cream
- 1 drop of pure MAGIC ORANGE intermixture.

Preparation: Place the creme fraiche and the quark in a bowl and cream together. Keep some rose leaves for decoration aside, process the rest of the leaves in a food processor until smooth, then transfer them into the bowl; add the acacia honey and stir to mix. Whisk in the rose water and either the cognac or the MAGIC ORANGE maple syrup. Fold in the whipped cream and the pure MAGIC ORANGE intermixture gently, being careful not to over mix. Pour the mixture into some small plastic containers, cover and freeze until the ice is firm. Transfer the ice to the refrigerator about 20 min before serving to allow it to soften a little. Serve in scoops decorated with rose leaves and berries.

Sweet Florentine

Sweet almond munchies.
Ingredients:

- 500 g butter
- 200 g sugar
- 2 packages organic bourbon vanilla sugar
- 250 mL cream
- 300 g sliced almonds
- 30 g spelt or wheat grain
- 15–20 drops MAGIC ORANGE or CAPRI intermixture emulsified in 1 tablespoon maple treacle
- 100 g chocolate couverture with 5–8 drops CAPRI or MAGIC ORANGE intermixture.

Preparation: Caramelize the sugar, then stir in the bourbon vanilla, butter until the sugar has been melted, then stir in almonds and flour. Preheat the oven to 180°C, then spoon the mixture on a baking tray and bake for 10 min. Do not worry, it is in their nature to melt. To serve, just cut them into diamonds after cooling down and remove them from the pan. Dive them halfway into the chocolate couverture only (the lower smooth side) then let them dry. Serve chilled or iced.

RÉSUMÉ

Aroma-vital cuisine is an aspect of aroma culture and therefore an art and cultivation of using the senses especially taste and smell.

19 Essential Oils Used in Veterinary Medicine

K. Hüsnü Can Başer and Chlodwig Franz

CONTENTS

19.1 INTRODUCTION

Essential oils are volatile constituents of aromatic plants. These liquid oils are generally complex mixtures of terpenoid and/or nonterpenoid compounds. Mono-, sesqui-, and sometimes diterpenoids, phenylpropanoids, fatty acids and their fragments, benzenoids, and so on may occur in various essential oils (Baser and Demirci, 2007).

Except for citrus oils obtained by cold pressing, all other essential oils are obtained by distillation. Products obtained by solvent extraction or supercritical fluid extraction are not technically considered as essential oils (Baser, 1995).

Essential oils are used in perfumery, food flavoring, pharmaceuticals, and sources of aromachemicals.

Essential oils exhibit a wide range of biological activities and 31 essential oils have monographs in the latest edition of the European Pharmacopoeia (Table 19.1).

TABLE 19.1
Essential Oil Monographs in the European Pharmacopoeia (6.5 Edition, 2009)

English Name	Latin Name	Plant Name
Anise oil	*Anisi aetheroleum*	*Pimpinella anisum* L. fruits
Bitter-fennel fruit oil	*Foeniculi amari fructus aetheroleum*	*Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*
Bitter-fennel herb oil	*Foeniculi amari herba aetheroleum*	*Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*
Caraway oil	*Carvi aetheroleum*	*Carum carvi* L.
Cassia oil	*Cinnamomi cassiae aetheroleum*	*Cinnamomum cassia* Blume (*Cinnamomum aromaticum* Nees)
Cinnamon bark oil, Ceylon	*Cinnamomi zeylanici corticis aetheroleum*	*Cinnamomum zeylanicum* Nees
Cinnamon leaf oil, Ceylon	*Cinnamomi zeylanici folium aetheroleum*	*Cinnamomum verum* J.S. Presl.
Citronella oil	*Citronellae aetheroleum*	*Cymbopogon winterianus* Jowitt
Clarysage oil	*Salviae sclareae aetheroleum*	*Salvia sclarea* L.
Clove oil	*Caryophylli aetheroleum*	*Syzigium aromaticum* (L.) Merill et L.M. Perry (*Eugenia caryophyllus* C.S. Spreng. Bull. et Harr
Coriander oil	*Coriandri aetheroleum*	*Coriandrum sativum* L.
Dwarf pine oil	*Pini pumilionis aetheroleum*	*Pinus mugo* Turra.
Eucalyptus oil	*Eucalypti aetheroleum*	*Eucalyptus globulus* Labill.
Juniper oil	*Juniperi aetheroleum*	*Juniperus communis* L. meyvesi
Lavender oil	*Lavandulae aetheroleum*	*Lavandula angustifolia* P. Mill. (*Lavandula officinalis* Chaix.)
Lemon oil	*Limonis aetheroleum*	*Citrus limon* (L.) Burman fil.
Mandarin oil	*Citri reticulatae aetheroleum*	*Citrus reticulata* Blanco
Matricaria oil	*Matricariae aetheroleum*	*Matricaria recutita* L. (*Chamomilla recutita* (L.) Ranschert)
Mint oil, partly dementholized	*Menthae arvensis aetheroleum partim mentholi privum*	*Mentha canadensis* L. (*Mentha arvensis* L. var. *glabrata* (Benth.) Fern, *Mentha arvensis* L. var. *piperascens* Malinv. ex Holmes) **Japanese mint**
Neroli oil (formerly bitter-orange flower oil)	*Neroli aetheroleum* (formerly *Aurantii amari floris aetheroleum*)	*Citrus aurantium* L. subsp. *aurantium* (*Citrus aurantium* L. subsp. *amara* Engl.)
Nutmeg oil	*Myristicae fragrantis aetheroleum*	*Myristica fragrans* Houtt.
Peppermint oil	*Menthae piperitae aetheroleum*	*Mentha* × *piperita* L.
Pine silvestris oil	*Pini silvestris aetheroleum*	*Pinus silvestris* L.
Rosemary oil	*Rosmarini aetheroleum*	*Rosmarinus officinalis* L.
Spanish sage oil	*Salviae lavandulifoliae aetheroleum*	*Salvia lavandulifolia* Vahl.
Spike lavender oil	*Spicae aetheroleum*	*Lavandula latifolia* Medik.
Star anise oil	*Anisi stellati aetheroleum*	*Illicium verum* Hooker fil.
Sweet orange oil	*Aurantii dulcis aetheroleum*	*Citrus sinensis* (L.) Osbeck (*Citrus aurantium* L. var. *dulcis* L.)
Tea tree oil	*Melaleucae aetheroleum*	*Melaleuca alternifolia* (Maiden et Betch) Cheel, *Melaleuca linariifolia* Smith, *Melaleuca dissitiflora* F. Mueller and other species
Thyme oil	*Thymi aetheroleum*	*Thymus vulgaris* L., *T. zygis* L.
Turpentine oil, *Pinus pinaster* type	*Terebinthini aetheroleum ab pinum pinastrum*	*Pinus pinaster* Aiton. (**Maritime pine**)

Antimicrobial activities of many essential oils are well documented (Bakkali et al., 2008). Such oils may be used singly or in combination with one or more oils. For the sake of synergism this may be necessary.

Although many are generally regarded as safe (GRAS), essential oils are generally not recommended for internal use. However, their much diluted forms (e.g., hydrosols) obtained during oil distillation as a by-product may be taken orally.

Topical applications of some essential oils (e.g., oregano and lavender) in wounds and burns bring about fast recovery without leaving any sign of cicatrix. By inhalation, several essential oils act as a mood changer and have effect especially on respiratory conditions.

Several essential oils (e.g., citronella oil) have been used as pest repellents or as insecticides and such uses are frequently encountered in veterinary applications.

In recent years, especially after the ban on the use of antibiotics in animal feed in the European Union since January 2006, essential oils have emerged as a potential alternative to antibiotics in animal feed.

Essential oils used in veterinary medicine may be classified as follows:

1. Oils attracting animals
2. Oils repelling animals
3. Insecticidal, pest repellent, and antiparasitic oils
4. Oils used in animal feed
5. Oils used in treating diseases in animals.

19.2 OILS ATTRACTING ANIMALS

Valeriana oils (and valerianic and isovalerianic acids) and nepeta oils (and nepetalactones) are well-known feline-attractant oils. Their odor attracts male cats.

Douglas fir oil and its monoterpenes have been claimed to attract deer and wild boar (Buchbauer et al., 1994).

Dogs are normally drawn to floral oils and usually choose to take these by inhalation only. Monoterpene-rich oils are usually too strong for dogs, with the exception of bergamot, *Citrus bergamia*.

Cats also usually select only floral oils for inhalation. Cats do not have metabolic mechanism to break down essential oils due to the lack of enzyme glucuronidase. Therefore, they should not be taken by mouth and should not be generally applied topically (http://www.ingraham.co.uk).

19.3 OILS REPELLING ANIMALS

Peppermint oil (*Mentha piperita*) repels mice. It can be applied under the sink in the kitchen or applied in staples to prevent mice annoying horses and livestock. A few drops of peppermint oil in a bucket of water used to scrub out a stall and sprinkling a few drops around the perimeter and directly on straw or bedding is said to eliminate or severely curtail the habitation of mice (Anonymous, 2001).

A patent (United States Patent 4961929) claims that a mixture of methyl salicylate, birch oil, wintergreen oil, eucalyptus oil, pine oil, and pine-needle oil repels dogs.

Another patent (United States Patent 4735803) claims the same using lemon oil and α-terpinyl methyl ether.

Another similar formulation (United States Patent 4847292) claims that a mixture of citronellyl nitrile, citronellol, α-terpinyl methyl ether, and lemon oil repels dogs.

A mixture of black pepper and capsicum oils and the oleoresin of rosemary is claimed to repel animals (United States Patent 6159474).

Citronella oil repels cats and dogs (Moschetti, 2003).

Repellents alleged to repel cats include allyl isothiocyanate (oil of mustard), amyl acetate, anethole, capsaicin, cinnamaldehyde, citral, citronella, citrus oil, eucalyptus oil, geranium oil, lavender

oil, lemongrass oil, menthol, methyl nonyl ketone, methyl salicylate, naphthalene, nicotine, paradichlorobenzene, and thymol. Oil of mustard, cinnamaldehyde, and methyl nonyl ketone are said to be the most potent.

Essential oils comprised of 10 g/L solutions of cedarwood, cinnamon, sage, juniper berry, lavender, and rosemary, each were potent snake irritants. Brown tree snakes exposed to a 2-s burst of aerosol of these oils exhibited prolonged, violent undirected locomotory behavior. In contrast, exposure to a 10 g L^{-1} concentration of ginger oil aerosol caused snakes to locomote, but in a deliberate, directed manner. The 10 g/L solutions delivered as aerosols of *m*-anisaldehyde, *trans*-anethole, 1,8-cineole, cinnamaldehyde, citral, ethyl phenylacetate, eugenol, geranyl acetate, or methyl salicylate acted as potent irritants for brown tree snakes (*Boiga irregularis*) (Clark and Shivik, 2002).

19.4 OILS AGAINST PESTS

19.4.1 Insecticidal, Pest Repellent, and Antiparasitic Oils

The essential oil of bergamot (*Citrus bergamia*), anise (*Pimpinella anisum*), sage (*Salvia officinalis*), tea tree (*Melaleuca alternifolia*), geranium (*Pelargonium* sp.), peppermint (*Mentha piperita*), thyme (*Thymus vulgaris*), hyssop (*Hyssopus officinalis*), rosemary (*Rosmarinus officinalis*), and white clover (*Trifolium repens*) can be used to control certain pests on plants. They have been shown to reduce the number of eggs laid and the amount of feeding damage by certain insects, particularly lepidopteran caterpillars. Sprays made from Tansy (*Tanacetum vulgare*) have demonstrated a repellent effect on imported cabbageworm on cabbage, reducing the number of eggs laid on the plants. Teas made from wormwood (*Artemisia absinthium*) or nasturtiums (*Nasturtium* spp.) are reputed to repel aphids from fruit trees, and sprays made from ground or blended catnip (*Nepeta cataria*), chives (*Allium schoenoprasum)*, feverfew (*Tanacetum parthenium*), marigolds (*Calendula*, *Tagetes*, and *Chrysanthemum* spp.), or rue (*Ruta graveolens*) have also been used by gardeners against pests that feed on leaves (Moschetti, 2003).

19.4.2 Fleas and Ticks

Dogs, cats, and horses are plagued by fleas and ticks. One to two drops of citronella or lemongrass oils added to the shampoo will repel these pests. Alternatively, 4–5 drops of cedarwood oil and pine oil is added to a bowl of warm water and a bristle hair brush is soaked with this solution to brush the pet down with it. Eggs and parasites gathered in the brush are rinsed out. This is repeated several times. This solution can be used similarly for livestock after adding citronella and lemon grass oils to this mixture.

Flea collar can be prepared by a mixture of cedarwood (*Juniperus virginiana*), lavender (*Lavandula angustifolia*), citronella (*Cymbopogon winterianus* (Java)), thyme oils, and 4–5 garlic (*Allium sativum*) capsules. This mixture is thinned with a teaspoonful of ethanol and soaked with a collar or a cotton scarf. This is good for 30 days (Anonymous, 2001).

Ticks can be removed by applying 1 drop of cinnamon or peppermint oil on Q-tip by swabbing on it.

Carvacrol-rich oil (64%) of *Origanum onites* and carvacrol was found to be effective against the tick *Rhipicephalus turanicus*. Pure carvacrol killed all the ticks following 6 h of exposure, while 25% and higher concentrations of the oil were effective in killing the ticks by the 24-h posttreatment (Coskun et al., 2008).

19.4.3 Mosquitoes

Catnip oil (*Nepeta cataria*) containing nepetalactones can be used effectively as a mosquito repellent. It is said to be 10 times more effective than DEET (Moschetti, 2003). *Juniperus communis* berry oil

is a very good mosquito repellent. Ocimum volatile oils including camphor, 1,8-cineole, methyl eugenol, limonene, myrcene, and thymol strongly repelled mosquitoes (Regnault-Roger, 1997).

Citronella oil repels mosquitoes, biting insects, and fleas.

Essential oils of *Zingiber officinale* and *Rosmarinus officinalis* were found to be ovicidal and repellent, respectively, toward three mosquito species (Prajapati et al., 2005). Root oil of *Angelica sinensis* and ligustilide was found to be mosquito repellent (Wedge et al., 2009).

19.4.4 Moths

Cedarwood oil is used in mothproofing. A large number of patents have been assigned to the preservation of cloths from moths and beetles: Application of a solution containing clove (*Syzigum aromaticum*) essential oil on woolen cloth; filter paper containing *Juniperus rigida* oil, and tablets of *p*-dichlorobenzene mixed with essential oils to be placed in wardrobe.

19.4.5 Aphids, Caterpillars, and Whiteflies

19.4.5.1 Garlic Oil

Essential oils effective in insect pest control (Regnault-Roger, 1997).

19.4.6 Ear Mites

Peppermint oil is applied to a Q-tip and swabbed inside of the ear.

19.4.7 Antiparasitic

A patent (United States Patent 6800294) on an antiparasitic formulation comprising eucalyptus oil (*Eucalyptus globulus*), cajeput oil (*Melaleuca cajeputi*), lemongrass oil, clove bud oil (*Syzigium aromaticum*), peppermint oil (*Mentha piperita*), piperonyl, and piperonyl butoxide. The formulation can be used for treating an animal body, in the manufacture of a medicament for treating ectoparasitic infestation of an animal, or for repelling parasites.

Two essential oils derived from *Lavandula angustifolia* and *Lavandula* × *intermedia* were investigated for any antiparasitic activity against the human protozoal pathogens *Giardia duodenalis* and *Trichomonas vaginalis* and the fish pathogen *Hexamita inflata*, all of which have significant infection and economic impacts. The study has demonstrated that low (≤1%) concentrations of *Lavandula angustifolia* and *Lavandula* × *intermedia* oil can completely eliminate *Trichomonas vaginalis*, *Giardia duodenalis*, and *Hexamita inflata in vitro*. At 0.1% concentration, *Lavandula angustifolia* oil was found to be slightly more effective than *Lavandula* × *intermedia* oil against *Giardia duodenalis* and *Hexamita inflata* (Moon et al., 2006).

The antiparasitic properties of essential oils from *Artemisia absinthium*, *Artemisia annua*, and *Artemisia scoparia* were tested on intestinal parasites, *Hymenolepis nana*, *Lambli intestinalis*, *Syphacia obvelata*, and *Trichocephalus muris* [*Trichuris muris*]. Infested white mice were injected with 0.01 ml/g of the essential oils (6%) twice a day for 3 days. The effectiveness of the essential oils was observed in 70–90% of the tested animals (Chobanov et al., 2004).

Parasites, such as head lice and scabies, as well as internal parasites, are repelled by oregano oil (86% carvacrol). The oil can be added to soaps, shampoos, and diluted in olive oil for topical applications. By taking a few drops daily under the tongue, one can gain protection from waterborne parasites, such as *Cryptosporidium* and *Giardia*. Internal dosages also are effective in killing parasites in the body (http://curingherbs.com/wild_oregano_oil.htm) (Foster, 2002).

Essential oils from *Pinus halepensis*, *Pinus brutia*, *Pinus pinaster*, *Pinus pinea*, and *Cedrus atlantica* were tested for molluscicidal activity against *Bulinus truncatus*. The oil from *Cedrus*

atlantica was found the most active (LC 50 = 0.47 ppm). Among their main constituents, α-pinene, β-pinene, and myrcene exhibited potent molluscicidal activity (LC 50 = 0.49; 0.54, and 0.56 ppm, respectively). These findings have important application of natural products in combating schistosomiasis (Lahlou, 2003).

Origanum essential oils have exhibited differential degrees of protection against myxosporean infections in gilthead and sharpsnout sea bream tested in land-based experimental facilities (Athanassopoulou et al., 2004a, 2004b).

19.5 ESSENTIAL OILS USED IN ANIMAL FEED

Essential oils can be used in feed as appetite stimulant, stimulant of saliva production, gastric and pancreatic juices production enhancer, and antimicrobial and antioxidant to improve broiler performance. Antimicrobial effects of essential oils are well documented. Essential oils due to their potent nature should be used as low as possible levels in animal nutrition. Otherwise, they can lead to feed intake reduction, gastrointestinal (GIT) microflora disturbance, or accumulation in animal tissues and products. Odor and taste of essential oils may contribute to feed refusal; however, encapsulation of essential oils could solve this problem (Gauthier, 2005).

Generally, Gram-positive bacteria are considered more sensitive to essential oils than Gram-negative bacteria because of their less complex membrane structure (Lis-Balchin, 2003).

Carvacrol, the main constituent of oregano oils, is a powerful antimicrobial agent (Baser, 2008). It asserts its effect through the biological membranes of bacteria. It acts through inducing a sharp reduction of the intercellular ATP pool through the reduction of ATP synthesis and increased hydrolysis. Reduction of the membrane potential (transmembrane electrical potential), which is the driving force of ATP synthesis, makes the membrane more permeable to protons. A high level of carvacrol (1 mM) decreases the internal pH of bacteria from 7.1 to 5.8 related to ion gradients across the cell membrane. 1 mM of carvacrol reduces the internal potassium (K) level of bacteria from 12 mmol/mg of cell protein to 0.99 mmol/mg in 5 min. K plays a role in the activation of cytoplasmic enzymes and in maintaining osmotic pressure and in the regulation of cytoplasmic pH. K efflux is a solid indication of membrane damage (Ultee et al., 1999).

It has been shown that the mode of action of oregano oils is related to an impairment of a variety of enzyme systems, mainly involved in the production of energy and the synthesis of structural components. Leakage of ions, ATP, and amino acids also explain the mode of action. Potassium and phosphate ion concentrations are affected at levels below the MIC concentration (Lambert et al., 2001).

19.5.1 Ruminants

A recent review compiled information on botanicals including essential oils used in ruminant health and productivity (Rochfort et al., 2008). Unfortunately, there are few reports on the effects of essential oils and natural aromachemicals on ruminants. It was demonstrated that the consumption of terpene volatiles such as camphor and α-pinene in "tarbush" (*Flourencia cernua*) effected feed intake in sheep (Estell et al., 1998). *In vitro* and *in vivo* antimicrobial activities of essential oils have been demonstrated in ruminants (Cardozo, 2005; Elgayyar et al., 2001; Moreira et al., 2005; Wallace et al., 2002). Synergistic antinematodal effects of essential oils and lipids were demonstrated (Ghisalberti, 2002). Other nematocidal volatiles reported are as follows: benzyl isothiocyanate (goat), ascaridole (goat and sheep) (Githiori et al., 2006; Ghisalberti, 2002), geraniol, eugenol (Githiori et al., 2006; Chitwood, 2002), and menthol, 1,8-cineole (Chitwood, 2002).

Methylsalicylate, the main component of the essential oil of *Gaultheria procumbens* (Wintergreen), is topically used as emulsion in cattle, horses, sheep, goats, and poultry in the treatment of muscular and articular pain. The recommended dose is 600 μg/kg bw twice a day. The duration of treatment is usually less than 1 week (EMEA, 1999). It is included in Annex II of

Council Regulation (EEC) N. 2377/90 as a substance that does not need an MRL level. *Gaultheria procumbens* should not to be used as flavoring in pet food since salicylates are toxic to dogs and cats. As cats metabolize salicylates much more slowly than other species, they are more likely to be overdosed. Use of methylsalicylate in combination with anticoagulants such as warfarin can result in adverse interactions and bleedings (Chow et al., 1989; Ramanathan, 1995; Tam et al., 1995; Yip et al., 1990).

The essential oil of *Lavandula angustifolia* (*Lavandulae aetheroleum*) is used in veterinary medicinal products for topical use together with other plant extracts or essential oils for antiseptic and healing purposes. The product is used in horses, cattle, sheep, goats, rabbits, and poultry. It is included in Annex II of Council Regulation (EEC) N. 2377/90 as a substance that does not need an MRL level (EMEA, 1999; Franz et al., 2005).

The outcomes of *in vitro* studies investigating the potential of *Pimpinella anisum* essential oil as a feed additive to improve nutrient use in ruminants are inconclusive, and more and larger preferably *in vivo* studies are necessary for evaluation of efficacy (Franz et al., 2005).

Carvacrol, carvone, cinnamaldehyde, cinnamon oil, clove bud oil, eugenol, and oregano oil have resulted in a 30–50% reduction in ammonia N concentration in diluted ruminal fluid with a 50:50 forage concentrate diet during the 24-h incubation (Busquet et al., 2006).

Carvacrol has been suggested as a potential modulator of ruminal fermentation (Garcia et al., 2007).

19.5.2 Poultry

19.5.2.1 Studies with CRINA Poultry

Dietary addition of essential oils in a commercial blend (CRINA® Poultry) showed a decreased *Escherichia coli* population in ileo-cecal digesta of broiler chickens. Furthermore, in high doses, a significant increase in certain digestive enzyme activities of the pancreas and intestine was observed in broiler chickens (Jang et al., 2007).

In another study, CRINA Poultry was shown to control the colonization of the intestine of broilers with *Clostridium perfringens* and the stimulation of animal growth was put down to this development (Losa, 2001).

Commercial essential oil blends CRINA Poultry and CRINA Alternate were tested in broilers infected with viable oocysts of mixed *Eimeria* spp. It was concluded that these essential oil blends may serve as an alternative to antibiotics and/or ionophores in mixed *Eimeria* infections in non-cocci-vaccinated broilers, but no benefit of essential oil supplementation was observed for vaccinated broilers against coccidia (Oviedo-Rondon et al., 2006).

19.5.2.1.1 Other Studies

Supplementation of 200 ppm essential oil mixture (EOM) that included oregano, clove, and anise oils (no species name or composition given!) in broiler diets was said to significantly improve the daily live weight gain and feed conversion ratio (FCR) during a growing period of 5 weeks (Ertas et al., 2006). Similar results were obtained with 400 mg/kg anise oil (composition not known!) (Ciftci et al., 2005).

A total of 50 and 100 mg/kg of feed of oregano oil* were tested on broilers. No growth-promoting effect was observed. At 100 mg/kg of feed, antioxidant effect was detected on chicken tissues (Botsoglou et al., 2002a).

Positive results were also reported for oregano oil added in poultry feed (Bassett, 2000).

* Oregano essential oil was in the form of a powder called Orego-Stim. This product contains 5% oregano essential oil (Ecopharm Hellas, SA, Kilkis, Greece) and 95% natural feed grade inert carrier. The oil of *Origanum vulgare* subsp. *hirtum* used in this product contains 85% carvacrol + thymol.

Antioxidant activities of rosemary and sage oils on lipid oxidation of broiler meat have been shown. Following dietary administration of rosemary and sage oils to the live birds, a significant inhibition of lipid peroxidation was reported in chicken meat stored for 9 days (Lopez-Bote et al., 1998). A dietary supplementation of oregano essential oil (300 mg/kg) showed a positive effect on the performance of broiler chickens experimentally infected with *Eimeria tenella*. Throughout the experimental period of 42 days, oregano essential oil exerted an anticoccidial effect against *Eimeria tenella*, which was, however, lower than that exhibited by lasalocid. Supplementation with dietary oregano oil to *Eimeria tenella*-infected chickens resulted in body weight gains and feed conversion ratios not differing from the noninfected group, but higher than those of the infected control group and lower than those of chickens treated with the anticoccidial lasalocid (Giannenas et al., 2003).

Inclusion of oregano oil at 0.005% and 0.01% in chicken diets for 38 days resulted in a significant antioxidant effect in raw and cooked breast and thigh muscle stored up to 9 days in refrigerator (Botsoglou et al., 2002b).

Oregano oil (55% carvacrol) exhibited a strong bactericidal effect against lactobacilli and following the oral administration of the oil MIC values of amicain, apramycin, and streptomycinand neomyc against *Escherichia coli* strains increased (Horosova et al., 2006).

An *in vitro* assay measuring the antimicrobial activity of essential oils of *Coridothymus capitatus*, *Satureja montana*, *Thymus mastichina, Thymus zygis*, and *Origanum vulgare* was carried out against poultry origin strains of *Escherichia coli, Salmonella enteritidis*, and *Salmonella essen*, and pig origin strains of enterotoxigenic *Escherichia coli* (ETEC), *Salmonella choleraesuis*, and *Salmonella typhimurium*. *Origanum vulgare* (MIC ≤ 1% v/v) oil showed the highest antimicrobial activity against the four strains of *Salmonella*. It was followed by *Thymus zygis* oil (MIC ≤ 2% v/v). *Thymus mastichina* oil inhibited all the microorganisms at the highest concentration, 4% (v/v). Monoterpenic phenols carvacrol and thymol showed higher inhibitory capacity than the monoterpenic alcohol linalool. The results confirmed potential application of such oils in the treatment and prevention of poultry and pig diseases caused by salmonella (Penalver et al., 2005).

In another study, groups of male, 1-day-old Lohmann broilers were given maize–soya bean meal diets, with oils extracted from thyme, mace, and caraway or coriander, garlic, and onion (0, 20, 40, and 80 mg/kg) for 6 weeks. The average daily gain and FCR were not different between the broilers fed with the different oils; meat was not tainted with flavor or smell of the oils (Vogt and Rauch, 1991).

19.5.2.2 Studies with Herbromix

Essential oils from oregano herb (*Origanum onites*), laurel leaf (*Laurus nobilis*), sage leaf (*Salvia fruticosa*), fennel fruit (*Foeniculum vulgare*), myrtle leaf (*Myrtus communis*), and citrus peel (rich in limonene) were mixed and formulated as feed additive after encapsulation. It is marketed in Turkey as poultry feed under the name Herbromix®.

The following three *in vivo* experiments with this product were recently accomplished.

19.5.2.2.1 In Vivo Experiment 1

In this study, 1250 sexed 1-day-old broiler chicks obtained from a commercial hatchery were randomly divided into five treatment groups of 250 birds each (negative control, antibiotic, and essential oil combination (EOC) at three levels). Each treatment group was further subdivided into five replicates of 50 birds (25 males and 25 females) per replicate. Commercial EOC at three different levels (24, 48, and 72 mg) and antibiotic (10 mg avilamycin) per kg were added to the basal diet. There were significant effects of dietary treatments on body weight, feed intake (except at day 42), FCR, and carcass yield at 21 and 42 days. Body weights were significantly different between the treatments. Birds fed on diet containing 48 mg essential oil/kg being the highest and this treatment was followed by chicks fed on the diet containing 72 mg essential oil/kg, antibiotic, negative control, and 24 mg essential oil/kg at day 42.

Supplementation with 48 mg EOC/kg to the broiler diet significantly improved the body weight gain, FCR, and carcass yield compared to other dietary treatments on 42 days of age. EOC may be considered as a potential growth promoter in the future of the new era, which agrees with producer needs for increased performance and today's consumer demands for environment-friendly broiler production. The EOC can be used cost effectively when its cost is compared with antibiotics and other commercially available products in the market.

19.5.2.2.2 In Vivo Experiment 2

In this study, 1250 sexed 1-day-old broiler chicks were randomly divided into five treatment groups of 250 birds each (negative control, organic acid, probiotic, and EOC at two levels). Each treatment group was further subdivided into five replicates of 50 birds (25 males and 25 females) per replicate. The oils in the EOC were extracted from different herbs growing in Turkey. The organic acid at 2.5 g/kg diet, the probiotic at 1 g/kg diet, and the EOC at 36 and 48 mg/kg diet were added to the basal diet.

The results obtained from this study indicated that the inclusion of 48 mg EOC/kg broiler diet significantly improved the body weight gain, FCR, and carcass yield of broilers compared to organic acid and probiotic treatments after a growing period of 42 days. The EOC may be considered as a potential growth promoter like organic acids and probiotics for environment-friendly broiler production.

19.5.2.2.3 In Vivo Experiment 3

The aim of the present study was to examine the effect of essential oils and breeder age on growth performance and some internal organs weight of broilers. A total of 1008 unsexed 1-day-old broiler chicks (Ross-308) originating from young (30 weeks) and older (80 weeks) breeder flocks were randomly divided into three treatment groups of 336 birds each, consisting of control and two EOMs at a level of 24 and 48 mg/kg diet. There were no significant effects of dietary treatments on body weight gain of broilers at days 21 and 42.

On the other hand, there were significant differences on the feed intake at days 21 and 42. The addition of 24 or 48 mg/kg EOM to the diet reduced significantly the feed intake compared to the control. The groups fed with the added EOM had significantly better FCR than the control at days 21 and 42. Although, there was no significant effect of broiler breeder age on body weight gain at day 21, significant differences were observed on body weight gain at 42 days of age. Broilers originating from young breeder flock had significantly higher body weight gain than those originating from old breeder flock at 42 days of age. No difference was noticed for carcass yield, liver, pancreas, proventriculus, gizzard, and small intestine weight. Supplementation with EOM to the diet in both levels significantly decreased mortality at days 21 and 42.

The results indicated that the Herbromix may be considered as a potential growth promoter. However, more trials are needed to determine the effect of essential oil supplementation to diet on the performance of broilers with regard to variable management conditions including different stress factors, essential oils and their optimal dietary inclusion levels, active substances of oils, dietary ingredients, and nutrient density (Cabuk et al., 2006a, 2006b; Alcicek et al., 2003, 2004; Bozkurt and Baser, 2002a, 2002b).

19.5.3 Pigs

CRINA® Pigs was tested on pigs. The results for the first 21-day period showed that males grew faster, ate less, and exhibited superior FCR compared to females. Although female carcass weight was higher, males had a significantly lower carcass fat than females (Losa, 2001).

The addition of fennel (*Foeniculum vulgare*) and caraway (*Carum carvi*) oils was not found beneficial for weaned piglets. In feed choice conditions, fennel oil caused feed aversion (Schoene et al., 2006).

Oregano oil was found to be beneficial for piglets (Molnar and Bilkei, 2005).

In a preliminary investigation, the effects of low-level dietary inclusion of rosemary, garlic, and oregano oils on pig performance and pork quality were carried out. Unfortunately, no information on the species from which the oils were obtained and their composition existed in the paper. The pigs appeared to prefer the garlic-treated diet, and the feed intake and the average daily gain were significantly increased although no difference in the feed efficiency was observed. Carcass and meat quality attributes were unchanged, although a slight reduction of lipid oxidation was noted in oregano-fed pork. Since the composition of the oils is not clear, it is not possible to evaluate the results (Janz et al., 2007).

A study revealed that the inclusion of essential oil of oregano in pigs' diet significantly improved the average daily weight gain and FCR of the pigs. Pigs fed with the essential oils had higher carcass weight, dressing percentage, and carcass length than those fed with the basal and antibiotic-supplemented diet. The pigs that received the essential oil supplementation had a significantly lower fat thickness. Also lean meat and ham portions from these pigs were significantly higher. Therefore, the use of *Origanum* essential oil as feed additive improves the growth of pigs and has greater positive effects on carcass composition than antibiotics (Onibala et al., 2001).

Ropadiar®, an essential oil of the oregano plant, was supplemented in the diet of weaning pigs as alternative for antimicrobial growth promoters (AMGPs), observing its efficacy on the performance of the piglets. Ropadiar liquid contains 10% oregano oil and has been designed to be added to water. Compared to the negative control (without AMGP), Ropadiar® improved performance only during the first 14 days after weaning. Based on the results of this trial, it cannot be argued about the usefulness of Ropadiar® as an alternative for AMGP in diets of weanling pigs. However, its addition in prestarter diets could improve performance of these animals (Krimpen and Binnendijk, 2001).

The objective of another trial was to ascertain the effect on nutrient digestibilities and N-balance, as well as on parameters of microbial activity in the gastrointestinal tract of weaned pigs after adding oregano oil to the feed. The apparent digestibility of crude nutrients (except fiber) and the N-balance of the weaned piglets in this study were not influenced by feeding piglets restrictively with this feed additive. By direct microbiological methods, no influence of the additive on the gut flora could be found (Moller, 2001).

The inclusion of essential oil of spices in the pigs' diet significantly improved the average daily weight gain and FCR of the pigs in Groups 3, 4, and 5, as compared to Groups 1 and 2 ($P < 0.01$). Furthermore, pigs fed with the essential oils had higher carcass weight ($P < 0.01$), dressing percentage ($P < 0.01$), and carcass length ($P < 0.01$) than those fed with the basal and antibiotic-supplemented diet. In Groups 3, 4, and 5, backfat thickness was significantly lower than those in Groups 1 and 2. Moreover, lean meat and ham portions from pigs in Groups 3, 4, and 5 were significantly higher than those from pigs in Groups 1 and 2. In conclusion, the use of essential oils as feed additives improves the growth of pigs and has greater positive effects on carcass composition than antibiotics (Onibala et al., 2001).

19.6 ESSENTIAL OILS USED IN TREATING DISEASES IN ANIMALS

There is scarce scientific information on the use of essential oils in treating diseases in animals. Generally, the oils used in treating diseases in humans are also recommended for animals.

Internet literature is abound with valid and/or suspicious information in this issue. We have tried to compile relevant information using the reachable resources. The information may not be concise or comprehensive but should be seen as an effort to combine the available information in a short period of time.

The oil of *Ocimum basilicum* has been reported as an expectorant in animals. The combined oils of *Ocimum micranthum* and *Chenopodium ambrosioides* is claimed to treat stomach ache and colic in animals (http://www.ansci.cornell.edu/plants/medicinal/basil.html).

Bad breath as a result of gum disease and bacterial buildup on the teeth of pets can be treated by brushing their teeth with a mixture of a couple of tablespoons of baking soda, 1 drop of clove oil and 1 drop of aniseed oil. Lavender, myrrh, and clove oils can also be directly applied to their gums.

For wounds, abscesses, and burns, lavender and tea tree oils are used by topical application. Skin rashes can be treated with tea tree, lavender, and chamomile oils.

Earache of pets can be healed by dripping a mixture of lavender, chamomile, and tea tree oils (1 drop each) dissolved in a teaspoonful of grapeseed or olive oil in the infected ears.

Hoof rot in livestock can be treated with a hot compress made up of 10 drops of chamomile, 15 drops of thyme, and 5 drops of melissa oils diluted in about 100 ml of vegetable oil (e.g., grapeseed oil).

Intestinal worms of horses can be expelled by applying 3–4 drops of thyme oil and tansy leaves to each feed. Melissa oil can be added to feed to increase milk production of both cows and goats (http://scentsnsensibility.com/newsletter/Apr0601.htm).

Aromatic plants such as *Pimpinella isaurica*, *Pimpinella aurea*, and *Pimpinella corymbosa* are used as animal feed to increase milk secretion in Turkey (Tabanca et al., 2003).

To calm horses, chamomile oil is added to their feed. Pneumonia in young elephants caused by *Klebsiella* is claimed to be healed by *Lippia javanica* oil. Rose and yarrow oils bring about emotional release in donkeys by licking them. Wounds in horses are treated with *Achillea millefolium* oil; sweet itch is treated with peppermint oil. *Matricaria recutita* and *Achillea millefolium* oils are used to heal the skin with inflammatory conditions (Anonymous, 2008).

A study evaluated the effect of dietary oregano etheric oils as nonspecific immunostimulating agents in growth-retarded, low-weight growing-finishing pigs. A group of pigs were fed with commercial fattening diet supplemented with 3000 ppm oregano additive (Oregpig®, Pecs, Hungary), composed of dried leaf and flower of *Origanum vulgare*, enriched with 500 g/kg cold-pressed essential oils of the leaf and flower of *Origanum vulgare*, and containing 60 g carvacrol and 55 g thymol/kg. Dietary oregano improved growth in growth-retarded growing-finishing pigs and had nonspecific immunostimulatory effects on porcine immune cells (Walter and Bilkei, 2004).

Menthol is often used as a repellent against insects and in lotions to cool legs (especially for horses) (Franz et al., 2005).

Milk cows become restless and aggressive each time a group of cows are separated and regrouped. This can last a few days putting cows in more stress resulting in a drop in milk production. Two Auburn University scientists could solve this problem by spraying anise oil (*Pimpinella anisum*) on the cows. Treated animals could not distinguish any differences among the cows in new or old groupings. They were mellower and kept their milk production up. Among many other oils tested but only anise seemed to work (Anonymous, 1990).

Essential oils have been found effective in honeybee diseases (Ozkirim, 2006; Ozkirim et al., 2007).

In this review, we tried to give you an insight into the use of essential oils in animal health and nutrition. Due to the paucity of research in this important area there is not much to report. Most information on usage exists in the form of not-so-well-qualified reports. We hope that this rather preliminary report can be of use as a starting point for more comprehensive reports.

REFERENCES

Alcicek, A., M. Bozkurt, and M. Cabuk, 2003. The effect of an essential oil combination derived from selected herbs growing wild in Turkey on broiler performance. *S. Afr. J. Anim. Sci.*, 33(2): 89–94.

Alcicek, A., M. Bozkurt, and M. Cabuk, 2004. The effect of a mixture of herbal essential oils, an organic acid or a probiotic on broiler performance. *S. Afr. J. Anim. Sci.*, 34(4): 217–222.

Anonymous, 1990. Bovine aromatherapy: Common herb quells cowcophony. *HerbalGram*, 22: 8.

Anonymous, 2001. Pet care & pest control—using essential oils. *Scents Sensibility Newsletter*, 2(12): 1 (http://scentsnsensibility.com/newsletter/Apr0601.htm). Accessed 2008.

Anonymous, 2008. Zoopharmacognosy—working with aromatic medicine (http://www.ingraham.co.uk/). Accessed 2008.

Anonymous, 2009. Treating livestock with medicinal plants: Beneficial or toxic?—*Ocimum basilicum, O. americanum* and *O. micranthum.* (http://www.ansci.cornell.edu/plants/medicinal/basil.html). Accessed September 2009.

Athanassopoulou, F., E. Karagouni, E. Dotsika, V. Ragias, J. Tavla, and P. Christofilloyani, 2004a. Efficacy and toxicity of orally administrated anticoccidial drugs for innovative treatments of *Polysporoplasma sparis* infection in *Sparus aurata* L. *J. Appl. Ichthyol.*, 20: 345–354.

Athanassopoulou, F., E. Karagouni, E. Dotsika, V. Ragias, J. Tavla, P. Christofilloyanis and I. Vatsos, 2004b. Efficacy and toxicity of orally administrated anticoccidial drugs for innovative treatments of *Myxobolus* sp. infection in *Puntazzo puntazzo. Dis. Aquat. Org.*, 62: 217–226.

Bakkali, F., S. Averbeck, D. Averbeck, and M. Idaomar, 2008. Biological effects of essential oils—a review. *Food Chem. Technol.*, 46: 446–475.

Baser, K.H.C., 1995. Analysis and quality assessment of essential oils. In: *A Manual on the Essential Oil Industry*, K.T. De Silva (ed.), pp. 155–177. Vienna: UNIDO.

Baser, K.H.C., 2008. Chemistry and biological activities of carvacrol and carvacrol-bearing essential oils. *Curr. Pharm. Des.*, 14: 3106–3120.

Baser, K.H.C. and F. Demirci, 2007. Chemistry of essential oils. In: *Flavours and Fragrances. Chemistry, Bioprocessing and Sustainability*, R.G. Berger (ed.), pp. 43–86. Berlin, Heidelberg: Springer.

Bassett, R., 2000. Oreganos positive impact on poultry production. *World Poultry-Elsevier*, 16: 31–34.

Botsoglou, N.A., E. Christaki, D.J. Fletouris, P. Florou-Paneri, and A.B. Spais, 2002a. The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. *Meat Sci.*, 62: 259–265.

Botsoglou, N.A., P. Floron-Paneri, E. Christaki, D.J. Fletouris, and A.B. Spais, 2002b. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid peroxidation of breast, thigh and abdominal fat tissues. *Br. Poult. Sci.*, 43(2): 223–230.

Bozkurt, M. and K.H.C. Baser, 2002a. The effect of antibiotic, Mannan oligosaccharide and essential oil mixture on the laying egg performance. *1st European Symp. on Bioactive Secondary Plant Products in Veterinary Medicine*, Vienna, Austria, 4–5 Ekim 2002.

Bozkurt, M. and K.H.C. Baser, 2002b. The effect of commercial organic acid, probiotic and essential oil mixture at two levels on the performance of broilers. *1st European Symp. on Bioactive Secondary Plant Products in Veterinary Medicine*, Vienna, Austria, 4–5 Ekim 2002.

Buchbauer, G., L. Jirovetz, M. Wasicky, and A. Nikiforov, 1994. Comparative investigation of Douglas fir headspace samples, essential oils, and extracts (needles and twigs) using GC-FID and GC-FTIR-MS. *J. Agric. Food Chem.*, 42: 2852–2854.

Busquet, M., S. Calsamiglia, A. Ferret, and C. Kamel, 2006. Plant extracts affect *in vitro* rumen microbial fermentation. *J. Dairy Sci*., 89: 761–771.

Cabuk, M., M. Bozkurt, A. Alcicek, A.U. Catli, and K.H.C. Baser, 2006a. Effect of dietary essential oil mixture on performance of laying hens in summer season, *S. Afr. J. Anim. Sci.*, 36(4): 215–221.

Cabuk, M., M. Bozkurt, A. Alcicek, Y. Akbas, and K. Kucukyilmaz, 2006b. Effect of a herbal essential oil mixture on growth and internal organ weight of broilers from young and old breeder flocks. *S. Afr. J. Anim. Sci.*, 36(2): 135–141.

Cardozo, P.W., S. Calsamiglia, A. Ferret, and C. Kamel, 2005. Screening for the effects of natural plant extracts at different pH on *in vitro* Rumen microbial fermentation of a high-concentrate diet for beef cattle. *J. Anim. Sci.*, 83: 2572–2579.

Chitwood, D.J., 2002. Phytochemical based strategies for nematode control. *Ann. Rev. Phytopathol.*, 40: 221–249.

Chobanov, R.E., A.N. Aleskerova, S.N. Dzhanahmedova, and L.A. Safieva, 2004. Experimental estimation of antiparasitic properties of essential oils of some *Artemisia* (Asteraceae) species of Azerbaijan flora. *Rastitel'nye Resursy*, 40(4): 94–98.

Chow, W.H., K.L. Cheung, H.M. Ling, and T. See, 1989. Potentiation of warfarin anticoagulation by topical methylsalicylate ointment. *J. R. Soc. Med.*, 82(8), 501–502.

Ciftci, M., T. Guler, B. Dalkilic, and O.N. Ertas, 2005. The effect of anise oil (*Pimpinella anisum* L.) on broiler performance. *Int. J. Poultry Sci*., 4(11): 851–855.

Clark, L. and J. Shivik, 2002. Aerosolized essential oils and individual natural product compounds as brown tree snake repellents. *Pest Manag. Sci.*, 58(8): 775–783.

Coskun, S., O. Giriskin, M. Kurkcuoglu, H. Malyer, A.O. Giriskin, N. Kirimer, and K.H.C. Baser, 2008. Acaricidal efficacy of *Origanum onites* L. essential oil against *Rhipicephalus turanicus* (Ixodidae). *Parasitol. Res.*, 103: 259–261.

Council Regulation (EEC) N. 2377/90 (1990), laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin (http://www.bsmi.gov.tw/wSite/public/Attachment/f1224040164375.pdf). Accessed 2008.

Elgayyar, M., F.A. Draughon, D.A. Golden, and J.A. Mount, 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *J. Food Prot.*, 64(7): 1019–1024.

EMEA, 1999. Salicylic acid, sodium salicylate, aluminium salicylate and methyl salicylate. Committee for Veterinary Medicinal Products (EMEA/MRL/696/99, 1999) (http://www.emea.europa.eu/pdfs/vet/mrls/069699en.pdf). Accessed 2008.

Ertas, O.N., T. Guler, M. Ciftci, B. Dalkilic, and U.G. Simsek, 2006. The effect of an essential oil mixture from oregano, clove and anis on broiler performance. *Int. J. Poultry Sci*., 4(11): 879–884.

Estell, R.E., E.L. Fredrickson, M.R. Tellez, K.M. Havstad, W.L. Shupe, D.M. Anderson, and M.D. Remmenga, 1998. Effects of volatile compounds on consumption of alfalfa pellets by sheep. *J. Anim. Sci.*, 76: 228–233.

Franz, Ch., R. Bauer, R. Carle, D. Tedesco, A. Tubaro, K. Zitterl-Eglseer, 2005. Study on the assessment of plants/herbs, plant/herb extracts and their naturally or synthetically produced components as "additives" for use in animal production. CFT/EFSA/FEEDAP/2005/01 (http://www.agronavigator.cz/UserFiles/File/Agronavigator/Kvasnickova_2/EFSA_feedap_report_plantsherbs.pdf). Accessed 2008.

Foster, S., 2002. The fighting power of Oregano: This versatile herb packs a powerful punch—earth medicine. *Better Nutr*., March: 1.

Garcia, V., P. Catala-Gregori, J. Madrid, F. Hernandez, M.D. Megias, H.M. Andrade-Montemayor, 2007. Potential of carvacrol to modify *in vitro* rumen fermentation as compared with monensin. *Animal*, 1: 675–680.

Gauthier, R., 2005. Organic acids and essential oils, a realistic alternative to antibiotic growth promoters in poultry, *I Forum Internacional de Avicultura*. Foz do Iguaçu, PR, Brazil, August 17–19, 2005.

Ghisalberti, E.L., 2002. Secondary metabolites with antinematodal activity. In: *Studies in Natural Products Chemistry*, Atta-ur-Rahman (ed.), Vol. 26, pp. 425–506. Amsterdam: Elsevier Science BV.

Giannenas, I., P.P. Florou, M. Papazahariadou, E. Christaki, and N.A. Botsoglou, A.B. Spais, 2003. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with Eimeria tenella. *Arch. Anim. Nutr.*, 57(2): 99–106.

Githiori, J.B., S. Athanasiadou, and S.M. Thamsborg, 2006. Use of plants in novel approaches for control of gastrointestinal helminthes in livestock with emphasis on small ruminants. *Vet. Parasitol*., 139: 308–320.

Horosova, K., D. Bujnakova, and V. Kmet, 2006. Effect of oregano essential oil on chicken lactobacilli and *E. coli. Folia Microbiol*., 51(4): 278–280.

Jang, I.S., Y.H. Ko, S.Y. Kang, and C.Y. Lee, 2007. Effect of commercial essential oil on growth performance digestive enzyme activity and intestinal microflora population in broiler chickens. *Anim. Feed Sci. Technol*., 134: 304–315.

Janz, J.A.M., P.C.H. Morel, B.H.P. Wilkinson, and R.W. Purchas, 2007. Preliminary investigation of the effects of low-level dietary inclusion of fragrant essential oils and oleoresins on pig performance and pork quality. *Meat Sci.*, 75: 350–355.

Krimpen, M.V. and G.P. Binnendijk, 2001. Ropadiar® as alternative for anti microbial growth promoter in diets of weanling pigs. Rapport Praktijkonderzoek Veehouderij, May 2001. ISSN 0169–3689.

Lahlou, M., 2003. Composition and molluscicidal properties of essential oils of five Moroccan Pinaceae. *Pharm. Biol*., 41(3): 207–210.

Lambert, R.J.W., P.N. Skandamis, P.J. Coote, and G.-J.E. Nychas, 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol*., 91: 453–462.

Lis-Balchin, M., 2003. Feed additives as alternatives to antibiotic growth promoters: Botanicals. *Proc. 9th Int. Symp. on Digestive Physiology in Pigs*, Vol. 1, pp. 333–352. Banff AB, Canada: University of Alberta.

Lopez-Bote, L.J., J.I. Gray, E.A. Gomaa, and C.I. Flegal, 1998. Effect of dietary administration of oil extracts from rosemary and sage on lipid oxidation in broiler meat. *Br. Poult. Sci.*, 39: 235–240.

Losa, R., 2001. The use of essential oils in animal nutrition. In: *Feed Manufacturing in the Mediterranean Region. Improving Safety: From Feed to Food*, J. Brufau (ed.), pp. 39–44. Zaragoza: CIHEAM-IAMZ, (Cahiers Options Méditerranéennes; v. 54), 3. Conf. of Feed Manufacturers of the Mediterranean. 2000/03/22–24, Reus (Spain).

Moller, T., 2001., Studies on the effect of an oregano-oil-addition to feed towards nutrient digestibilities, N-balance as well as towards the parameters of microbial activity in the alimentary tract of weaned piglets; thesis, http://www.agronavigator.cz/UserFiles/File/Agronavigator/Kvasnickova_2/EFSA_feedap_report_plantsherbs.pdf, accessed July 2009.

Molnar, C.and G. Bilkei, 2005. The influence of an oregano feed additive on production parameters and mortality of weaned piglets, Tieraerzliche Praxis, Ausgabe Grosstiere. *Nutztiere*, 33: 42–47.

Moon, T., J. Wilkinson, and H. Cavanagh, 2006. Antiparasitic activity of two Lavandula essential oils against *Giardia duodenalis*, *Trichomonas vaginalis* and *Hexamita inflata*.*Parasitol. Res*., 99(6): 722–728.

Moreira, M.R., A.G. Ponze, C.E. del Valle, and S.I. Roura, 2005. Inhibitory parameters of essential oils to reduce a foodborne pathogen. *LWT*, 38: 565–570.

Moschetti, R., 2003. Pesticides made with botanical oils and extracts (http://www.plantoils.in/uses/other/other.html). Accessed September 2009.

Onibala, J.S.I.T., K.D. Gunther, and Ut. Meulen, 2001. Effects of essential oil of spices as feed additives on the growth and carcass characteristics of growing-finishing pigs. Sustainable development in the context of globalization and locality: Challenges and options for networking in Southeast Asia, EFSA.

Oviedo-Rondon, E.O., S. Clemente-Hernandez, F. Salvador, R. Williams, and R. Losa, 2006. Essential oils on mixed coccidia vaccination and infection in broilers, *Int. J. Poultry Sci.*, 5(8): 723–730.

Ozkirim, A., 2006. The detection of antibiotic resistance in the American and European Foulbrood diseases of honey bees (*Apis mellifera* L.). PhD thesis, Hacettepe University, Ankara, Turkey.

Ozkirim, A., N. Keskin, M. Kurkcuoglu, and K.H.C. Baser, 2007. Screening alternative antibiotics-essential oils from *Seseli* spp. against *Paenibacillus larvae* subsp. *larvae* strains isolated from different regions of Turkey, *40th Apimondia Int. Apicultural Congr*., Melbourne, Australia, September 9–14, 2007.

Penalver, P., B. Huerta, C. Borge, R. Astorga, R. Romero, and A. Perea, 2005. Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. *APMIS*, 113: 1–6.

Prajapati, V., A.K. Tripathi, K.K. Aggarwal, and S.P.S. Khanuja, 2005. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus. Bioresource Technol.*, 96(16): 1749–1757.

Ramanathan, M., 1995. Warfarin–topical salicylate interactions: Case reports. *Med. J. Malaysia*, 50(3): 278–279.

Regnault-Roger, C., 1997. The potential of botanical essential oils for insect pest control. *Int. Pest Manag. Rev.*, 2: 25–34.

Rochfort, S., A.J. Parker, and F.R. Dunshea, 2008. Plant bioactives for ruminant health and productivity. *Phytochemistry*, 69: 299–322.

Schoene, F., A. Vetter, H. Hartung, H. Bergmann, A. Biertuempfel, G. Richter, S. Mueller, and G. Breitschuh, 2006. Effects of essential oils from fennel (Foeniculi aetheroleum) and caraway (Carvi aetheroleum) in pigs. *J. Anim. Physiol. Anim. Nutr*., 90: 500–510.

Tabanca, N., E Bedir, N. Kirimer, K.H.C. Baser, S.I. Khan, M.R. Jacob, and I.A. Khan, 2003. Antimicrobial compounds from *Pimpinella* species growing in Turkey. *Planta Med.*, 69: 933.

Tam, L.S., T.Y. Chan, W.K. Leung, and J.A. Critchley, 1995. Warfarin interactions with Chinese traditional medicines: Danshen and methyl salicylate medicated oil. *Aust. N. Z. J. Med.*, 25(3): 258.

Ultee, A., E.P.W. Kets, and E.J. Smid, 1999. Mechanisms of action of carvacrol in the food-borne pathogen *Bacillus cereus. Appl. Environ. Microbiol*., 65: 4606–4610.

U.S. Patent 4961929. Process of repelling dogs and dog repellent material (http://www.freepatentsonline.com/4961929.html). Accessed 2008.

U.S. Patent 4735803. Naturally-odoriferous animal repellent (http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article =1151&context=icwdm_usdanwrc). Accessed 2008.

U.S. Patent 4847292. Repelling animals with compositions comprising citronellyl nitrile, citronellol, alpha-terpinyl methyl ether and lemon oil (http://www.freepatentsonline.com/4847292.html). Accessed 2008.

U.S. Patent 6159474. Animal repellant containing oils of black pepper and/or capsicum (http://www.freepatentsonline.com/6159474.html). Accessed 2008.

Vogt, H. and H.W. Rauch, 1991. The use of several essential oils in broiler diets. *Landbauforschung Volkenrode*, 41: 94–97.

Wallace, R.J., N.R. McEwan, F.M. McIntosh, B. Teferedegne, and C.J. Newbold, 2002. Natural products as manipulators of rumen fermentation. *Asian-Aust. J. Anim. Sci.*, 15: 1458–1468.

Walter, B.M. and G. Bilkei, 2004. Immunostimulatory effect of dietary oregano etheric oils on lymphocytes from growth-retarded, low-weight growing-finishing pigs and productivity. *Tijdschrift voor Diergeneeskunde*, 129(6): 178–181.

Wedge, D.E., J.A. Klun, N. Tabanca, B. Demirci, T. Ozek, K.H.C. Baser, Z Liu, S. Zhang, C.L. Cantrell, and J. Zhang, 2009. Bioactivity-guided Fractionation and GC-MS Fingerprinting of *Angelica sinensis* and *A. archangelica* root components for antifungal and mosquito deterrent activity. *J. Agric. Food Chem.*, 57: 464–470.

Yip, A.S., W.H. Chow, Y.T. Tai, and K.L. Cheung, 1990. Adverse effect of topical methylsalicylate ointment on warfarin anticoagulation: An unrecognized potential hazard. *Postgrad. Med. J*., 66(775): 367–369.

20 Trade of Essential Oils

Hugo Bovill

The essential oil industry is highly complex and fragmented. There are at least 100 different producing countries, as can be seen from the map Essential Oils of the World (Figure 20.1). Many of these producing countries have been active in these materials for many decades. They are often involved in essential oils due to historical colonization, for example, clove oil from Madagascar has traditionally been purchased via France, nutmeg from Indonesia through Holland, and West Indian and Chinese products through Hong Kong and the United Kingdom. The main markets for essential oils are the United States (New Jersey), Germany, the United Kingdom, Japan, and France (Paris and Grasse). Within each producing country, there is often a long supply chain starting with the small peasant artisanal producer, producing just a few kilos, who then sells it to a collector who visits different producers and purchases the different lots that are then bulked together to form an export lot, which is then often exported by a firm based in the main capital or main seaport of that country. This exporter is equipped with the knowledge of international shipping regulations, in particular for hazardous goods, which applies to many essential oils. They also are able to quote in US$ or Euros, which is often not possible for small local producers (Figure 20.2).

Producers of essential oils can vary from the very large, such as an orange juice factory where orange oil is a by-product, down to a small geranium distiller (Figures 20.3 and 20.4).

The business is commenced by sending type samples that are examples of the production from the supplier and should be typical of the production that can be made going forward. Lot samples are normally provided to the purchaser in the foreign country to enable them to chemically analyze the quality organoleptically both on odor and flavor. It is essential that the qualities remain constant as differing qualities are not acceptable and there is normally no such thing as a "better" quality; it is either the same or it is not good. This is the key to building close relationships between suppliers in the country of origin and the purchaser.

Many suppliers try to improve their processes by adapting their equipment and modernizing. In Paraguay, petitgrain distillers replaced wooden stills with stainless steel stills on the advice of overseas aid noncommercial organizations (NCOs). This led to a change in quality and the declining usage of petitgrain oil. The quality issues made customers unhappy, and in fact the Paraguayan distillers reverted back to their traditional wooden stills (Figure 20.5).

Market information, as provided by the processor, is essential to developing long-term relationships. To enable the producer to understand market pricing, he should appreciate that when receiving more enquiries for an oil, it is likely that the price is moving upward and it is by these signs of demand that he can establish that there are potential shortages in the market (Figure 20.6).

Producers and dealers exporting oil should be prepared to commit to carry inventory to ensure carryover and adequate delivery reliability. It is important to note that with climate change, weather and market conditions are becoming increasingly important, and prior to planting, advice should be sought from the buyer as to their intentions, for short, medium, and long term. Long- and medium-term contracts are unusual and it is becoming increasingly common for flavor and fragrance companies not to commit over 1 year but to buy hand to mouth and purely give estimated volume needs going forward. This strengthens the role of the essential oil dealers, of whom there are very few remaining in the main trading centers of the world, such as the United States, France, the United Kingdom, Germany, and Japan.

FIGURE 20.1 **(See color fold-out insert at the back of the book)** World map showing production centers of essential oils. Courtesy of Treatt PLC.

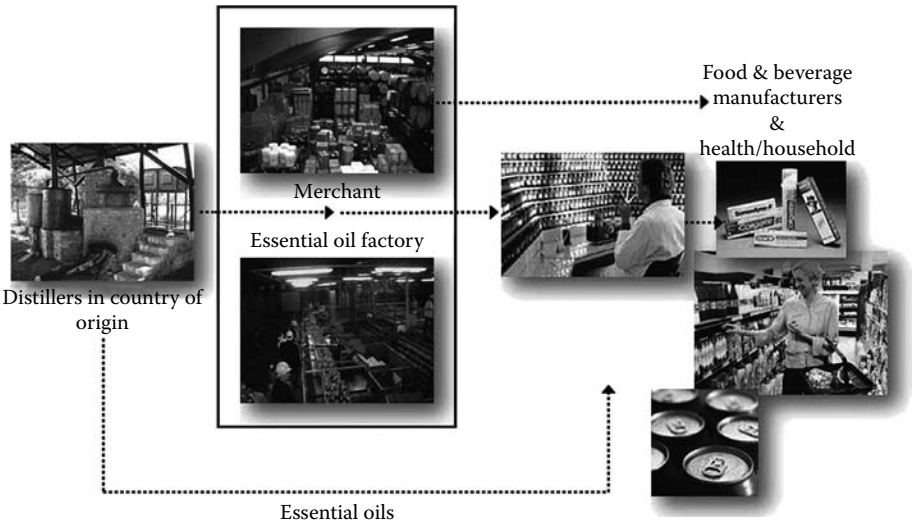


FIGURE 20.2 Flowchart showing the supply chain from distiller to finished product.

FIGURE 20.3 South American orange juice factory. (Photograph by kind permission of Sucocitrico Cutrale Ltd.)

FIGURE 20.4 Copper Still in East Africa.

FIGURE 20.5 Petitgrain still.

TREATT
PLC

Treatt Market Report
July 2007

Orange Oil Sweet

Strong demand currently for orange oil of all origins as we reach a period of the year where Florida plants are off season and Brazil is just beginning processing but oil of acceptable aldehyde is not yet available in volume for shipment from Brazil. The Brazilian crop this year is expected to be a very similar size to last season which is the first time the bi-annual cycle has been broken for 8 years. Better crop management including increased irrigation of groves and favourable weather conditions are two reasons cited for the better than expected crop in Brazil. Prices are moderately firm due to strong demand but this may subside as volume begins to come through in Brazil.

The 2006/07 crop in Florida was very low indeed at just 129 million boxes which contrasts markedly with the record crop of 1997/98 at 244 million boxes for example. As regularly reported in this column a significant hurricane event in Florida could result in a very firm market.

Lemon Oil

As volume availability improves, thanks to South American new crop the market price is showing signs of stabilising.

High quality oil continues to be in strong demand and discerning buyers are advised to carefully monitor the quality of their oils.

Lime Oil Distilled

Better fruit availability at the peak of the crop in Mexico has moved prices to lower levels as the market comes off the top of the cycle. However, strong fresh fruit demand is expected to keep the market firm compared with what we have seen in the last decade.

FIGURE 20.6 Market information.

To quote from *Marketing Essential Oils* (n.d.) by W.A. Ennever of R.C. Treatt & Co. Ltd, London in the 1960s, "The dealer serves as a buffer between these two interests (producer and essential oil merchant house) by purchasing and carrying stocks of oils for his own account and risk when the producer and/or merchant house is unable to wait for the user's demand and hold stocks until the latter is ready to purchase. The risk of market fluctuations to the essential oil dealer or merchant in this practice, is quite considerable, but naturally, is reduced by his knowledge and experience of the trade. He is equipped to handle large or small quantities and a range of qualities, as a buyer or seller. Thus through the dealer's participation, the producer has a larger number of outlets for his production and the user can be reasonably certain of finding supplies of the oils required when he considers it necessary to purchase." The dealer is aware of world markets and potential shortages that other producers may not be aware of, as these are happening in different continents. They can also have the knowledge of increasing demand and movements in consumer tastes.

Some essential oils are produced for their chemical constituents, whereas most are produced for their aromatic parts, and it is important that suppliers understand what is expected of them by their customer, whether it is chemical constituents naturally occurring or whether it is the aroma and flavor. Examples of this are turpentine oil, litsea cubeba oil, sassafras oil, clove leaf oil, and coriander oil.

There is greater demand for ethical supplies, but it should be borne in mind that these surprisingly often do not receive a premium and when entering the essential oil industry it is important to note that it is not always the highest priced oils that give the best return as these are often those that are the most popular for new entrants to produce. Before entering into production of an essential oil, it is important to fully verify the market. It may be that there is good supply locally of the herb, for example, but maybe this is for a traditional purpose such as local medicinal use, producing local foodstuffs, or liqueurs.

Origins are constantly changing and moving, as can be seen from the following: peppermint oil Mitcham production went from England to the United States; mint came from China, then went to Brazil and Paraguay, back to China and now to India.

Within the essential oil market, there are generally four different types of buyers: aromatherapy, the flavor and fragrance industries, and dealers. Many of these can be contacted through agents who would not pay for the goods themselves but would take a nominal commission of, say, 5%. The end users range from aromatherapists selling very small volumes of high, fine quality, natural essential oils to flavor and fragrance companies, and in a few cases, consumer product companies. The main markets are the essential oils dealers, of which there are probably 10 or 20 major companies remaining in the world, some of which are also involved in the manufacture of flavors or fragrances. To avoid conflicts of interest, it is perhaps better to work with those who concentrate solely on raw materials. Several of these companies have been established for many years and have a good trading history. Some information about them can be gained from their websites, but without meeting them in person, it is not easy to establish their credentials.

Conditions of trade are normally done on a FOB or a CIF basis, and the price should be given before samples are sent. With each sample, a Material Safety Data Sheet (MSDS), a Child Labor Certificate, and a Certificate of Analysis should be sent. It should be noted that the drums should be sealed and that the sample should be fully topped with nitrogen or be full to ensure that there is no oxygen present, in order to make sure that oxidation is avoided. The sample bottles should be made from glass and not from plastic to avoid contamination by phthalates. The lots should be bulked before sampling and a flashpoint test should be obtained to guarantee that it is within the law to send the sample by mail or by air freight with the correct labeling.

Many customers are able to give advice on production, but dealers in particular are best placed to advise. To enable contact with such dealers, it is worthwhile attending international meetings such as the International Federation of Essential Oils and Aroma Trades (IFEAT) annual conference or reading the *Perfumer and Flavorist* magazine, which gives full details of brokers, dealers, and essential oil suppliers. There is no reference site that is 100% reliable in pricing for essential

oils; this information should be gained by working with a variety of buyers, and from this a knowledge of the market can be acquired.

The essential oil industry is very traditional and even though there have been changes in analytical methods and demands, the knowledge required in 1950 by buyers such as Mr Ennever of Treatt (as can be seen from his quotation earlier in this chapter) are not too different from today. There is greater demand for organically certified, Kosher, Halal, and other standards. The market can change far quicker now than in the past, thanks to the worldwide web. Producers are often their own worst enemies and can destroy their own successful markets by communicating with their neighboring farmers, thereby encouraging them to enter the market. This can depress prices as a result of increased supply, but on the other hand, it can sometimes be in the interest of a sole producer to have other producers participating in the supply, to ensure guarantees of supply and to lower the costs of production, which in turn encourages buyers to use the oil. Oils such as patchouli and grapefruit have had significant changes in price, as can be seen in the price graphs in Figures 20.7 through 20.9.

These price movements have reduced demand as major buyers of these products have had to look for alternatives to replace them as they are unable to cope with the massively increased prices from US$10 to US$100 for grapefruit and from US$12.5 to US$70 per kilo for peppermint oil. It can be seen, therefore, that stable pricing can lead to increased demand. Unstable pricing can lead to the death of essential oils. This is an important reason for holding inventory so that producers can enter into long-term associations with essential oil buyers to ensure good relationships.

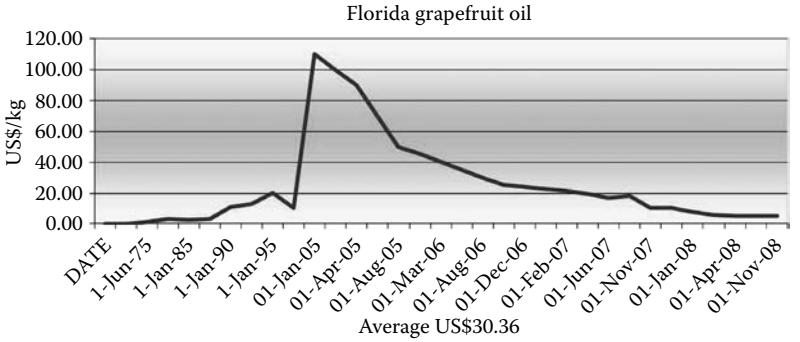


FIGURE 20.7 Price graph of grapefruit oil.

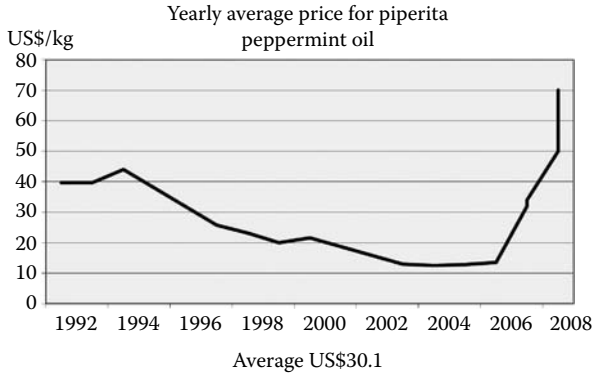


FIGURE 20.8 Price graph of peppermint oil (piperita).

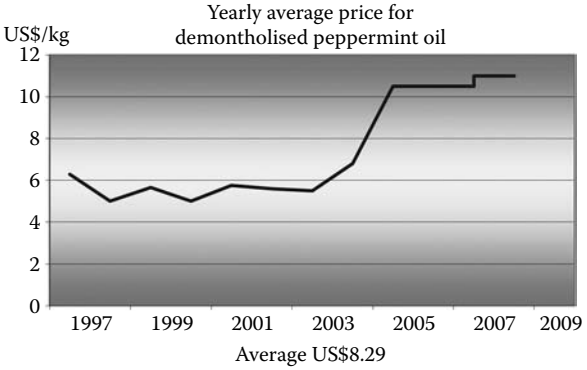


FIGURE 20.9 Price graph of peppermint oil (arvensis).

In the 1970s, there was considerable fraud of millions of dollars, caused by the shipment of essential oils from Indonesia to the major buyers. The oils were in fact water, despite analysis certificates from Indonesian Government laboratories showing them to be the named essential oil. Payment had been made by letters of credit and this fraudulent practice has discouraged buyers from opening letters of credit to suppliers today. Terms of trade should normally be cash against shipping documents or payment after receipt and quality control of goods.

The United States produces import statistics for essential oils and these can often be useful sources of information, and the European Union (EU) also has such statistics. The EU statistics cover a wide range of essential oils in each tariff; therefore the information is very vague and should not be used to make decisions. These statistics give no clues as to the quality of the product and it is that which can determine the price. The production of essential oils, as can be seen in the quotation by V.A. Beckley OBE, MC, Senior Agricultural Chemist, Kenya, who said during a meeting in 1931 in Nairobi, is perhaps more chancy than most farming propositions; it most certainly requires more attention and supervision than most, and, with certain rare exceptions, does not pay much more highly is still valid to this day, despite this being said in 1935.

The essential oil industry is a very small, tightly knit circle of traders, dealers, producers and consumers, and apart from some notable exceptions there is a very strong trade ethos. As it is a relatively small industry in terms of global commodities, statistics are not produced and it is by relationships with customers that information becomes available. Much that is on the Internet is misleading as it is for small quantities or is often written by consultants, and this information can be rapidly out-of-date as prices can move extremely quickly in either direction.

21 Storage and Transport of Essential Oils

Klaus-Dieter Protzen

CONTENTS

21.1 MARKETING OF ESSENTIAL OILS: THE FRAGRANT GOLD OF NATURE POSTULATES PASSION, EXPERIENCE, AND KNOWLEDGE

The trade of essential oils is affected more and more by Legal regulations related to Safety Aspects. The knowledge and the compliance with these superseding regulations have today become a *Conditio Sine Qua Non* (precondition) to ensure trouble-free global business relation as far as regulatory requirements are concerned as these requirements often may adversely affect usual commercial aspects.

When placing essential oils on the market in the EU for use as flavors and fragrances in foods, animal feed, cosmetic pharmaceuticals, aromatherapy, and so on, among others, the following regulations have to be observed (Dueshop, 2008):

- Council Directive 79/831/EEC—Dangerous Substances (see Section 21.6)
- Dangerous Preparations 99/45/EEC
- EU Flavouring Directive No. 88/388/EEC and a new EU flavor regulation in the stage of announcement
- Novel Food Directive No. 258/97/EEC
- Labelling Directive 2000/13/EC—food allergens
- EU Food Regulation No. 178/2002/EU
- New Pesticide Provisions—Regulation No. 396/2005/EU
- New EU Cosmetic Regulations Amending Directive No. 76/768/EEC—restrictions and bans (see Table 21.2 at the end of this chapter)
- Detergent Use Regulation No. 648/2004/EC
- EU Pharmaceutical Legislation—GMP aspects
- Biocide Use Directive No. 98/8/EEC
- Dangerous Substance Directive DSD 67/584/EEC

Essential oils are agro-based products that are generally manufactured by or collected from small individual producers. A large-scale production would require capital investment, which is rarely attracted as investors evidently realize the problems that no quick return of money is ensured because of too many factors influencing the market negatively like the dependency on weather conditions affecting the size of a crop over the whole vegetation period, competing crops challenging the acreage, a keen global competition striving for market shares, and narrow margins that do not compensate the involved risks. These aggravating factors also have an impact on the trade of essential oils.

A major part of the essential oil industry and the trade of these articles are dominated by small-scale and medium-sized family enterprises as only entrepreneurs with passion, a personal engagement and a persistent dedication as well as a long-standing experience nerve themselves to stay successfully in this business of the liquid gold of nature.

Success in the field of essential oils depends on enthusiasm and hard work, on a broad knowledge of the market situation, in spending a lot of time and cost to investigate new ideas of state-of-the-art conditions of processing raw materials that affect yield and quality and the return of investment, the adherence to comply with ever changing administrative regulations.

Essential oils are natural substances mainly obtained from vegetable raw materials either by distillation with water or steam or by mechanical process (expression) from the epicarp of citrus fruits. They are concentrated fragrance and flavor materials of complex composition, in general volatile alcohols, aldehydes, ethers, esters, ketones, hydrocarbons, and phenols of the group of mono- and sesquiterpenes or phenylpropanes as well as nonvolatile lactones.

A definition of the term essential oils and related fragrance/aromatic substances is given in the ISO-Norm 9235 Aromatic Natural Raw Materials (International Standard Organization, Geneva, 1997).

Because of their composition essential oils are classified by regulatory authorities in the EU as "Natural" but also as "Chemical Substances" (Dueshop, 2007).

The classification of chemical Substances is laid down in the Council Directive 67/548 and subsequent amendments but in particular in Council Directive 79/831/EEC of 18-09-1979. This 6th amendment is the basis of all existing regulations for dangerous/hazardous chemicals as it earmarked the beginning of a new era.

The topic REACH will not be covered in this chapter because of its complexity and too many open questions and answers respectively at the time of this writing. I hope, however, that in exchange a brief introduction to the historic development of the existing regulatory framework can be of help to understand the Safety Aspects, which are the background of the actual regulations as well as the forthcoming impediments in connection with REACH.

REACH is the abbreviation for *R*egistration, *E*valuation, *A*uthorization of *C*hemicals. It is another impeding Regulation in Europe—the consistent continuation of the existing rules to satisfy the EU administration of a perfect system to safeguard absolute security to protect humans and the environment regarding the use of chemicals within the EU.

For the trade, that is, the industry as well as importers and dealers of essential oils, REACH is a heavy burden demanding, already in the forefront, an unbelievable amount of time to clarify questions regarding the required product information for an appropriate registration of the so-called natural complex substances (NCS).

Before the publication of Directive 79/831/EEC only a few people were aware of the aftermath of a centralized European administration. Regulations regarding transport of dangerous goods were adhered—the trade of essential oils was well aware of the risk of flammability of many of the oils but most people, however, were caught more or less unprepared with regard to the new classification that natural essential oils have to be considered as "chemicals." The new Directive with its detailed regulations came as a surprise. It terminated the familiar view that essential oils because of their natural origin (and the fact they were used for centuries in medicines, flavors, and fragrances) could continue to exist as a special group of natural products like a sleeping beauty in the reality of a hostile world of administrative regulations. Now, all of a sudden it caused essential

oils to be considered as chemical substances of which a major part had to be classified as hazardous "chemical" substances.

21.2 THE IMPACT AND CONSEQUENCES ON THE CLASSIFICATION OF ESSENTIAL OILS AS NATURAL BUT CHEMICAL SUBSTANCES

The bell for the new era sounded when chemical substances in use within the EC during a reference period of 10 years had to be notified for European Inventory of Existing Commercial Chemical Substances (EINECS).

At that time EINECS enabled the EC administration not only to dispose of, for the first time, a survey of all chemical substances that had been in use in the EC between January 1, 1971 and September 18, 1981, but also to distinguish between "known substances" and "new substances."

"KNOWN" substances are all chemicals notified for EINECS, whereas all chemical substances that were not notified (and subsequently registered as "known substances" in EINECS) are considered by the EU administration as "new chemicals."

EINECS is a "closed list"—"New" chemical substances to be placed on the market in the EU after the deadline of September 18, 1981, therefore have to be notified for the European List of Notified Chemical Substances (ELINCS), the list complementing EINECS.

NEW chemical substances can be placed on—and used in—the market of the EU only after clearance according to uniform EC standards by competent (national) authorities. Thus, from the beginning, all potential risks of a (new) chemical substance are ascertained for a proper labeling for handling to avoid risks for humans as well as to protect the environment.

"Known" chemical substances (notified for EINECS) enjoyed, in a transitional phase, temporary exemption from the obligation to furnish the same safety data required for new chemical substances. Based on the experience gathered during their use, for quite a while it was assumed (Dueshop, 2007) that the temporary continuation of their use could be tolerated according to the hitherto used older standards of safety—and in view of the fact that a short-term clearance of approximately 100,000 chemical substances registered in EINECS could not be effected overnight.

Because these products have been notified for EINECS and therefore known to the regulative agencies in the EC, they are screened step by step either depending on their potential risk or according to the volumes produced or imported respectively to make sure that the known substances also comply with the new safety standards according to the following volume bands:

<100 kg
100–1000 kg
1–10 tons
10–100 tons
100–1000 tons
1000 tons plus.

Once new chemical substances have been cleared by the competent EC authorities, an ELINCS notification number—and later on an ELINCS registration number—is allocated. The names of the substances are published in regular intervals as newly registered chemical substances in ELINCS.

Responsible for the registration of substances in EINECS—and later on for ELINCS—was (is) the ECB/JRC (the European Chemical Bureau/Joint Research Centre of the European Commission at ISPRA). This agency was commissioned by the EC administration to allocate an EINECS registration number after having collected, evaluated, and arranged in proper order all notified substances.

To perform this task, the EU administration made use of the principles of the CAS system and arranged for the majority of essential oils and other UVCBs notified for EINECS the allocation of (new) CAS numbers.

But ATTENTION—the CAS number is an identification number for a chemical substance allotted by a private enterprise in the United States and must not be confused with the EINECS registration number.

EINECS and ELINCS numbers are registration numbers allocated by the EU administration, that is, ECB/JRC at ISPRA.

CAS numbers are assigned by the (private) CAS organization in the United States with the purpose of identification of (defined) chemical substances. A CAS number is allocated to a new (defined) chemical substance only after thorough examination of the product as per IUPAC Rules by the CAS organization to make sure that irrespective of different chemical descriptions and/or coined names that have been given to a product, a substance can be clearly related by the allocated CAS number according to the (CAS) principle "one substance—one number."

Using the CAS number system to register also chemical substances in EINECS that are not defined chemicals, the problem had to be sorted out how to register, for example, essential oils as they are products of complex composition. It was therefore necessary to extend the CAS system for this reason to allot a CAS number also to the so-called UVCBs, that is, substances that have been summed up under this abbreviation as substances of "*u*nknown or *v*ariable composition, *c*omplex reaction products, and *b*iological materials."

Essential oils are eventually registered as NCS by their botanical origin as for example:

Lavender oil: Lavender—*Lavandula angustifolia* ext.
EINECS registration no. 289-995-2—CAS no. (Einecs) 90063-37-9 extractives and their physically modified derivatives such as tinctures, concretes, absolutes, essential oils, terpenes, terpene-free fractions, distillates, and residues from *Lavandula angustifolia*—Labiatae (Lamiaceae)

Lavender oil: Lavender—*Lavandula angustifolia* ext.
EINECS registration no. 283-994-0—CAS no. (Einecs) 84776-65-8 extractives … from *Lavandula angustifolia angustifolia*— Labiatae (Lamiaceae)

Lavender concrete/absolute: Lavender—*Lavandula angustifolia* ext.
EINECS registration no. 289-995-2—CAS no. (Einecs) 90063-37-9 extractives and their physically modified derivatives such as tinctures, *concretes, absolutes*, essential oils, terpenes, terpene-free fractions, distillates, and residues from *Lavandula angustifolia*—Labiatae (Lamiaceae)

Lavandin oil: *Lavandula hybrida* ext.
EINECS registration no. 294-470-6—CAS no. (Einecs) 91722-69-9 extractives and their physically modified derivatives such as tinctures, concretes, absolutes, essential oils, terpenes, terpene-free fractions, distillates, and residues from *Lavandula hybrida*—Labiatae (Lamiaceae)

Lavandin oil abrialis: *Lavandula hybrida abrial* ext.
EINECS registration no. 297-384-7—CAS no. (Einecs) 93455-96-0 extractives and … from *Lavandula hybrida abrial*—Labiatae (Lamiaceae)

Lavandin oil grosso: *Lavandula hybrida grosso* ext.
EINECS registration no. 297-385-2—CAS no. (Einecs) 93455-97-1 extractives and … from *Lavandula hybrida grosso*—Labiatae (Lamiaceae).

Since essential oils are registered as extractives under their botanical origin, concretes/absolutes and other natural extractives of the same botanical origin have the same EINECS and CAS numbers as the essential oil.

When checking an EINECS number it is important to investigate in the official original documentation as in the secondary literature there exist too many inaccuracies.

TABLE 21.1
Examples of Different CAS-Numbers used in USA and EINECS in EU

	CAS No. USA	CAS No. EINECS	EC Registration No.
Eucalyptus oil	8000-48-4	84625-32-1	283-406-2
Eucalyptus globulus Lab.—Myrtaceae			
Lavender oil	8000-28-0	90063-37-9	289-995-2
Lavandula angustifolia—Labiatae			
Lavandula angustifolia angustifolia—Labiatae		84776-65-8	283-994-0
Lemon oil	8008-56-8	8028-48-6	284-515-8
		84929-31-7	284-515-8
Citrus limon L.—Rutaceae			
Orange oil	8008-52-9	8028-48-6	232-433-8
Citrus sinensis—Rutaceae			
Peppermint oil	8006-90-4	98306-02-6	308-770-2
Mentha piperita L.—Lamiaceae			
Manuka oil tairawhiti	—	223749-44-8	425-630-7
Leptospermum scoparium—Myrtaceae			(ELINCS)

Due to the lack of rules for an uniform classification of UVCBs (as an example the correct identification of the botanical origin of an essential oil), it happened that against the principles of the CAS organization in some cases several CAS numbers had been allocated to essential oils of the same denomination and in addition:

- An older CAS number allocated for an (earlier) registration of the product in the USA.
- A new CAS number allocated for registration in the EC for EINECS/ELINCS, respectively.

Once again, a CAS number does not mean that the product is registered in the European EINECS—the CAS number is just an identification number of a chemical substance allotted upon request by the (private) CAS organization.

Table 21.1 is exemplifying the allocation of several CAS numbers for the same essential oils but in connection with EINECS only the CAS number (EINECS) is of relevance.

Manuka Oil from New Zealand is the first (and only) essential oil that had to be notified for ELINCS as a new chemical substance after the Council Directive 79/831/EEC became effective on September 18, 1981 (Dueshop, 2007). It is quoted here only for the sake of completeness and curiosity.

This brief reflection on the background of EINECS and ELINCS is made as an introduction of the actual situation with regard to safety requirements and to alert new players in the field of essential oils to make sure that before intending to place a fragrance or flavor raw material on the European market they check whether or not this product is listed in EINECS or ELINCS respectively or is marketed in compliance with the Regulations of REACH for new substances. Placing of chemical substances in the states of the EU that are not meeting these requirements is a breach of law that can even be prosecuted as an offense with a penalty or a fine up to euros 100,000.

21.3 DANGEROUS SUBSTANCES AND DANGEROUS GOODS

There is a significant difference between the similar sounding words and regulations regarding DANGEROUS SUBSTANCES and DANGEROUS (HAZARDOUS) GOODS.

Both regulations are targeted to protect humans and the environment, but the term “Dangerous Substance” refers to the risks connected with the properties of the substance, that is, the potential risk of a direct contact with the product during production, packaging, and use.

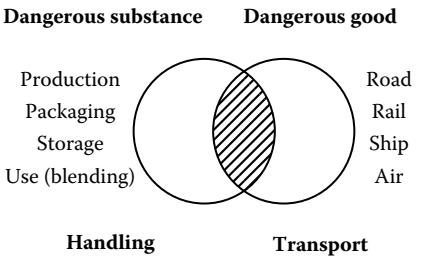


FIGURE 21.1 Interrelationship between dangerous substances and dangerous goods. (Friendly permission, Paul Kaders, Hamburg.)

Dangerous Substances are chemicals that fall into the categories quoted in article 2 of the already mentioned Council Directive 79/831/EEC—the 6th amendment of Directive 67/548. They are categorized as

Explosive
Oxidizing
Flammable (extremely flammable—highly flammable—flammable)
Toxicity (very toxic—toxic—harmful)
Corrosive (corrosive—irritant)
Dangerous for the environment (ecotoxicity)
Carcinogenic—teratogenic—mutagenic (CMR).

To protect humans and the environment—but principally the workers using them—articles that fall in these categories have to be classified as "Dangerous Substances" and labeled as per the subsequent Dangerous Substances Directive.

The term **Dangerous Goods** refer to dangerous substances properly packed and labeled for storage and transport by road, rail, sea, or air (Figure 21.1).

As per the rules and recommendations developed by a UN Committee of Experts regarding the transport of dangerous goods or substances they are defined as articles or substances that are capable of posing a risk to health, safety, property, or the environment.

Dangerous goods are classified into the following groups (classes of relevance for essential oils have been marked in bold font):

Class 1: Explosives
Class 2: Gases
Class 3: Flammable liquids
Class 4: Flammable solids
Class 5: Oxidizing substances and organic peroxides
Class 6: Toxic and infectious substances—eventually "***poison***"
Class 7: Radioactive material
Class 8: Corrosives
Class 9: Miscellaneous dangerous goods.

21.4 PACKAGING OF DANGEROUS GOODS

Dangerous goods must be transported in UN-approved packaging, which has been tested for sufficient stability and graded in the packing groups (PGs) I, II, and III.

PG III (low risk)—Suitable for dangerous goods having a low-risk classification only.
PG III corresponds to the UN packing code "Z"

PG II (medium risk)—This type of packing matches the requirements for most of the essential oils.
PG II corresponds to UN packing code "Y"—PG II includes PG III.
PG I (high risk) corresponds to UN packing code "X".
PG I includes PG II and III—this type of packing has the highest stability.

All dangerous goods have to be packed in the so-called UN-approved packing.

Essential oils that are classified as dangerous goods and shipped in bulk, that is, drum lots for example, will only be accepted for transport if they are packed in UN-approved iron drums. These drums with a bunghole for example bear the following UN code:

UN 1A1/Y/1.4/150/(06)/(NL)/(VL824)

This specification reveals the following details:

1A1	Steel drum—nonremovable head
Y	PG II
1.4	Maximum relative density at which the packing has been tested
150	Test pressure
(0.6)	Year of manufacture
(NL)	State (country)
(VL 123)	Code number of manufacturer

The potential risks of dangerous substances or goods respectively have to be declared in the relevant transport documentation. In addition to this information, also warning labels have to be used on the packages to alert workers regarding the nature of the goods they are handling.

The aim of dangerous goods regulations is not only to protect persons occupied with the conveyance of dangerous substances but to also serve, for example, fire brigades, who in case of an accident or fire are called and have to be aware of the risks.

In this connection, a few words are due on the so-called UN/ID numbers for dangerous goods. These UN numbers are assigned to dangerous goods according to their hazard classification and composition. These UN (hazard identification) numbers should not be confused with the number of UN packaging.

UN numbers are listed in all regulations for transport of dangerous goods and are identical for all types of transport.

Approximately 170 essential oils have to be classified as dangerous substances/goods. According to their composition, the following UN numbers have been assigned to these oils:

65	UN no. 1169—extracts, aromatic, and liquid
52	UN no. 3082—environmentally hazardous substance, liquid, n.o.s.
14	UN no. 1272—pine oil(s)
6	UN no. 1992—flammable liquid, toxic, n.o.s.
6	UN no. 2810—toxic liquid organic n.o.s.
5	UN no. 2319—terpene hydrocarbons

and others are distributed among the UN nos. 2811 (3), 2924 (3), 1545 (2), 1130 (1), 1197 (1), 1201 (1), 1299 (1), 1990 (1), and 3077 (1).

Details can be found in EFFA's Code of Practice (CoP, 2008, et seq.), which is described later on.

Consignments of dangerous substances (and dangerous goods respectively) must be accompanied by a so-called Material Safety Data Sheet. For this purpose, the International Standard

Organization (ISO) has developed a standard form that—divided into 16 headings—provides basically information on

Name of the supplier
Name and identification of the substance/preparation
Composition/components of the article
Hazard identification
First aid measures
Fire fighting measures
Accidental release measures
Ecological information
Transport information
Regulatory information and so on

to inform users and forwarders about the risks in connection with the chemical substance.

Not only producers but also suppliers have the responsibility that the MSDS Form (material safety datasheet) is properly completed.

21.5 LABELING

IFRA and IOFI have regularly informed their members as well as stakeholders in the industry and trade for more than five decades about potential health risks that have been assessed for natural and synthetic raw materials used in flavors and fragrances in research and tests.

Since a couple of years ago, the European Association of the Flavour and Fragrance Industry in Europe (EFFA) has been publishing a Code of Practice (CoP, 2008) with recommendations regarding a proper classification and labeling of aromatic chemicals and essential oils.

This "CoP" is complementing the information of IFRA and IOFI. It is continuously updated by experts of the industry and the trade by the Hazard Communication Working Group (HCWG) and furnishes for the disposal of people all over the world occupied in handling essential oils and aromatic chemicals; an up-to-date recommendation for a proper classification and labeling of hazardous fragrance and flavor raw materials (Protzen, 1989).

The actual version of this CoP 2009 is available on the internet free of charge from the homepage of EFFA: **http://www.effa.be/**.

Because of the compiled state-of-the-art expertise, EFFA's CoP has almost obtained in practice the quality of an official documentation. Therefore not only the trade but also the port and transport authorities who are in charge of controlling the compliance of safety regulations for transport of dangerous goods are today referring to this guideline (Protzen, 1998).

For approximately. 1200 aromatic chemicals used in the flavor and fragrance industry and 220 commercially used essential oils as well as information on 60 natural extracts like absolutes and resinoids, the CoP contains a guideline detailing information on

EC registration number
CAS number relevant in the EC/EU
CAS number relevant in the USA
Commercial name
Content of hydrocarbons (%)
Warning labels
UN Transport Regulations (dangerous goods class, required class of packing group class, appropriate UN number)

R (Risk) phrases
S (Safety) phrases.

Before the Council Directive 79/831/EEC was issued, flammability of essential oils was considered the main danger emanating from these articles. Today's knowledge of potential risks of essential oils is extended. As a precaution very rigid safety regulations that consider extreme conditions often exceeding empirical and practical experience require that from the 220 essential oils listed in the CoP 2008, approximately 70%, that is, 170 essential oils, are classified as dangerous Substances and therefore must be labeled accordingly for storage, use, and transport, as for example:

Harmful

Dangerous for the environment

Irritant

The following warning labels cover the majority of risks:

190	Xn	Harmful—a St. Andrew's Cross (Xn)
174	N	Dangerous for the environment
60	Xi	Irritant—a St. Andrew's Cross (Xi)
12	T	Toxic—a skull and cross-bones (T)
3	C	Corrosive—the symbol showing the damaging effect of an acid

In addition to this information also R-phases and R labels must be used on the packaging. A list that explains the meaning of R + S phrases required for labeling essential oils as per the CoP is enclosed for further perusal.

A statistical evaluation of the R (Risk) labels to be used is shown in the following differentiation to have a better and detailed idea of the potential risks:

205	R-43	May cause sensation by skin contact
158	R-65	Harmful—may cause lung damage if swallowed
103	R-51/53	Toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment
95	R-38	Harmful if swallowed
88	R-50/53	Very toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment
80	R-10	Flammable
40	R-52/53	Harmful to aquatic organisms—may cause long-term adverse effects on the aquatic environment
38	R-22	Harmful if swallowed.

Flammability as a major risk of essential oils is today outnumbered by the potential risks emanating from these concentrated fragrances and flavors causing harm to the skin, to the health risk if swallowed, and their ecotoxicity.

The cumulative frequency of occurrence reveals that the majority of essential oils have to be handled with care and workers should use a protection particularly when a contact of these concentrated volatile natural fragrance and flavor materials with the skin is possible.

Special care and attention should be given when handling essential oils labeled with

R-50/53	Very toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment
R-34	Causes burns (oils containing thymol)
R-45	May cause cancer (oils containing safrol)
R-68	Possible risk of irreversible effects

Safety starts at the point of production but in the chain of supply each party involved is directly responsible for proper handling, that is, declaration and labeling of goods. In Europe, a special transport police is in the ports and on the roads intensifying the controls for correct declaration, packaging and labeling of dangerous goods and heavy fines are imposed:

Risk phrases applicable for storage and transport of essential oil—data as per EFFA CoP 2008:

R-10	Flammable
R-20	Harmful by inhalation
R-21	Harmful in contact with the skin
R-22	Harmful if swallowed
R-23	Toxic by inhalation
R-24	Toxic in contact with the skin
R-25	Toxic if swallowed
R-26	Very toxic by inhalation
R-27	Very toxic in contact with the skin
R-34	Causes burns
R-36	Irritating to eyes
R-37	Irritating to the respiratory system
R-38	Irritating to the skin
R-41	Risk of serious damage to eyes
R-43	May cause sensation by skin contact
R-45	May cause cancer
R-65	Harmful—may cause lung damage if swallowed
R-66	Repeated exposure may cause skin dryness or cracking
R-68	Possible risk of irreversible effects
R-21/22	Harmful in contact with skin and if swallowed
R-36/38	Irritating to eyes and skin
R-50/53	Very toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment
R-51/53	Toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment
R-52/53	Harmful to aquatic organisms—may cause long-term adverse effects on the aquatic environment
R-68/22	Harmful—possible risk of irreversible effects if swallowed

21.6 LIST OF REGULATIONS FOR THE CONSIDERATION OF DOING BUSINESS IN THE EU

- Council Directive 79/831/EEC of 18 September 1979 amending for the sixth time Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances
  OJ L 259, 15.10.1979, p. 10–28 (DA, DE, EN, FR, IT, NL)
- Directive 1999/45/EC of the European Parliament and of the Council of 31 May 1999 concerning the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous preparations
  OJ L 200, 30.7.1999, p. 1–68 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Council Directive 88/388/EEC of 22 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production
  OJ L 184, 15.7.1988, p. 61–66 (ES, DA, DE, EL, EN, FR, IT, NL, PT)
- Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients
  OJ L 43, 14.2.1997, p. 1–6 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs
  OJ L 109, 6.5.2000, p. 29–42 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety
  OJ L 31, 1.2.2002, p. 1–24 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EECText with EEA relevance.
  OJ L 70, 16.3.2005, p. 1–16 (ES, CS, DA, DE, ET, EL, EN, FR, IT, LV, LT, HU, MT, NL, PL, PT, SK, SL, FI, SV)
- Regulation (EC) No 648/2004 of the European Parliament and of the Council of 31 March 2004 on detergents (Text with EEA relevance)
  OJ L 104, 8.4.2004, p. 1–35 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market
  OJ L 123, 24.4.1998, p. 1–63 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances
  OJ 196, 16.8.1967, p. 1–98 (DE, FR, IT, NL) English special edition: Series I Chapter 1967 P. 0234
- Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products
  (OJ L 262, 27.9.1976, p. 169)

For amendments see Table 21.2.

TABLE 21.2

COUNCIL DIRECTIVE
of 27 July 1976
on the approximation of the laws of the Member States relating to cosmetic products
(76/768/EEC)
(OJ L 262, 27.9.1976, p. 169)

Amended by		Official Journal No	Page	Date
▶ **M1**	Council Directive 79/661/EEC of 24 July 1979	L 192	35	31.7.1979
▶ **M2**	Commission Directive 82/147/EEC of 11 February 1982	L 63	26	6.3.1982
▶ **M3**	Council Directive 82/368/EEC of 17 May 1982	L 167	1	15.6.1982
▶ **M4**	Commission Directive 83/191/EEC of 30 March 1983	L 109	25	26.4.1983
▶ **M5**	Commission Directive 83/341/EEC of 29 June 1983	L 188	15	13.7.1983
▶ **M6**	Commission Directive 83/496/EEC of 22 September 1983	L 275	20	8.10.1983
▶ **M7**	Council Directive 83/574/EEC of 26 October 1983	L 332	38	28.11.1983
▶ **M8**	Commission Directive 84/415/EEC of 18 July 1984	L 228	31	25.8.1984
▶ **M9**	Commission Directive 85/391/EEC of 16 July 1985	L 224	40	22.8.1985
▶ **M10**	Commission Directive 86/179/EEC of 28 February 1986	L 138	40	24.5.1986
▶ **M11**	Commission Directive 86/199/EEC of 26 March 1986	L 149	38	3.6.1986
▶ **M12**	Commission Directive 87/137/EEC of 2 February 1987	L 56	20	26.2.1987
▶ **M13**	Commission Directive 88/233/EEC of 2 March 1988	L 105	11	26.4.1988
▶ **M14**	Council Directive 88/667/EEC of 21 December 1988	L 382	46	31.12.1988
▶ **M15**	Commission Directive 89/174/EEC of 21 February 1989	L 64	10	8.3.1989
▶ **M16**	Council Directive 89/679/EEC of 21 December 1989	L 398	25	30.12.1989
▶ **M17**	Commission Directive 90/121/EEC of 20 February 1990	L 71	40	17.3.1990
▶ **M18**	Commission Directive 91/184/EEC of 12 March 1991	L 91	59	12.4.1991
▶ **M19**	Commission Directive 92/8/EEC of 18 February 1992	L 70	23	17.3.1992
▶ **M20**	Commission Directive 92/86/EEC of 21 October 1992	L 325	18	11.11.1992
▶ **M21**	Council Directive 93/35/EEC of 14 June 1993	L 151	32	23.6.1993
▶ **M22**	Commission Directive 93/47/EEC of 22 June 1993	L 203	24	13.8.1993
▶ **M23**	Commission Directive 94/32/EC of 29 June 1994	L 181	31	15.7.1994
▶ **M24**	Commission Directive 95/34/EC of 10 July 1995	L 167	19	18.7.1995
▶ **M25**	Commission Directive 96/41/EC of 25 June 1996	L 198	36	8.8.1996
▶ **M26**	Commission Directive 97/1/EC of 10 January 1997	L 16	85	18.1.1997
▶ **M27**	Commission Directive 97/18/EC of 17 April l997	L 114	43	1.5.1997
▶ **M28**	Commission Directive 97/45/EC of 14 July 1997	L 196	77	24.7.1997
▶ **M29**	Commission Directive 98/16/EC of 5 March 1998	L 77	44	14.3.1998
▶ **M30**	Commission Directive 98/62/EC of 3 September 1998	L 253	20	15.9.1998
▶ **M31**	Commission Directive 2000/6/EC of 29 February 2000	L 56	42	1.3.2000
▶ **M32**	Commission Directive 2000/11/EC of 10 March 2000	L 65	22	14.3.2000
▶ **M33**	Commission Directive 2000/41/EC of 19 June 2000	L 145	25	20.6.2000
▶ **M34**	Commission Directive 2002/34/EC of 15 April 2002	L 102	19	18.4.2002
▶ **M35**	Commission Directive 2003/1/EC of 6 January 2003	L 5	14	10.1.2003
▶ **M36**	Commission Directive 2003/16/EC of 19 February 2003	L 46	24	20.2.2003
▶ **M37**	Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003	L 66	26	11.3.2003
▶ **M38**	Commission Directive 2003/80/EC of 5 September 2003	L 224	27	6.9.2003

continued

TABLE 21.2 (continued)

Amended by		Official Journal No	Page	Date
▶ **M39**	Commission Directive 2003/83/EC of 24 September 2003	L 238	23	25.9.2003
▶ **M40**	Commission Directive 2004/87/EC of 7 September 2004	L 287	4	8.9.2004
▶ **M41**	Commission Directive 2004/88/EC of 7 September 2004	L 287	5	8.9.2004
▶ **M42**	Commission Directive 2004/94/EC of 15 September 2004	L 294	28	17.9.2004
▶ **M43**	Commission Directive 2004/93/EC of 21 September 2004	L 300	13	25.9.2004
▶ **M44**	Commission Directive 2005/9/EC of 28 January 2005	L 27	46	29.1.2005
▶ **M45**	Commission Directive 2005/42/EC of 20 June 2005	L 158	17	21.6.2005
▶ **M46**	Commission Directive 2005/52/EC of 9 September 2005	L 234	9	10.9.2005
▶ **M47**	Commission Directive 2005/80/EC of 21 November 2005	L 303	32	22.11.2005
▶ **M48**	Commission Directive 2006/65/EC of 19 July 2006	L 198	11	20.7.2006
▶ **M49**	Commission Directive 2006/78/EC of 29 September 2006	L 271	56	30.9.2006
▶ **M50**	Commission Directive 2007/1/EC of 29 January 2007	L 25	9	1.2.2007
▶ **M51**	Commission Directive 2007/17/EC of 22 March 2007	L 82	27	23.3.2007
▶ **M52**	Commission Directive 2007/22/EC of 17 April 2007	L 101	11	18.4.2007
▶ **M53**	Commission Directive 2007/53/EC of 29 August 2007	L 226	19	30.8.2007
▶ **M54**	Commission Directive 2007/54/EC of 29 August 2007	L 226	21	30.8.2007
▶ **M55**	Commission Directive 2007/67/EC of 22 November 2007	L 305	22	23.11.2007
▶ **M56**	Commission Directive 2008/14/EC of 15 February 2008	L 42	43	16.2.2008
▶ **M57**	Commission Directive 2008/42/EC of 3 April 2008	L 93	13	4.4.2008
▶ **M58**	Commission Directive 2008/88/EC of 23 September 2008	L 256	12	24.9.2008
▶ **M59**	Commission Directive 2008/123/EC of 18 December 2008	L 340	71	19.12.2008
▶ **M60**	Directive 2008/112/EC of the European Parliament and of the Council of 16 December 2008	L 345	68	23.12.2008
▶ **M61**	Commission Directive 2009/6/EC of 4 February 2009	L 36	15	5.2.2009
Amended by				
▶ **A1**	Act of Accession of Greece	L 291	17	19.11.1979
▶ **A2**	Act of Accession of Spain and Portugal	L 302	23	15.11.1985
Corrected by				
▶ **C1**	Corrigendum, OJ L 255, 25.9.1984, p. 28 (84/415/EEC)			
▶ **C2**	Corrigendum, OJ L 157, 24.6.1988, p. 38 (88/233/EEC)			
▶ **C3**	Corrigendum, OJ L 199, 13.7.1989, p. 23 (89/174/EEC)			
▶ **C4**	Corrigendum, OJ L 273, 25.10.1994, p. 38 (94/32/EC)			
▶ **C5**	Corrigendum, OJ L 341, 17.12.2002, p. 71 (2002/34/EC)			
▶ **C6**	Corrigendum, OJ L 151, 19.6.2003, p. 44 (2002/34/EC)			
▶ **C7**	Corrigendum, OJ L 58, 26.2.2004, p. 28 (2003/83/EC)			
▶ **C8**	Corrigendum, OJ L 97, 15.4.2005, p. 63 (2004/93/EC)			
▶ **C9**	Corrigendum, OJ L 258, 4.10.2007, p. 44 (2007/54/EC)			
▶ **C10**	Corrigendum, OJ L 136, 24.5.2008, p. 52 (2008/42/EC)			

REFERENCES

EFFA Code of Practice 2008, 2008. The European Flavour & Fragrance Association. Accessed October 2008 from http://www.effa.be/

Dueshop, L., 2007. Personal communications.

Dueshop, L., 2008. Personal communications.
Protzen, K-D., 1989. *Guideline for Classification and Labelling of Essential Oils for Transport and Handling* distributed during IFEAT CONFERENCE, London.
Protzen, K-D., 1998. Transportation/Safety Regulations Update, International Conference on Essential Oils and Aromas, November 8–12, 1998, London.

22 Recent EU Legislation on Flavors and Fragrances and Its Impact on Essential Oils

Jan C.R. Demyttenaere

CONTENTS

22.1 INTRODUCTION

In the last years, several new European Regulations and Directives have been adopted or announced in relation to flavors and fragrances. As essential oils and extracts are very important ingredients for flavoring and fragrance applications, these new regulations will have a major impact on the trade and use in commerce of these essential oils and extracts.

This chapter will focus on some pieces of legislation that are of major importance for the Flavour and Fragrance (F&F) Industry, such as the Cosmetic Directive 76/768/EC and especially its Seventh Amendment (2003/15/EC) and the first amendment of the Detergent Regulation (June 2006), which make the labeling of 26 specific fragrance ingredients (the so-called 26 "alleged" allergens) mandatory: the presence of these materials above the given threshold has to be declared irrespective of the way they are added (as such or as being part of "complex ingredients" such as extracts and essential oils).

Some attention will be paid to the new Flavouring Regulation (part of the so-called Food Improvement Agents Package) that will replace the current Flavouring Directive 88/388/EEC and that is currently under discussion at the EU Commission, EU Parliament and Council levels.

Also the issue of hazard classification and labeling of dangerous substances and preparations, and essential oils containing hazardous components will be addressed and some examples will be given. This relates to the recent publication of the Commission Directive 2006/8/EC amending the Dangerous Preparations Directive 1999/45/EC.

22.2 COSMETIC AND DETERGENT LEGISLATION AND ALLERGEN LABELING

22.2.1 History and Background

In recent years (the late 1980s and 1990s), there has been a scientific debate on the safety of fragrance (perfumery) ingredients. Dermatologists have highlighted the risk of contact allergy from fragrance ingredients (Santussi et al., 1987; Becker et al., 1994), and actions to prevent the disease have been requested (Frosch et al., 1995; Larsen et al., 1996; SCCNFP 0017/98).

As a result of this and in response to a question from a Member State (MS) and members of the European parliament, the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP) has been asked by DG Enterprise (EU Commission) to respond to the following mandate in relation to the safety of fragrance ingredients and to answer (among others) the following questions:

- It is proposed that all known fragrance allergens are labeled on cosmetics if used in the products. Does the SCCNFP agree to this proposal? If so,
  - Which chemicals fall under this classification?
  - Is there a maximum concentration of each chemical permissible without the requirement for labeling?
- Restrictions are proposed for the three most common fragrance allergens (cinnamic aldehyde, isoeugenol, and hydroxycitronellal). Does the SCCNFP agree to restriction on the use of common fragrance allergens (Annex III listing)? If so
  - Which fragrance materials should be subject to restrictions?
  - What are the conditions for restrictions (maximum concentration, fields of applications, etc.)?

Other questions were related to industry-restricted and industry-prohibited substances.

In its Interim position on Fragrance allergy SCCNFP/0202/99 adopted at the SCCNFP session of June 23, 1999, the SCCNFP already stated: "Contact allergy to fragrance substances is an important clinical problem. Up to 10% of individuals with eczema are allergic to fragrance substances and possibly 1–2% of the general population."

In the same Interim position, SCCNFP considered that the mandate from the European Commission could be usefully divided into the following two sections:

1. Identification of those fragrance ingredients that are of concern as allergens for the consumer. Recommendations on informing the consumer of the presence of important allergens to permit the consumer with a known fragrance allergy as a means to avoid contact with an allergen. An opinion as to whether such an identification can be related to concentrations present in a product when elicitation levels are known.
2. An opinion on the adoption of industry-prohibited substances into Annex 2 and adoption of industry-restricted substances into Annex 3. Consideration as to whether the concentration limits or other restrictions suggested by industry can be supported or need to be changed if there is such an inclusion in Annex 22.3. Whether there are additional substances that should be subject to inclusion in an annex.

Taking into account the importance and enormity of the mandate, it was concluded that the first section should be considered initially.

As a result, the SCCNFP published, as a follow-up to the Interim position, its opinion SCCNFP/0017/98 (adopted by the SCCNFP during the plenary session of December 8, 1999) entitled "Fragrance allergy in consumers—A review of the problem: Analysis of the need for appropriate consumer information and Identification of consumer allergens."

This opinion relates to the first section mentioned above and consists of

- A critical review of the problem of fragrance allergy in consumers.
- Identification of those fragrance ingredients that are well recognized as consumer allergens.
- An opinion as to whether such identification can be related to concentrations present in a product when elicitation levels are known.

Allergy to natural ingredients (such as oakmoss) was not addressed in this opinion but was analyzed separately (see SCCNFP opinion of October 24, 2000).

It was the opinion of the SCCNFP that

- Fragrance ingredients have to be considered as an important cause of contact allergy.
- Based on criteria restricted to dermatological data reflecting the clinical experience, it was possible to identify 24 fragrance ingredients, which correspond to the most frequently recognized allergens. Thirteen of these have been reported more frequently; these are well-recognized contact allergens in consumers and are thus of most concern; 11 others are less well documented.

In the opinion (SCCNFP/0017/98), two lists were given: a List A with 13 fragrance chemicals, which according to existing knowledge, are most frequently reported and well-recognized consumer allergens, and a List B with 11 fragrance chemicals, which are less frequently reported and thus less documented as consumer allergens.

Tables 22.1 and 22.2 review the substances of Lists A and B.

In addition, the SCCNFP stated in its opinion that information should be provided to consumers about the known presence in cosmetic products of fragrance ingredients with a well-recognized potential to cause contact allergy: "Information regarding these fragrance chemicals should be given to consumers if deliberately added to a fragrance formulation either in the form of a chemical or as an identified constituent of an ingredient."

TABLE 22.1
List A (SCCNFP/0017/98)—13 Most Frequently Reported Allergens (CAS No.)

Amyl cinnamal (122-40-7)	Amylcinnamyl alcohol (101-85-9)
Benzyl alcohol (100-51-6)	**Benzyl salicylate (118-58-1)**
Cinnamyl alcohol (104-54-1)	**Cinnamal (104-55-2)**
Citral (5392-40-5)	**Coumarin (91-64-5)**
Eugenol (97-53-0)	**Geraniol (106-24-1)**
Hydroxycitronellal (107-75-5)	Hydroxymethylpentylcyclohexene-carboxaldehyde (HMPCC) (31906-04-4)
Isoeugenol (97-54-1)	

Note: Substances highlighted in bold are naturally occurring fragrance materials and the other substances are synthetic fragrance ingredients that are not known to occur in nature.

TABLE 22.2
List B (SCCNFP/0017/98)—11 Less Frequently Reported Allergens (CAS No.)

Anisyl alcohol (105-13-5)	**Benzyl benzoate (120-51-4)**
Benzyl cinnamate (103-41-3)	**Citronellol (106-22-9)**
Farnesol (4602-84-0)	Hexyl cinnamaldehyde (101-86-0)
Lilial (80-54-6)	***d*-Limonene (5989-27-5)**
Linalool (78-70-6)	Methyl heptine carbonate (111-12-6)

3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one (=alpha-iso-methylionone) (127-51-5)

Note: Substances highlighted in bold are naturally occurring fragrance materials and the other substances are synthetic fragrance ingredients that are not known to occur in nature.

Additionally, as mentioned above, also two natural ingredients, oakmoss and tree moss extracts, were addressed in a separate SCCNFP opinion (adopted during the 14th plenary meeting of October 24, 2000).

These two natural mosses are identified as follows: oakmoss extracts derived from the lichen, *Evernia prunastri* (L.) Arch. (Usneaceae), growing primarily on oak trees, and tree moss extracts derived from a mixture of lichens, mainly *Evernia furfuracea* (L.) Arch. (Usneaceae) growing on *Pinus* species.

Oakmoss extract has CAS no. 90028-68-5 and EINECS no. 289-861-3.

Tree moss extract has CAS no. 90028-67-4 and EINECS no. 289-860-8.

The term "labeling" comes from the EU Commission (DG Enterprise) and whether a fragrance ingredient should be labeled or not is a Risk Manager's decision. In its *memorandum* of 2001 (SCCNFP/0450/01), the SCCNFP (being the Risk Assessor) clearly states that "because of the lack of dose/elicitation data for these substances, the SCCNFP has been unable to provide recommendations on levels above which the information to the consumer would be necessary." Nevertheless, SCCNFP mentions in its memorandum that it is "aware that for practical risk management reasons there is a need for threshold levels for the provision of information." There is a proposal that for leave-on products, this threshold level should be 10 ppm in the finished cosmetic product, whereas for rinse-off products, the SCCNFP would consider a working level 10 times higher than that recommended for leave-on products to be reasonable, being 100 ppm.

22.2.2 Cosmetic Directive and its Seventh Amendment

The EU Commission has implemented the above-mentioned SCCNFP opinions in the 7th Amendment of the Cosmetic Directive 76/768/EC (2003/15/EC) by adding the following restrictions [limitations and requirements (for labeling)] to 26 fragrance substances in Annex III, Part 1: "The presence of the substance must be indicated in the list of ingredients referred to in Article 6(1)(g) when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products."

However, no further restrictions (such as maximum authorized concentrations in the finished cosmetic products), except the labeling requirements were introduced at that time.

This means that the presence of any of the 26 alleged allergens (sensitizers) must be indicated (labeled) in the list of ingredients on the packaging of the finished cosmetic products when its concentration exceeds 10 ppm (leave-on products) or 100 ppm (rinse-off products), according to Art. 6.1(g) of the Cosmetic Directive, 7th Amendment.

However, it is important to note here that the Fragrance Industry is self-regulating by issuing the International Fragrance Association Code of Practice (IFRA CoP), which is published by the IFRA. This CoP consists of Standards (the so-called IFRA Standards) for the fragrance ingredients with certain restrictions/limitations and in some cases bans to which the International Fragrance Industry should comply. The last amendment of the IFRA CoP is the 44th Amendment. The IFRA CoP and

its 44th Amendment and the IFRA Standards can be found on the homepage of the International Fragrance Association: www.ifraorg.org.

22.2.3 Impact on Extracts and Essential Oils and Aromatic Natural Raw Materials

The mandatory labeling requirement for the 26 alleged allergens is irrespective of the source of the allergen or the way by which it has been introduced in the final cosmetic product. In other words, the presence of these materials above the given threshold has to be declared irrespective of the way they are added (as such or as being part of "complex ingredients" such as extracts and essential oils). This means that the use of essential oils containing them in formulations may lead to the presence of such allergens and the labeling requirement will apply.

Sixteen of the 24 alleged allergenic substances are naturally occurring (see substances indicated in bold in Tables 22.1 and 22.2), the other eight substances are synthetic fragrance ingredients that do not occur in nature as far as known.

The structures of the 16 naturally occurring allergenic substances are depicted in Figures 22.1 and 22.2.

The remaining two alleged allergens are aromatic natural raw materials by themselves: oakmoss (*E. prunastri*) and tree moss (*E. furfuracea*).

According to the current knowledge of the F&F Industry, these 16 allergens occur in about 180 natural raw materials (extracts and essential oils) (EFFA CoP, 2007).

A list of aromatic natural raw materials containing any of the 16 naturally occurring sensitizers and their presence (if >0.1%) or concentration can be found in Annex 22.1 to this chapter—this is based on earlier internal communication (2004) of the F&F industries related to a former version of the EFFA CoP.

One of the key challenges for the Fragrance Industry is the analysis and identification of the 16 naturally occurring allergens in the natural raw materials (extracts and essential oils) and fragrance compounds (mixtures and preparations). To address this work, the Fragrance Industry has established an Analytical Working Group of IFRA where methods of analysis are developed. A recommended method of analysis for gas chromatography-mass spectrometry (GC-MS) quantification of suspected allergens in fragrance compounds has been published by this group in 2003 (Chaintreau et al., 2003). Some further work on the investigation of the GC-MS determination of allergens (GC-MS quantification of allergens in fragrances and data treatment strategies and method performances) was published more recently by the same group (Chaintreau et al., 2007).

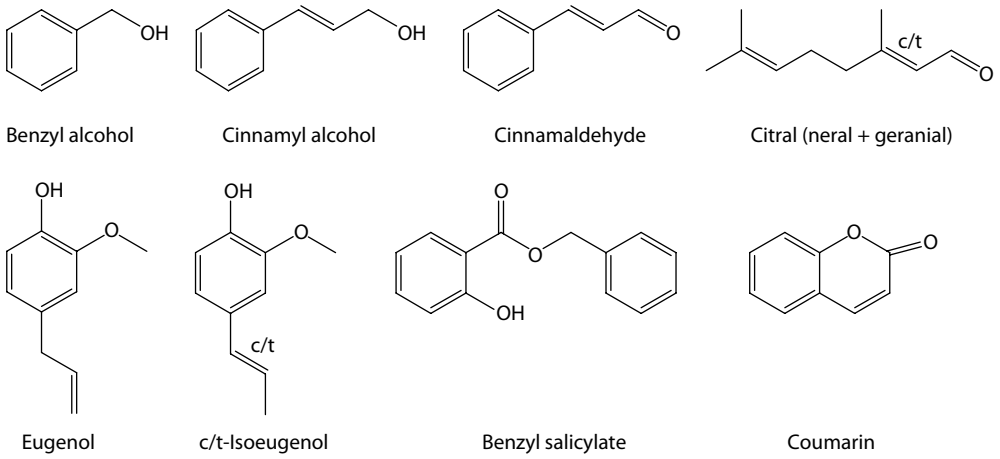


FIGURE 22.1 Structures of the 16 naturally occurring alleged allergenic substances (part 1).

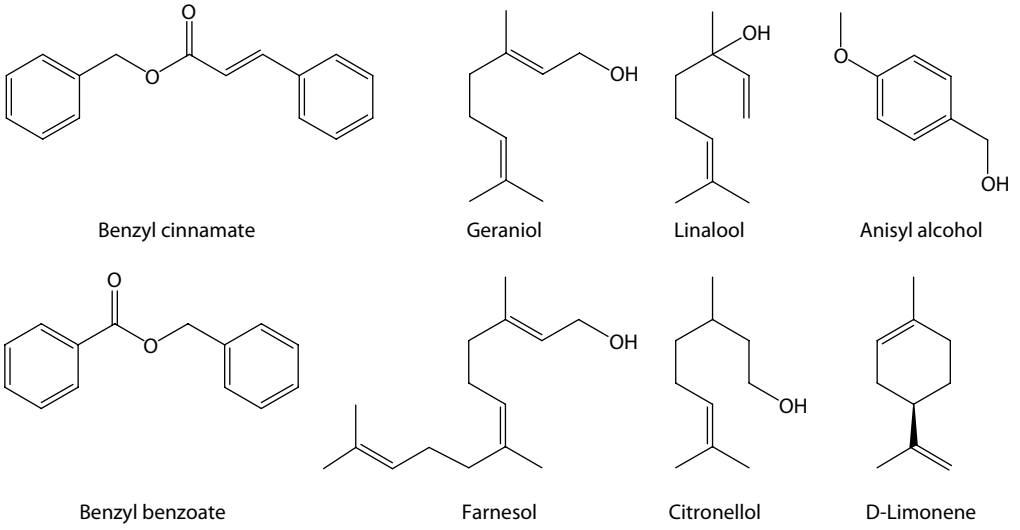


FIGURE 22.2 Structures of the 16 naturally occurring alleged allergenic substances (part 2).

22.2.4 Recent Data on Sensitization to Fragrances

Recently a new study on the sensitization to the 26 fragrance ingredients (24 single substances and two natural extracts) that have to be labeled according to the European Regulation was published by the group of Schnuch et al. (2007). This study was part of the multicenter project Information Network of Departments of Dermatology (IVDK) (Schnuch et al., 1997, 2004). The aim was to study the frequency of sensitization to these 26 alleged allergenic fragrances, in particular the actual frequencies of contact allergy to these 26 fragrances. To test this, the fragrance ingredients were patch tested in consecutive, unselected patients (in total 21,325 patients) by the IVDK network during a 2-year period, consisting of four periods of 6 months. The number of patients tested with each of the fragrance substances ranged from 1658 to 4238.

The frequency of sensitization was expressed by the proportion of patients reacting allergic (% pos.), that is, the number of allergic patients compared to the number of patients tested (*n* pos./*n* tested, %) and the frequency of allergic reactions was then standardized for age and sex.

The "allergenic" fragrances were divided into three groups, depending on the frequency of sensitization, based on the 95% confidence interval (CI).

The first group of ingredients with the upper CI > 1.0% could be regarded as important allergens and was called Group I. This group includes the two natural extracts, oakmoss and tree moss, and the substances HMPCC, hydroxycitronellal, isoeugenol, cinnamic acid, and farnesol.

Another group of ingredients with an upper CI between 0.5% and 1.0% was found to be clearly allergenic but less important in terms of sensitization frequency (Group II). This group comprises cinnamic alcohol, citral, citronellol, geraniol, eugenol, coumarin, lilial, amyl-cinnamic alcohol, and benzyl cinnamate.

On the other hand, the third group (Group III) comprises substances that have turned out to be (extremely) rare sensitizers in this study, or which in other instances may even be considered as nonsensitizers, according to the authors. This group with an upper CI of less than 0.5% contains 10 materials: benzyl alcohol, linalool, methylheptin carbonate, α-amyl-cinnamic aldehyde, α-hexyl-cinnamic aldehyde, limonene, benzyl salicylate, γ-methylionone, benzyl benzoate, and anisyl alcohol. It was further concluded that sensitization to allergens of the first group is significantly more frequent than sensitization to allergens of the third group.

Regarding Group III it is also worth noting that some molecules are not allergens as such, but only turn into allergens after substantial oxidation, for example, limonene and linalool (Karlberg and Dooms-Goossens, 1992; Karlberg et al., 1992; Hagvall and Karlberg, 2006).

It is interesting to note that there is a difference in the classification of the allergens (reported frequency) according to the opinion of the SCCP (SCCP/0017/98) and the classification in groups by Schnuch et al. For example one substance that is an important allergen according to the study of Schnuch (Group I), farnesol, is according to SCCP "less frequently reported." The same applies to two substances of Group II that are according to SCCP "less frequently reported," namely citronellol and benzyl cinnamate. On the other hand, two materials that are according to the study of Schnuch (extremely) rare sensitizers (Group III) are according to SCCP "frequently reported," namely benzyl alcohol and benzyl salicylate. A comparison of the classifications is given in Table 22.3. Differences in classification according to the two sources are highlighted in bold.

It is important to focus in some more depth on the allergenic potential of the natural ingredients, oakmoss and tree moss. In contrast to oakmoss, which is known to be a potent sensitizer since a long time ago, tree moss had not been systematically tested in cosmetic patch test series in the past, and the study by Schnuch et al. (2007) is claimed to be the first study in which tree moss was tested in a larger population. In this study, tree moss was found to be the most frequent allergen. Earlier study reports had already identified atranol and chloroatranol (degradation products of atranorin and chloroatranorin) as the most potent allergens (Johansen et al., 2003, 2006). The chemical structures of atranol and chloroatranol are depicted in Figure 22.3.

TABLE 22.3
Classification of Alleged Allergens According to SC Opinion (Frequently or Less Frequently Reported)

Name	Frequency (SCCNFP/0017/98) (Except for the Mosses (SCCNFP opinion of October 24, 2000; SCCP (SCCP/00847/04))	Group (Schnuch et al., 2007)	Frequency of Sensitization[a]
Tree moss extract 1	SCCNFP/0202/99 and SCCP/00847/04: potent	Group I	2.4
Oakmoss extract 1	SCCNFP/0202/99 and SCCP/00847/04: potent	Group I	2.0
Isoeugenol	Frequently reported	Group I	1.1
Cinnamal	Frequently reported	Group I	1.0
Farnesol	**Less frequently reported**	Group I	0.9
Cinnamyl alcohol	Frequently reported	Group II	0.6
Citral	Frequently reported	Group II	0.6
Citronellol	**Less frequently reported**	Group II	0.5
Eugenol	Frequently reported	Group II	0.4
Coumarin	Frequently reported	Group II	0.4
Geraniol	Frequently reported	Group II	0.4
Benzyl cinnamate	**Less frequently reported**	Group II	0.3
Benzyl alcohol	**Frequently reported**	Group III	0.3
Linalool	Less frequently reported	Group III	0.2
Benzyl salicylate	**Frequently reported**	Group III	0.1
d-Limonene	Less frequently reported	Group III	0.1
Anisyl alcohol	Less frequently reported	Group III	0.0
Benzyl benzoate	Less frequently reported	Group III	0.0

Source: SCCNFP opinions versus the publication by Schnuch et al., 2007.

[a] Frequency of sensitization (%) = *n* test/*n* pos. (standardized for age and sex).

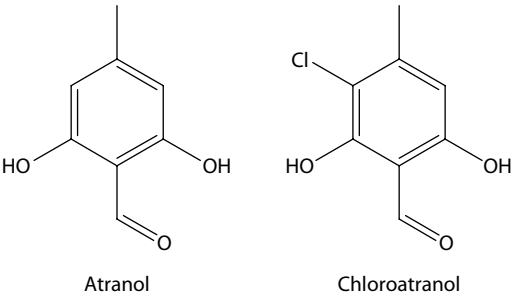


FIGURE 22.3 Structures of atranol and chloroatranol, most potent allergens in oakmoss and tree moss.

This also seems to be in line with the opinion of the SCCP (SCCP/00847/04) on atranol and chloroatranol present in natural extracts (e.g., oakmoss and tree moss extract) where both constituents were regarded as very potent allergens. Because chloroatranol was shown to cause elicitation of reactions by repeated open exposure at the ppm level (0.0005%) and at the ppb level on patch testing (50% elicit at 0.000015%), the SCCP concluded that "chloro-atranol and atranol should not be present in cosmetic products."

As a result, today the Fragrance Industry is producing oakmoss and tree moss with reduced levels of atranol and chloroatranol (i.e., oakmoss and tree moss absolutes treated for the selective removal of atranol and chloroatranol).

The authors concluded in their paper that the study again emphasizes the need for a "different look" on fragrances as contact allergens, which is a confirmation of previous findings (Schnuch et al., 2002). The authors propose a differentiated evaluation of ingredients of each group for overall evaluation, considering not only frequency of sensitization, but also the amount of exposure or use, as well as allergenic potency—also exposure to (highly) oxidized materials could be taken into account.

In particular, for Group I substances the authors agree that a regulation in terms of restrictions (or even ban) and labeling is needed, whereas for Group II substances labeling alone may be adequate enough for the purpose of prevention. But according to the authors, for some of the ingredients of Group III, neither restrictions nor labeling seems justified.

Thus, the authors express their opinion, justified by the findings of this study, that the Commission Decision on the labeling of all 26 "alleged allergens" should be revised.

The Fragrance Industry in turn has taken note of this study and comes to the same conclusion. Based on this, the Industry would now like to propose a pragmatic and different approach for the three Groups of "alleged fragrance ingredients": for example for Group III materials, Industry would advocate no labeling requirements and only restrictions where needed based on scientifically justified concern and for Group II and Group I materials, the Industry would propose appropriate and adequate measures based on scientific data. The Industry would like to avoid overregulation and overlabeling for alleged sensitizers.

Also the Commission took note of the publication of this study and as a consequence DG Enterprise sent a mandate to the SCCP with a request for an updated scientific opinion on the fragrance substances hydroxycitronellal (CAS 107-75-5), isoeugenol (CAS 97-54-1), and *d*-limonene (CAS 5989-27-5).

Currently, the presence of hydroxycitronellal and isoeugenol needs to be labeled in the final cosmetic product according to Annex III, Part 1 of the Cosmetic Directive (Entries 72 and 73, respectively). However, in the future, restrictions to these fragrance ingredients may be proposed, because the Commission is considering a maximum concentration of 1.0% of hydroxycitronellal and of 0.02% of *cis*- and *trans*-isoeugenol (or their sum) in finished cosmetic products (except oral care products). DG Enterprise has asked SCCP its opinion whether they consider these concentrations to be safe for

consumers when used in cosmetic products taking into account the scientific data provided. In fact such restrictions would be more in line with the self-regulating policy and principles of the Fragrance Industry, as applied through the IFRA CoP and its Standards, as explained above.

Regarding limonene, DG Enterprise has asked SCCP to re-evaluate the level of peroxides for the limonenes in cosmetic products. In parallel, the Fragrance Industry through the Research Institute for Fragrance Materials (RIFM) is conducting some local lymph node assay (LLNA) work on limonene and some other key materials for a better scientific substantiation of the maximum peroxide level. RIFM is planning to test limonene with different (low) levels of peroxide to determine the EC3 value (equivalent to the human NOEL). This is a project with the University of Göteburg, Sweden (Professor. A.-T. Karlberg). Some of the goals of this research are to investigate the fundamental scientific basis of the auto-oxidation of four important structurally related fragrance ingredients (e.g., limonene) and one essential oil; to look more closely at the sensitization potential of limonene (and hence to challenge the current sensitization hazard classification of R43 of limonene, which itself is not a sensitizer, and essential oils rich in limonene such as orange oil); and to challenge the sensitization hazard classification of other essential oils, containing another important fragrance ingredient, labeled as allergen, namely linalool. This is possible if it can be demonstrated that linalool oxidizes differently in an essential oil as compared to the pure compound.

The impact of hazard classification (e.g., R43 Risk Phrase) of fragrance ingredients on the classification of essential oils containing them will be discussed in more detail in the section on Hazard Classification and Labeling.

The impact of allergen labeling requirements on essential oils is very important and a different approach of the regulators toward labeling based on the new scientific data available could be very high. If for example no further labeling requirements would apply to Group III materials (which include the following six naturally occurring substances: benzyl alcohol, linalool, benzyl salicylate, *d*-limonene, anisyl alcohol, and benzyl benzoate), the number of affected natural raw materials (extract and essential oils) would be reduced from about 180 to only 80 extracts/essential oils that would need to be taken into account for labeling purposes (see Annex 22.2 to this chapter), according to the EFFA CoP. This would also have a favorable impact on the analytical burden: much less essential oils would have to be analyzed for the presence and concentration of the allergens; also the number of target analytes (allergens) would be reduced from 16 to 10 naturally occurring ones.

The issue on allergen labeling can also have detrimental business impact as some customers (clients) of the fragrance industry (being the cosmetic and detergent industry) are requesting fragrances (i.e., perfume mixtures and preparations) that are "allergen-free." This would mean that the suppliers of essential oils would need to produce "allergen-free" essential oils and extracts, that is, natural materials that do not contain any of the 16 naturally occurring "alleged allergens." This is of course practically impossible. Moreover, generally producing extracts and essential oils without or even with reduced levels of the 16 naturally occurring "allergens" (which are very important fragrance constituents by themselves), for example, by selectively removing them, would have a very high and negative impact on the organoleptic and sensory properties of the essential oils and hence on the fine fragrances and perfumes containing them. As mentioned above, only in particular cases (e.g., for oakmoss and tree moss extracts, which are of high importance to the perfumer but also very potent allergens) the Industry is successfully producing new qualities with reduced levels of allergens (*in casu* atranol and chlorotranol) to reduce the sensitization potential of the mosses. It is worthwhile to mention here that a considerable number of IFRA Standards (IFRA prohibited materials) are part of the Cosmetics Directive (banned materials under Annex II of the Cosmetics Directive).

22.2.5 First Amendment of the Detergent Regulation and Allergen Labeling

In line with the 7th Amendment of the Cosmetic Directive 76/768/EEC as just discussed above in the previous paragraph, the first amendment of the Detergent Regulation (from June 2006) makes

the labeling of the 26 alleged "allergenic" materials mandatory: the presence of these materials above the given threshold has to be declared irrespective of the way they are added (as such or as being part of "complex ingredients" such as essential oils).

This first amendment is the Commission Regulation (EC) No. 907/2006 (20/06/06) amending Regulation (EC) No. 648/2004 on detergents. The recital (whereas) (4) of this regulation states the following:

> *(4) There is a requirement to declare allergenic fragrances if they are added in the form of pure substances. However there is no requirement to declare them if they are added as constituents of complex ingredients such as essential oils or perfumes. To ensure better transparency to the consumer, allergenic fragrances in detergents should be declared irrespective of the way they are added to the detergent.*

The threshold for labeling is defined as 0.01% by weight (100 ppm), according to the adaptation of Annex VII (for labeling and ingredient data sheet) as follows:

> *If added at concentrations exceeding 0.01% by weight, the allergenic fragrances that appear on the list of substances in Annex III, Part 1 to Directive 76/768/EEC, as a result of its amendment by Directive 2003/15/EC of the European Parliament and of the Council to include the allergenic perfume ingredients from the list first established by the Scientific Committee on Cosmetics and Non-food Products (SCCNFP) in its opinion SCCNFP/0017/98, shall be listed using the nomenclature of that Directive, as shall any other allergenic fragrances that are subsequently added to Annex III, Part 1 to Directive 76/768/EEC by adaptation of that Annex to technical progress.*

This text for Annex VII is written in such a way to ensure that the presence of the 26 alleged fragrance materials above the given threshold has to be declared irrespective of the way they are added (i.e., as such or as being part of "complex ingredients" such as essential oils).

In that way according to the first amendment of the Detergent Regulation, the same rules apply for detergents as for cosmetic end products for the requirements of allergen labeling.

22.3 CURRENT FLAVOURING DIRECTIVE AND FUTURE FLAVOURING REGULATION: IMPACT ON ESSENTIAL OILS

In the European Union for flavorings, the current Flavouring Directive 88/388/EC still applies. This is the Council Directive of June 22, 1988, on the approximation of the laws of the MS relating to flavorings for use in foodstuffs and to source materials for their production, as published in the Official Journal on 15/07/88 (OJ L 184, p. 61). It has been amended once by the Commission Directive 91/71/EEC of 16/01/91 (OJ L 42, p. 25, 15/02/91). As this is a Directive, it is up to the EU MS to take the necessary measures to ensure that flavorings may not be marketed or used if they do not comply with the rules laid down in this Directive, as stated in Art. 3 of this Directive.

However since recent years (around 2002) a Proposal for a new Regulation of the European Parliament and of the Council on flavorings and certain food ingredients with flavoring properties for use in and on foods is under discussion. The last version of the Commission Proposal that was the basis for further discussions and Amendments from EU Parliament was issued in July 2006. As many essential oils and extracts either contain flavoring substances or are regarded as "food ingredients with flavoring properties," this new Flavouring Regulation will have an impact on essential oils and their use as flavoring ingredients for food products. Extracts and essential oils contain certain constituents (substances) that according to this regulation "should not be added as such to food" or to which maximum levels apply. They are often referred to as "biologically active substances" or "active principles." Especially the application of maximum levels of these substances will have an impact on how and when extracts, essential oils but also herbs and spices may or can be applied to food. Also the definitions for "natural" have drastically changed. The difference

between the current Directive 88/388/EC and the future Flavour Regulation will be outlined in the next paragraphs.

22.3.1 Current Flavouring Directive 88/388/EC

22.3.1.1 Maximum Levels of "Biologically Active Substances"

In the current Flavouring Directive 88/388/EC, Annex II sets maximum levels (limits) for certain substances obtained from flavorings and other food ingredients with flavoring properties in foodstuffs as consumed in which flavorings have been used. Art. 4 (c) stipulates that

> *(c) the use of flavourings and of other food ingredients with flavouring properties does not result in the presence of substances listed in Annex II in quantities greater than those specified therein.*

The limits apply to Foodstuffs and Beverages (mg/kg) — exceptions apply: for example, alcoholic beverages and confectionaries. In Table 22.4, the maximum levels (without the exceptions) for these substances for foodstuffs in general and beverages are given. A more detailed table (with all the exceptions) is given in Annex 22.3 to this chapter.

This means that for essential oils, extracts, complex mixtures containing these "biologically active substances" (e.g., nutmeg, cinnamon, peppermint, and sage oils) and when added to food and flavorings, maximum levels apply. The same applies to herbs and spices containing these "biologically active substances" as herbs and spices are also "food ingredients with flavoring properties."

22.3.1.2 Definition of "Natural"

Also important is how the current Flavouring Directive addresses "naturalness" of flavors and how "natural" is defined for the purpose of labeling. This is stipulated by Art. 9a.2 (amending the original Art. 9.2 of 88/388/EC by 91/71/EEC):

> *2. the word 'natural', or any other word having substantially the same meaning, may be used only for flavourings in which the flavouring component contains exclusively flavouring substances as defined in Article 1 (2) (b) (i) and/or flavouring preparations as defined in Article 1 (2) (c). If the sales description of the flavourings contains a reference to a foodstuff or a flavouring source, the word 'natural' or any other word having substantially the same meaning, may not be used unless the flavouring component has been isolated by appropriate physical processes, enzymatic or microbiological processes or traditional food-preparation processes solely or almost solely from the foodstuff or the flavouring source concerned.*

TABLE 22.4
Annex II of 88/388/EC—Maximum Levels (mg/kg) for Certain Substances in Foodstuffs and Beverages

Substance	Foodstuffs and Beverage	Substance	Foodstuffs	Beverages
Agaric acid	20	Aloin	0.1	0.1
β-Asarone	0.1	Berberine	0.1	0.1
Coumarin	2	Hydrocyanic acid	1	1
Hypericine	0.1	Pulegone	25	100
Quassine	5	Safrole and isosafrole	1	1
Santonin	0.1	Thujone (α and β)	0.5	0.5

How a "natural flavoring substance" can be obtained is thus defined in Art. 1.2 (b) (i):

(b) 'flavouring substance' means a defined chemical substance with flavouring properties which is obtained:
(i) by appropriate physical processes (including distillation and solvent extraction) or enzymatic or microbiological processes from material of vegetable or animal origin either in the raw state or after processing for human consumption by traditional food-preparation processes (including drying, torrefaction and fermentation),

How a "flavoring preparation" can be obtained is defined in Art. 1.2 (c):

(c) 'flavouring preparation' means a product, other than the substances defined in (b) (i), whether concentrated or not, with flavouring properties, which is obtained by appropriate physical processes (including distillation and solvent extraction) or by enzymatic or microbiological processes from material of vegetable or animal origin, either in the raw state or after processing for human consumption by traditional food-preparation processes (including drying, torrefaction and fermentation);

The above means that a "flavoring preparation" is by default always "natural" and that extracts and essential oils (obtained by appropriate physical processes such as distillation and solvent extraction) from material of vegetable origin (e.g., plant material) can be considered as "flavoring preparation" and thus "natural."

22.3.2 Future Flavouring Regulation

As mentioned above, a Proposal for a new Flavouring Regulation is under discussion since the last years at three levels: the EU Commission, the EU Parliament, and the Council (MS-level). The full title of this proposal is *Proposal for a Regulation of the European Parliament and of the Council on flavourings and certain food ingredients with flavouring properties for use in foods and amending Council Regulation (EEC) No. 1576/89, Council Regulation (EEC) No. 1601/91, Regulation (EC) No. 2232/96 and Directive 2000/13/EC*. The last (amended) proposal from the Commission dates from 24/10/2007 (Commission Directive 91/71/EEC).

With this new Regulation, the former Council Directive 88/388/EEC of June 22, 1988, as well as its amendment Directive 91/71/EEC and the Commission Decision 88/389/EEC will be repealed.

This Flavouring Regulation is part of a larger package, called the "Food Improvement Agents Package" (FIAP), comprising the Flavouring, Additives and Enzymes Regulation and the Common Authorisation Procedure. The drafting of the entire package started at Commission level, has undergone a tremendous amount of amendments, as issued and adopted by the European Parliament, and was at the time of the preparation of the manuscript for this chapter under discussion with three parties: the EU Commission, the EU Parliament, and the Council under Portuguese Presidency. The last chance for the parties to come to a political agreement and to reach a common position under the first Reading was in December 2007. However, a common position could not be reached by the end of 2007 and there was a second Reading (Plenary session) in July 2008: under Slovenian Presidency.

For a long time (at the time of the preparation of the manuscript of this chapter) there was not one final document but two major draft versions: the last amended Commission Proposal of 24/10/2007 (Commission Directive 91/71/EEC) and the last Council Proposal for Political Agreement of 10/12/2007 (Commission Directive 93/21/EEC), which were the basis for discussion for the Council meeting (Agriculture and Fisheries) on December 17–18, 2007. The major differences between the two versions (Commission Proposal and Council Proposal) available at the end of 2007 (at the time of the preparation of this manuscript), and the impact on essential oils will be outlined below. As a final Proposal of the European Parliament and the Council had just come available shortly before submission of this manuscript for publication (Council Proposal, July 15, 2008), also this final

Council Proposal will be discussed briefly, in order to be as much as possible up-to-date. Meanwhile at the time of the publication of this book, the final version of the new Flavouring Regulation has been published in the Official Journal on 31 December 2008 (OJ L 354, 31.12.2008, p. 34): Regulation (EC) No 1334/2008. In essence this Regulation as published is the same as the final Council Proposal which was published on July 15, 2008. It has entered into force on January 20, 2009 and will apply as from January 20, 2011. As of this application date, the current Flavouring Directive 88/388/EEC will be repealed.

22.3.2.1 Maximum Levels of "Biologically Active Substances"

Apart from the fact that the current Directive 88/388/EC will turn into a Regulation, there are many changes that will have an impact on how essential oils and extracts will be used as source of flavors.

The most important issue is how the so-called biologically active substances are addressed.

This is addressed by Art. 5 of the Draft Council Proposal (Art. 6 of Commission Proposal): "Presence of certain substances," which refers to Annex III with the same title. Both Council and Commission proposals clearly state in the first paragraph that "Substances listed in Part A of Annex III shall *not* be added *as such* to food."

However, when it comes to the levels of these substances coming from the use of flavorings and food ingredients with flavoring properties (such as extracts, essential oils, herbs, and spices), the Commission and Council proposals differ slightly.

Art. 6.2 in the Commission Proposal reads as follows:

> *2. Maximum levels of certain substances, naturally present in flavourings and food ingredients with flavouring properties, in the compound foods listed in Part B of Annex III shall not be exceeded as a result of the use of flavourings and food ingredients with flavouring properties in and on those foods.*
>
> *The maximum levels shall apply to the compound foods as offered ready for consumption or as prepared according to the instructions of the manufacturer.*

Art. 5.2 in the Council Proposal (December 10, 2007) reads as follows:

> *2. Without prejudice to Council Regulation No. 1576/89 maximum levels of certain substances, naturally present in flavourings and/or food ingredients with flavouring properties, in the compound foods listed in Part B of Annex III shall not be exceeded as a result of the use of flavourings and/or food ingredients with flavouring properties in and on those foods.*
>
> *The maximum levels of the substances set out in Annex III apply to foods as marketed, unless otherwise stated. By way of derogation from this principle, for dried and/or concentrated foods which need to be reconstituted the maximum levels apply to the food as reconstituted according to the instructions on the label, taking into account the minimum dilution factor.*

The wording in the latest Council Proposal of July 15 (Art. 6) is essentially the same as the wording of the Council Proposal of December 10 (Art. 5).

This means that maximum levels of these substances also apply when the substances come from any type of food ingredients with flavoring properties; the only difference between the Commission Proposal and the Council proposals is that in the Council proposals an exception is given to dried and/or concentrated foods that can have higher levels before they are diluted/reconstituted. Upon dilution/reconstitution, the normal maximum levels apply again.

The main difference between the current Flavouring Directive 88/388 and the future Flavouring Regulation is that in the Directive 88/388 there is only one list (Annex II) of substances to which the maximum levels apply—all those substances may not be added *as such* to food. In contrast, in the future Flavouring Regulation, the Annex III is split into two parts: Part A with "Substances which may *not* be added *as such* to food" and Part B establishing "Maximum levels of certain substances, naturally present in flavourings and food ingredients with flavouring properties, in certain compound food as consumed to which flavourings and/or food ingredients with flavouring properties have been added."

TABLE 22.5
Annex III, Part A: Substances that May *Not* be Added *As Such* to Food

Agaric Acid	Aloin	**Capsaicin**
1,2-Benzopyrone, coumarin	**Hypericine**	**β-Asarone**
1-Allyl-4-methoxybenzene, estragole	**Hydrocyanic acid**	**Menthofuran**
4-Allyl-1,2-dimethoxybenzene, methyleugenol	**Pulegone**	**Quassin**
1-Allyl-3,4-methylene dioxy benzene, safrole	**Teucrin A**	**Thujone (α and β)**

Note: Substances in bold are those that are in Part A, Annex III of both the Council and Commission proposals—aloin and coumarin are *not* included in Annex III, Part A of the Commission proposal.

Part A contains 15 substances (according to the Council proposals) or 13 substances (according to the Commission Proposal—aloin and coumarin are not in), whereas Part B contains 11 substances (according to the Council proposals) or 10 substances (according to the Commission Proposal—coumarin is not in).

Table 22.5 lists the Substances of Part A of Annex III "which may not be added as such to food" and Table 22.6 lists the 11 substances of Part B with their respectively maximum levels in the various compound foods according to the Council proposals.

There are some major differences between the Part B of Annex III in the Council proposals and the list in the Commission Proposal:

- As mentioned above, maximum levels for coumarin are only set in the Council proposals and not in the Commission Proposal.
- For Teucrin A different levels are set in the Council proposals for different compound foods, whereas in the Commission Proposal only for one category, namely alcoholic beverages, a maximum level of 2 mg/kg applies.
- Regarding the chemical names, in the Commission Proposal no trivial name is given for 1-allyl-4-methoxybenzene (estragol) and 4-allyl-1,2-dimethoxy-benzene (methyleugenol) in contrast to the Council Proposal (synonyms given).
- But the most important and major difference is the statement in the Council Proposal of December 10 on top of the table, which is *not* in the Commission Proposal, which reads as follows:

"These maximum levels shall not apply to compound foods which are prepared and consumed on the same site, contain no added flavourings and contain only herbs and spices as food ingredients with flavouring properties."

This statement is to allow the unrestricted use of herbs and spices to foods that are prepared and consumed on the same site, for example, restaurants and catering services.

However, in the latest Council Proposal of July 15, this statement has disappeared.

Instead another footnote has been introduced applying to three of the substances that are marked with an asterisk (*): estragol, safrole, and methyl eugenol. This footnote reads as follows (Council Proposal, July 15):

**The maximum levels shall not apply where a compound food contains no added flavourings and the only food ingredients with flavouring properties which have been added are fresh, dried or frozen herbs and spices. After consultation with the Member States and the Authority, based on data made available by the Member States and on the newest scientific information, and taking into account the*

TABLE 22.6
Maximum Levels of Certain Substances, Naturally Present in Flavorings and Food Ingredients with Flavoring Properties, in Certain Compound Foods Consumed to which Flavorings and/or Food Ingredients with Flavoring Properties have been Added

Name of the Substance	Compound Food in which the Presence of the Substance is Restricted	Maximum Level (mg/kg)
β-Asarone	Alcoholic beverages	1.0
1-Allyl-4-methoxybenzene, estragol	Dairy products	50
	Processed fruits, vegetables (including mushrooms, fungi, roots, tubers, pulses, and legumes), nuts, and seeds	50
	Fish products	50
	Nonalcoholic beverages	10
Hydrocyanic acid	Nougat, marzipan, or its substitutes or similar products	50
	Canned stone fruits	5
	Alcoholic beverages	35
Menthofuran	Mint/peppermint containing confectionery, except micro breath freshening confectionery	500
	Micro breath freshening confectionery	3000
	Chewing gum	1000
	Mint/peppermint containing alcoholic beverages	200
4-Allyl-1,2-dimethoxy-benzene, methyleugenol	Dairy products	20
	Meat preparations and meat products, including poultry and game	15
	Fish preparations and fish products	10
	Soups and sauces	60
	Ready-to-eat savouries	20
	Nonalcoholic beverages	1
Pulegone	Mint/peppermint containing confectionery, except micro breath freshening confectionery	250
	Micro breath freshening confectionery	2000
	Chewing gum	350
	Mint/peppermint containing nonalcoholic beverages	20
	Mint/peppermint containing alcoholic beverages	100
Quassin	Nonalcoholic beverages	0.5
	Bakery wares	1
	Alcoholic beverages	1.5
1-Allyl-3,4-methylene dioxy benzene, safrole	Meat preparations and meat products, including poultry and game	15
	Fish preparations and fish products	15
	Soups and sauces	25
	Nonalcoholic beverages	1
Teucrin A	**Bitter-tasting spirit drinks or bitter[a]**	**5**
	Liqueurs[b] with a bitter taste	**5**
	Other alcoholic beverages	**2**
Thujone (α and β)	Alcoholic beverages, except those produced from *Artemisia* species	10
	Alcoholic beverages produced from *Artemisia* species	35
	Nonalcoholic beverages produced from *Artemisia* species	0.5

continued

TABLE 22.6 (continued)
Maximum Levels of Certain Substances, Naturally Present in Flavorings and Food Ingredients with Flavoring Properties, in Certain Compound Foods Consumed to which Flavorings and/or Food Ingredients with Flavoring Properties have been Added

Name of the Substance	Compound Food in which the Presence of the Substance is Restricted	Maximum Level (mg/kg)
COUMARIN	Traditional and/or seasonal bakery ware containing cinnamon in the labeling	50
	"Breakfast cereals" including muesli	20
	Fine bakery ware with exception of traditional and/or seasonal bakery ware containing cinnamon in the labeling	15
	Desserts	5

[a] As defined by Article 1.4 (p) of EC Regulation 1576/89.
[b] As defined by Article 1.4 (r) of EC Regulation 1576/89.

use of herbs and spices and natural flavouring preparations, the Commission, if appropriate, proposes amendments to this derogation.

This means that the maximum levels do not apply to estragol, safrole, and methyl eugenol when only fresh, dried, or frozen herbs and spices are added! However when "food ingredients with flavoring properties" such as essential oils are added, or when essential oils and/or other flavorings are added in combination with herbs and spices, the levels do apply.

It is anticipated that nothing will change anymore in Art. 6 of the Council Proposal of July 15 until the adoption of the final text and that also the Annex III will remain as it is now. However, as stipulated in the footnote under the Annex III (applying to the three substances with an asterisk) amendments to the current derogations for herbs and spices can be expected.

It is also important to note that according to Art. 30 of the Flavouring Regulation (*Entry into Force*), which will only apply 24 months after its Entry into Force, Art. 22 shall apply from the date of its Entry into Force. Art. 22 concerns the Amendments to Annexes II through V. This means that if the Flavouring Regulation would be adopted by the end of 2008 and hence would apply end of 2010, the Annexes can be amended immediately, if necessary.

Whereas the Art. 6 of the Council Proposal of July 15 discussed above (i.e., Art. 6 of the Commission Proposal) relates to "certain substances," Art. 7 of the Council Proposal of July 15 (i.e., Art. 7 of the Commission Proposal) relates to "Use of certain source materials," which is even more important in relation to herbs, spices, extracts, and essential oils. This article refers to Annex IV of the Regulation, which is a new annex that was not in the current Flavouring Directive 88/388/EC entitled "List of source materials to which restrictions apply for their use in the production of flavourings and food ingredients with flavouring properties." This Annex IV consists of two parts:

- Part A: Source materials that shall not be used for the production of flavorings and food ingredients with flavoring properties.
- Part B: Conditions of use for flavorings and food ingredients with flavoring properties produced from certain source materials.

The complete Annex IV according to the current Draft proposals (there is no difference between the Draft Council and Commission proposals regarding the content of Annex IV) is given in Annex 22.4 to this chapter.

Art. 7 of the Council Proposal of July 15 (Art. 7 of the Commission Proposal) stipulates the following:

1. *Source materials listed in Part A of Annex IV shall not be used for the production of flavourings and/or food ingredients with flavouring properties.*
2. *Flavourings and/or food ingredients with flavouring properties produced from source materials listed in Part B of Annex IV may only be used under the conditions indicated in that Annex.*

With the exception of the fact that "and/or" in "flavourings *and/or* food ingredients" in both paragraphs in the Council Proposal is replaced by "and" ("flavourings *and* food ingredients") in the Commission Proposal, the remainder of the article is exactly the same. It is anticipated that nothing will change anymore on this article and the related Annex IV for the final version of the Flavouring Regulation.

22.3.2.2 Definition of "Natural"

Regarding "naturalness" of flavors and how "natural" is defined for the purpose of labeling, the situation has drastically changed since the current Flavouring Directive 88/388/EC.

For example today according to 88/388/EC there are three categories of flavoring substances: natural, nature-identical (NI), and artificial. However, with the new Flavouring Regulation there will be only two categories: natural and not natural, meaning that the difference between the former categories NI and artificial will disappear and these two will merge in one category of "synthetic flavorings."

Also the position of the Council is clearly different from the position of the Commission. In both proposals, "natural flavoring substance" is defined by Art. 3.2 (c).

This definition according to the *Commission* proposals (December 10 and July 15) reads as follows:

> *(c) 'natural flavouring substance' shall mean a flavouring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II.*

This definition according to the Council Proposal reads as follows:

> *(c)'natural flavouring substance' shall mean a flavouring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II; Natural flavouring substances correspond to substances that are naturally present and have been identified in nature;*

Important is the additional line, according to the Council proposals, stating that a substance has to be identified in nature before it can be regarded as "natural," so it is not only sufficient to produce it "in a natural way": it has to be identical to something that is present in nature. This is to avoid the problem that arises when an enzymatic or microbial process is developed by which a flavoring substance can be produced "by enzymatic or microbial processes from material of vegetable origin" (i.e., natural source materials) that up to then has never been identified in nature (and is not naturally occurring), such as ethylvanillin, then such a material would be labeled as a "natural flavoring substance."

More important than the definition of "natural" as such (Art. 3.2 (c)), however, is how "appropriate physical process" is defined.

Annex II to the Flavouring Regulation gives a list of "traditional food preparation processes" by which (natural) flavoring substances and *natural* flavoring preparations are obtained (in the title of

Annex II in the Commission Proposal the wording "*natural* flavouring preparations" is used, which is confusing since flavoring preparations are, as per definition, natural, see below). The full list of *traditional food preparation processes* is given in Annex 22.5 to this chapter.

The definition of "appropriate physical process" is different between the two proposals and is described in Art. 3.2 (k).

This definition according to the *Commission* Proposal reads as follows:

(k) 'appropriate physical process' shall mean a physical process which does not intentionally modify the chemical nature of the components of the flavouring, without prejudice to the listing of traditional food preparation processes in Annex II, and does not involve the use of singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation.

This definition according to the *Council* Proposal (December 10, 2007) reads as follows:

(k) 'appropriate physical process' shall mean a physical process which does not intentionally modify the chemical nature of the components of the flavouring and does not involve among others the use of singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation.

It can be noted that the definition according to the Council Proposal does no longer refer to the processes listed in Annex II. In contrast to the Commission Proposal where all *traditional food preparation processes* as listed in Annex II also fall under the definition of "appropriate physical processes" (*cf.* the wording "without prejudice to the listing of … "), according to the Council Proposal only processes that *do not intentionally modify the chemical nature of the components* of the flavoring are considered to be "appropriate physical processes" in order to obtain a "natural flavouring substance."

This means that distillation and certain extraction techniques that *do* modify the chemical nature of the components are not regarded as "appropriate physical processes" for obtaining natural flavoring substances.

Fortunately thanks to very strong and effective, successful lobbying by the European Flavour Industry, this has been rectified and an amendment to this definition (Art. 3.2 (k)) has been accepted by Council, Commission, and European Parliament.

According to the latest Council Proposal (July 15, 2008), this definition reads as follows:

(k) "appropriate physical process" shall mean a physical process which does not intentionally modify the chemical nature of the components of the flavouring, ***without prejudice to the listing of traditional food preparation processes in Annex II****, and does not involve, inter alia, the use of singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation.*

This definition again refers to the Annex II, which means that all processes listed in Annex II also fall again under the definition of "*appropriate physical processes*."

When looking at the situation for "flavouring preparations" (such as essential oils and extracts), the wording of the Commission Proposal is slightly different than that of the Council Proposal.

According to Art. 16.2 of the Council Proposal (July 15) and Art. 15.2 of the Commission Proposal, the term "natural" may be used for the description of a flavoring if the flavoring component comprises only flavoring preparations, which means that a "flavoring preparation" can be regarded as "natural" by definition. In other words, there is no such thing as a "synthetic flavoring preparation."

The definition for "flavouring preparation" according to the *Commission* Proposal reads as follows (Art. 3.2 (d)):

(d) 'flavouring preparation' shall mean a product, other than a flavouring substance, obtained from:

(i) food by appropriate physical, enzymatic or microbiological processes either in the raw state of the material or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II and/or appropriate physical processes;

and/or

> *(ii) material of vegetable, animal or microbiological origin, other than food, obtained by one or more of the traditional food preparation processes listed in Annex II and/or appropriate physical, enzymatic or microbiological processes;*

The definition for "flavouring preparation" according to the *Council* proposals (versions December 10 and July 15 being identical) reads as follows (Art. 3.2(d)):

> *(d) 'flavouring preparation' shall mean a product, other than a flavouring substance, obtained from:*
>
> *(i) food by appropriate physical, enzymatic or microbiological processes either in the raw state of the material or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II;*

and/or

> *(ii) material of vegetable, animal or microbiological origin, other than food, by appropriate physical, enzymatic or microbiological processes, the material being taken as such or prepared by one or more of the traditional food preparation processes listed in Annex II;*

Although the wording of Commission and Council differ on this definition, it could be concluded that according to the latter definition (according to Council Proposal) essential oils and extracts obtained from plant material (*material of vegetable origin*) prepared by distillation (which is a *traditional food preparation process* listed in Annex II) followed by an *appropriate physical process* can be considered as a "flavoring preparation" and thus natural as long as the chemical nature of the components is not intentionally modified during the physical process.

However, it can also be argued that if the physical process after the distillation (e.g., extraction, drying, evaporation, condensation, dilution, etc.) intentionally modifies the chemistry of the components (which is often the case), then the end product can no longer be regarded as *flavoring preparation* and thus natural, according to the Council Proposal.

In that respect, the wording of the Commission Proposal (part (ii)) is much broader and less ambiguous and will not lead to these restrictions. According to the Commission Proposal, an extract, essential oil, absolute or concrete, … obtained from material of vegetable origin (flower, root, fruit, etc.) by one or more of the processes listed in Annex II complies with the definition of "flavoring preparation" and can thus be regarded as a "natural."

However, it is anticipated that the latest version, being the Council Proposal of July 15, 2008, is the blueprint of the final Flavouring Regulation, and will be the text as it will most probably be adopted by the end of the year and published. Entry into force of the new EU Flavouring Regulation will be on the twentieth day following that of its publication in the Official Journal of the European Union. It is anticipated that this will be at the earliest by the end of 2008 or the beginning of 2009. As stated above, it shall apply 24 months after the entry into force of this Regulation.

22.4 HAZARD CLASSIFICATION AND LABELING OF FLAVORS AND FRAGRANCES

This section describes the rules for hazard classification and labeling of F&F substances and preparations, including natural raw materials, such as extracts and essential oils, containing hazardous constituents, according to the EU regulations.

For trade of F&F (including pure substances and mixtures or preparations thereof and natural raw materials) within the European Union, certain rules apply within the European Industry which are established by the European Flavour and Fragrance Association Code of Practice (EFFA CoP). The following general considerations are taken over from the Introductory note to the EFFA CoP, which is published yearly on the EFFA website: www.effa.be. It should be noted that the most recent

version of the EFFA CoP (version of 2009) for the first time also takes into account the Globally Harmonized System of Classification and Labeling of Chemicals (UN-GHS). This GHS has now also been implemented in the EU with the Publication of the EU-GHS Regulation (so-called "CLP Regulation": Classification, Labelling and Packaging of substances and mixtures) [Regulation (EC) No. 1336/2008, OJ L 354, 31.12.2008, p. 60]. It has entered into force on 20 January 2009 and the current Directive 67/548/EEC (DSD) and Directive 1999/45/EC (DPD) shall be repealed with effect from 1 June 2015. However, Annex I of the DSD has already been repealed and transferred into Annex VI of the EU-CLP Regulation, with exception of the last two technical adaptations (ATP 30 and ATP 31) to the DSD.

However, the following section on classification on labeling is still based on the currently applicable DSD and DPD.

Within the European Union, substances and preparations have to be classified and, if dangerous according to criteria laid down in the regulations, have to be labeled according to certain rules. The classification and labeling of substances are either prescribed in Annex I to the Dangerous Substances Directive 67/548/EEC (DSD) or have to be done by the supplier using the criteria of Annex VI of this Directive. For preparations, like F&F compounds, it is done according to the Dangerous Preparations Directive 1999/45/EC (DPD).

Several substances of interest to the fragrance and flavor industry are mentioned in Annex I of the DSD. They are included in the respective attachments to the EFFA CoP with their Annex I number next to their CAS and EU numbers. The label mentioned in the attachment has to be used in the MS of the European Union.

Special emphasis is put on Classification of aspiration hazard (Xn; R65) of both substances that can easily reach the lungs upon ingestion and cause lung damage (substances with low viscosity and low surface tension) and mixtures/preparations with a high hydrocarbon (HC) content and low kinematic viscosity that will pose the same hazard.

Based on measurement results for a number of natural raw materials (e.g., extracts and essential oils) with HC contents between 10% and 90+% and on similar measurements of some F&F compounds, a dedicated Working Group of the F&F Industry has come to the conclusion that in practice, substances and preparations containing more than 10% of HC(s) fall within the criteria for viscosity and surface tension.

Therefore, the European F&F Industry through its EFFA CoP recommends

- To determine the HC content of substances (supplier information, analysis) and preparations (including extracts and essential oils) (calculation) and to classify as Xn; R65 if more than 10% HC is present.
- That nonclassification should only be possible if viscosity and/or surface tension measurement results are available for a specific substance or preparation (including extracts and essential oils).

In addition, classification and labeling of skin sensitizers is addressed in the CoP: the issue on skin sensitization (and labeling of the alleged allergens for the purpose of the Cosmetic Directive, 7th Amendment) has been in depth discussed in the first section. However, it should be noted here that classification of substances and essential oils or extracts as sensitizers (R43) has nothing to do with the requirement to label the 26 alleged allergens on the final cosmetic products according to the Cosmetic Directive (7th Amendment).

Following the EFFA CoP, skin sensitizers are labeled Xi, R43. According to the CoP, it is recommended to use the administrative limit concentration of 1% when classifying preparations (including extracts and essential oils) containing them in all cases, unless a different threshold is laid down in Annex I to the DSD.

In the EFFA CoP, special attention is paid to the hazard classification and labeling of natural raw materials, referred to as "natural complex substances" (NCSs) in the CoP. The terminology *Natural*

Complex Substance is used because in some cases the natural raw material (the complex) is regarded as a single substance, rather than a complex mixture.

NCSs (e.g., essential oils, and extracts from botanical and animal sources) require special procedures due to the fact that they might have quite different chemical compositions (and therefore hazard classifications) under the same designation. This may occur even when this differentiates between species, cultivars and chemotypes and different production procedures (e.g., absolutes, resinoids, and distilled oils).

There are two ways of classifying and labeling NCSs such as extracts and essential oils: either based on the data known and available on the natural raw material as such (NCS is regarded as a single "substance") or based on the hazardous constituents they are composed of (NCS is regarded as a complex mixture).

In the first case, an NCS may be classified on the basis of the data obtained by testing the NCS. The test results of an NCS, even if containing classified constituents, are evaluated in accordance with the DSD. The health and environmental hazard classifications derived following this approach are quality dependent, which is also indicated in the EFFA CoP.

In the second case, for grades of NCSs and for endpoints for which reliable test data are lacking, the EU's Labelling Guide (Annex VI to the DSD) incorporates a requirement introduced by Commission Directive 93/21/EEC, whereby the hazard classification of complex substances shall be evaluated on the basis of levels of their known chemical constituents. Where knowledge about constituents exists, for example, on substances limited as per Annex II of Directive 88/388/EC (the so-called biologically active substances—see above) or on substances with sensitizing, toxic, harmful, corrosive, and environmentally hazardous properties, the classification and labeling of these NCSs according to the requirements of the European Union should follow the rules for preparations (= mixtures) as prescribed by the DPD.

One dedicated section of the EFFA CoP also provides a list with the composition of the NCSs (extracts, essential oils, concretes, absolutes, etc.) in terms of the presence (content in %) of hazardous constituents and HCs in the NCSs that have to be taken into account for the classification and labeling of the NCSs or a preparation containing these NCSs, based on the DPD.

F&F compounds that are preparations (i.e., compounded mixtures, formulations, or compositions) should be classified and labeled according to the EU's DPD 1999/45/EC and its articles 6 and 7.

In practice, test data on the flavor or fragrance compounds (preparations) are not available or collected. Therefore the classification of these preparations should be based on the chemical composition and should include the contributions of hazardous substances present as constituents in the NCSs present in the formulation. This is another reason why the composition of the NCSs in terms of presence of the hazardous constituents is also part of the EFFA CoP.

Examples of important constituents to take into account for classification:

- Sensitizers (R43) → NCSs (essential oils and extracts) to be classified as R43 if the content (%) of the sensitizer (if one) or the content of their sum (if more than one) is greater than or equal to 1%.
- CMRs (carcinogenic, mutagenic, and reprotoxic materials: R45; R46; R68) → NCSs to be classified as CMR if the content of the CMR substance(s) is greater than or equal to 0.1%.

The final classification and labeling of an essential oil can be totally different depending on the approach used for the classification, either based on data on the essential oil as such (the first case described above) or based on the hazardous constituents in the essential oil (the second case described above). This is illustrated below with two examples: orange oil, containing mainly limonene [which is classified as both a sensitizer (R43) and very toxic for the environment (R50/53)], and nutmeg oil, containing safrole which is a CMR (T; R45-22-68).

TABLE 22.7
Composition of Orange Oil with Main Hazardous Constituents

Constituent	Concentration (%)	Classification
d-Limonene	96.2	R10-38-43-50/53
Linalool	0.5	Not classified
Citral	0.2	R38-43

Orange oil: Table 22.7 below depicts the composition of orange oil with the classification of the main constituents.

Resulting classification of orange oil:

- Classification and labeling "as a single substance" (based on data on the oil as such):

Xn	Harmful
R10	Flammable
R65	Harmful: may cause lung damage if swallowed

- Classification based on its constituents:

R10	Flammable
R38	**Irritating to skin**
R43	**May cause sensitization by skin contact**
R50/53	**Very toxic to aquatic organisms (environment)**
R65	Harmful: may cause lung damage if swallowed

- Labeling (pictograms) based on its constituents:

Xn: Harmful 

N: dangerous for the environment 

Nutmeg oil: Table 22.8 below depicts the composition of nutmeg oil with the classification of the main constituents.

Resulting classification of nutmeg oil:

- Classification and labeling "as a single substance" (based on data on the oil as such):

Xn	Harmful
R10	Flammable
R65	Harmful: may cause lung damage if swallowed

TABLE 22.8
Composition of Nutmeg Oil with Main Hazardous Constituents

Constituent	Concentration (%)	Labeling and Classification
Pinenes	40	Xi; R43, N; R50/53
Limonene	7	Xi; R38-43, N; R50/53
Safrole	2	T; R45, Xn; R22-68
Isoeugenol	1	Xn; R21/22, Xi; R36/38-43

- Classification based on its constituents:

R10	Flammable
R43	**May cause sensitization by skin contact**
R45	**May cause cancer**
R50/53	**Very toxic to aquatic organisms (environment)**
R65	Harmful: may cause lung damage if swallowed
R68	**Possible risk of irreversible effects**

- Labeling (pictograms) based on its constituents:

T: Toxic (CMR) 

N: Dangerous for the environment 

So with these examples, it can be demonstrated that the final Classification and Labeling (C&L) of essential oils will change significantly depending on the approach used: based on existing data (for the various endpoints) on the essential oil as such or based on the hazardous constituents. It should however be underlined that according to the rules of the EFFA CoP, C&L of natural raw materials or NCSs can only be done for the endpoints for which data (on the NCS as such) are available (e.g., skin irritation, sensitization, environmental toxicity, etc.)—if no data are available, then the constituents must be taken into account for the classification for these endpoints.

22.5 CONCLUSION

As described and outlined above, several new European Regulations and Directives have been adopted during the last years, and other regulations are currently under discussion and will soon enter into force in relation to flavors and fragrances and cosmetics. NCSs or raw materials (such as essential oils and extracts) are very important ingredients for flavoring and fragrance applications. As a result, these new regulations will have a major impact on the trade and use in commerce of these essential oils and extracts, in particular on labeling issues, as has been demonstrated with the 7th Amendment of the Cosmetic Directive (labeling of alleged allergens) and the coming new Flavouring Regulation (labeling of "natural"). The labeling issue is especially important because of its impact on consumer behavior: consumers do not want to buy cosmetic end products that are labeled with potentially allergenic ingredients. Overlabeling should always be avoided. Therefore it is essential to lobby for a pragmatic approach toward allergen labeling and to advocate no labeling requirements for fragrance ingredients that are proven to be (extremely) rare sensitizers or no sensitizers at all, especially if these are naturally occurring constituents of a wide variety of essential oils and extracts. With respect to food, consumers prefer natural flavorings to synthetic ones. Good and pragmatic definitions in the Flavouring Regulation that will soon replace the current Flavouring Directive are essential to ensure that all natural raw materials such as essential oils and extracts can be labeled as natural.

ANNEX 22.1
Aromatic Natural Raw Materials Containing Any of the 16 Naturally Occurring Alleged Aensitizers

Aromatic Natural Raw Materials	Type	Benzyl Alcohol	Benzyl Salicylate	Cinnamyl Alcohol	Cinnamal	Citral	Coumarin	Eugenol	Geraniol	Isoeugenol	Anisyl Alcohol	Benzyl Benzoate	Benzyl Cinnamate	Citronellol	Farnesol	Limonene	Linalool	Total %
Ambrette		*	*	*	*	*	*	*	*	*	*	*	*	*	5	*	1	6
Angelica root		*	*	*	*	*	*	*	*	*	*	*	*	*	*	18	0.3	18.3
Angelica seed		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Star Anise		*	*	*	*	*	*	*	*	*	*	*	*	*	*	3	1.5	4.5
Anise		*	*	*	*	*	*	*	*	*	*	*	*	*	*	2	0.1	2.1
Armoise		*	*	*	*	*	*	*	*	*	*	*	*	0.2	*	2	*	2.2
Basil	Linalol	*	*	*	*	*	*	15	0.2	*	*	*	*	0.3	*	1	62	78.5
Basil	Me-chavicol	*	*	*	*	*	*	0.5	*	*	*	*	*	*	*	1	1.1	2.6
Bay		*	*	*	*	*	*	56	*	*	*	*	*	*	*	4	3	63
Benzoin	note 1	*	*	*	*	*	*	*	*	*	*	0.2	0.8	*	*	*	*	1
Bergamot (s) cold press		*	*	*	*	0.7	*	*	*	*	*	*	*	*	*	45	15	60.7
Bergamot bergapten-free		*	*	*	*	0.7	*	*	*	*	*	*	*	*	*	45	15	60.7
Bergamot distilled			*	*	*	0.4	*	*	*	*	*	*	*	*	*	40	40	80.4
Bitter orange		*	*	*	*	0.1	*	*	*	*	*	*	*	*	*	95	0.2	95.3
Buchu (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	30	0.5	30.5
Cabreuva		*	*	*	*	*	*	*	*	*	*	*	*	*	3	*	*	3
Cajuput		*	*	*	*	*	*	*	0.4	*	*	*	*	*	*	10	3.6	14
Camphor		*	*	*	*	*	*	*	*	*	*	*	*	*	*	25	0.5	25.5
Cananga		*	3	*	*	*	*	0.7	1.5	*	*	5	*	*	2	*	3	15.2
Caraway		*	*	*	*	*	*	*	*	*	*	*	*	*	*	45	*	45
Cardamom		*	*	*	*	0.6	*	*	1.2	*	*	*	*	*	*	4	4	9.8
Carrot		*	*	*	*	*	*	*	2	*	*	*	*	*	*	3	2	7
Cascarilla		*	*	*	*	*	*	0.3	*	*	*	*	*	*	*	5	5	10.3
Cassia		*	*	1	90	*	4	0.5	*	*	*	1	*	*	*	0.1	*	96.6
Cedarwood (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Celery		*	*	*	*	*	*	*	*	*	*	*	*	*	*	79	0.1	79.1
Chamomile	Roman	*	*	*	*	*	*	*	0.7	*	*	*	*	0.7	*	5	0.8	7.2

Chamomile	Blue	*	*	*	*	*	*	*	*	*	*	*	*	*	1	0.4	1.4
Cinnamon bark		*	*	0.2	75	*	0.3	6	*	*	*	1.5	*	*	*	1	6	90
Cinnamon leaf		*	*	1	3	*	0.3	85	*	*	*	4	*	*	*	*	4	97.3
Cistus (Rockrose)		*	*	*	*	*	*	*	1.2	*	*	*	*	*	*	4	0.5	5.7
Sri Lanka Citronella		*	*	*	*	1.1	*	*	23	*	*	*	*	8.5	*	12	1	45.6
Java Citronella		*	*	*	*	1.3	*	1	25	*	*	*	*	14	1	5	1.5	48.8
Clary Sage (s)		*	*	*	*	*	*	*	2.2	*	*	*	*	*	*	1	24	27.2
Clove (s)		*	*	*	*	*	*	92	*	0.5	*	*	*	*	*	*	*	92.5
Copaiba		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Coriander	fruit	*	*	*	*	*	*	*	3	*	*	*	*	*	*	5	78	86
Coriander	leaf	*	*	*	*	*	*	*	0.4	*	*	*	*	*	*	1	25	26.4
Corn mint	Arvensis	*	*	*	*	*	*	*	*	*	*	*	*	*	*	7	*	7
Cubeb		*	*	*	*	*	*	*	*	*	*	*	*	*	*	1	1.2	2.2
Cypress (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	14	0.8	14.8
Davana		*	*	*	*	*	*	0.1	*	*	*	*	*	*	*	0.1	1	1.2
Dill (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	45	4	49
Elemi		*	*	*	*	*	*	*	*	*	*	*	*	*	*	72	*	72
Estragon	Tarragon	*	*	*	*	*	*	0.2	*	*	*	*	*	*	*	6	*	6.2
Eucalyptus globulus		*	*	*	*	*	*	*	*	*	*	*	*	*	*	8	*	8
Eucalyptus dives		*	*	*	*	*	*	*	*	*	*	*	*	*	*	1	1	2
Eucalyptus citriodora		*	*	*	*	*	*	*	*	*	*	*	*	0.7	*	1	0.3	2
Fennel	Bitter	*	*	*	*	*	*	*	*	*	*	*	*	*	*	35	*	35
Fennel	Sweet	*	*	*	*	*	*	*	*	*	*	*	*	*	*	4	*	4
Galbanum		*	*	*	*	*	*	*	*	*	*	*	*	*	*	3.4	*	3.4
Geranium	N. Africa	*	*	*	*	1.5	*	*	18	*	*	*	*	36	*	1	8.5	65
Geranium	Bourbon	*	*	*	*	1.5	*	*	20	*	*	*	*	26	*	1	11	59.5
Geranium	China	*	*	*	*	1.2	*	*	12	*	*	*	*	43	*	1	4.5	61.7
Ginger (s)		*	*	*	*	0.7	*	*	*	*	*	*	*	*	*	2	0.6	3.3
Grapefruit (s)		*	*	*	*	0.15	*	*	*	*	*	*	*	*	*	95	0.1	95.25
Guaiac wood		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Gurjun		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Hay	note 1	*	*	*	*	*	8	*	*	*	*	*	*	*	*	*	*	8
Ho (s)		*	*	*	*	*	*	*	0.4	*	*	*	*	*	*	0.2	90	90.6
Hop		*	*	*	*	*	*	*	0.2	*	*	*	*	*	*	1	0.6	1.8

continued

ANNEX 22.1 (continued)

Aromatic Natural Raw Materials	Type	Benzyl Alcohol	Benzyl Salicylate	Cinnamyl Alcohol	Cinnamal	Citral	Coumarin	Eugenol	Geraniol	Isoeugenol	Anisyl Alcohol	Benzyl Benzoate	Benzyl Cinnamate	Citronellol	Farnesol	Limonene	Linalool	Total %
Hyssop		*	*	*	*	*	*	*	*	*	*	*	*	*	*	4	*	4
Immortelle		*	*	*	*	*	*	*	*	*	*	*	0.2	*	*	3.8	6	10
Juniper berry		*	*	*	*	*	*	*	*	*	*	*	*	*	*	20	0.2	20.2
Labdanum		*	*	*	*	*	*	*	*	*	*	*	*	*	*	4	*	4
Laurel (s)		*	*	*	*	*	*	2	0.3	*	*	*	*	*	*	5	11	18.3
Lavandin (s)		*	*	*	*	*	0.1	*	0.6	*	*	*	*	*	*	2	38	40.7
Lavandin absolute		*	*	*	*	*	5	*	1.5	*	*	*	*	*	*	1	42	49.5
Lavender absolute		*	*	*	*	*	8	*	1.5	*	*	*	*	*	*	0.3	42	51.8
Lavender (s)		*	*	*	*	*	*	*	1.1	*	*	*	*	*	*	1	45	47.1
Spike lavender		*	*	*	*	*	0.2	0.1	0.2	*	*	*	*	0.3	*	3	50	53.8
Lemon (s)		*	*	*	*	3	*	*	0.2	*	*	*	*	*	*	73	0.3	76.5
Lemongrass (s)		*	*	*	*	90	*	0.3	7	*	*	*	*	0.8	*	4	2	100
Lime (s) distilled		*	*	*	*	3	*	*	0.4	*	*	*	*	*	*	50	0.2	53.6
Lime (s) cold press		*	*	*	*	6.5	*	*	0.4	*	*	*	*	*	*	55	0.2	62.1
Litsea cubeba		*	*	*	*	78	*	*	1.5	*	*	*	*	1.5	*	15	**3**	99
Mace		*	*	*	*	*	*	1.1	*	0.2	*	*	*	*	*	4	0.4	5.7
Mandarin (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	75	0.3	75.3
Marjoram sweet		*	*	*	*	*	*	*	0.3	*	*	*	*	*	*	5	25	30.3
Marjoram wild	Spanish	*	*	*	*	*	*	*	*	*	*	*	*	*	*	6	48	54
Mentha citrata (s)		*	*	*	*	*	*	*	2	*	*	*	*	*	*	1	50	53
Myrrh		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Myrtle		*	*	*	*	*	0.2	0.7	0.8	*	*	*	*	0.3	*	12	2	16
Neroli		*	*	*	*	0.3	*	*	3.5	*	*	*	*	*	4	18	44	69.8
Niaouli		*	*	*	*	*	*	*	*	*	*	*	*	*	*	10	0.2	10.2
Nutmeg		*	*	*	*	*	*	0.6	0.4	1	*	*	*	0.2	*	7	0.4	9.6
Oakmoss	note 1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Olibanum	note 1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	18.5	*	18.5
Opoponax	note 1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0

Sweet orange (s)		*	*	*	*	0.1	*	*	*	*	*	*	*	*	*	95	0.4	95.5
Origanum	Spanish	*	*	*	*	0.5	*	0.3	*	0.3	*	*	*	*	*	1	15	17.1
Palmarosa (s)		*	*	*	*	1	*	*	85	*	*	*	*	*	1.2	1	4	92.2
Parsley (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	5	*	5
Patchouli (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Pepper (s)	Black	*	*	*	*	*	*	*	*	*	*	*	*	*	*	23	1	24
Peppermint	Piperita	*	*	*	*	*	*	*	*	*	*	*	*	*	*	3	0.4	3.4
Peru oil	note 1	4.5	*	*	*	*	*	*	*	*	*	78	9	*	*	*	*	91.5
Bitter orange petitgrain		*	*	*	*	0.3	*	*	4	*	*	*	*	*	*	6	30	40.3
Lemon petitgrain		*	*	*	*	26	*	*	3.5	*	*	*	*	*	*	42	2.5	74
Mandarin petitgrain		*	*	*	*	0.1	*	*	*	*	*	*	*	*	*	11	0.7	11.8
Bergamot petitgrain		*	*	*	*	1	*	*	4	*	*	*	*	*	*	1.5	30	36.5
Petitgrain	Paraguay	*	*	*	*	1	*	*	4.5	*	*	*	*	*	*	2	30	37.5
Pimento (s)		*	*	*	*	*	*	85	*	1	*	*	*	*	*	2	0.5	88.5
Pine (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	6	0.2	6.2
Rose oil	Bulgaria	*	*	*	*	1	*	1.5	22	*	*	*	*	34	1	*	1.4	60.9
Rose oil	Maroc	*	*	*	*	1	*	1.5	47	*	*	*	*	20	1	*	1.4	71.9
Rose oil	China	*	*	*	*	1.5	*	1.5	18.3	*	*	*	*	31	1	*	1.3	54.6
Rose oil (s)	Turkey	*	*	*	*	2	*	1.5	20	*	*	*	*	49	1	*	1.3	74.8
Rosemary (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	6	0.8	6.8
Rosewood		*	*	*	*	*	*	*	2.5	*	*	1.6	*	*	*	1	90	95.1
Sage (s)		*	*	*	*	*	*	*	0.4	*	*	*	*	*	*	5	9	14.4
Sandalwood		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Schinus molle (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	15	0.5	15.5
Spearmint (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	24	0.2	24.2
Spikenard		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Styrax resin	note 1	0.1	*	3.5	*	*	*	*	*	*	*	*	1.5	*	*	*	*	5.1
Styrax oil		*	*	54	1.1	*	*	*	*	*	*	*	*	*	*	*	*	55.1
Tagete (s)		*	*	*	*	*	*	0.2	*	*	*	*	*	3	*	9	0.5	12.7
Tangerine (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	95	0.5	95.5
Tarragon	Estragon	*	*	*	*	*	*	0.2	*	*	*	*	*	*	*	6	*	6.2
Tea tree		*	*	*	*	*	*	*	*	*	*	*	*	*	*	4	*	4
Red thyme	Spanish	*	*	*	*	0.5	*	*	0.5	*	*	*	*	*	*	1	6.5	8.5
Tolu	note 1	0.5	*	0.6	1.7	*	*	0.1	*	*	*	37.5	4.6	*	*	*	*	45

continued

ANNEX 22.1 (continued)

Aromatic Natural Raw Materials	Type	Benzyl Alcohol	Benzyl Salicylate	Cinnamyl Alcohol	Cinnamal	Citral	Coumarin	Eugenol	Geraniol	Isoeugenol	Anisyl Alcohol	Benzyl Benzoate	Benzyl Cinnamate	Citronellol	Farnesol	Limonene	Linalool	Total %
Tonka		*	*	*	*	*	65	*	*	*	*	*	*	*	*	*	*	65
Treemoss	note1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Turpentine (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	7	*	7
Valerian		*	*	*	*	*	*	*	*	*	*	*	*	*	*	2	*	2
Vetiver (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Ylang extra super		0.5	3.5	*	*	*	*	0.5	0.7	0.5	*	6	*	*	2	*	13	26.7
Ylang extra (s)		0.5	4	*	*	*	*	0.5	3	0.5	*	8	*	*	3	*	24	43.5
Ylang I (s)		0.5	4	*	*	*	*	0.5	2.6	0.5	*	9.2	*	*	3	*	19	39.3
Ylang II (s)		0.5	4	*	*	*	*	0.5	2.4	0.5	*	10	*	*	4	*	9.5	31.4
Ylang III (s)		0.5	5	*	*	*	*	0.5	0.8	0.5	*	8.5	*	*	4	*	40	59.8
Legend		* = <0.1%			% = Actual value					ISO standardized essential oils, INCI chemical names used in this list								

Source: Internal communication (2004) regarding former version of EFFA CoP.

ANNEX 22.2
Natural Raw Materials (Natural Complex Substances) Containing the 10 Naturally Occurring Alleged Allergens of Groups I and II According to Schnuch et al. (2007)

Ambrette	Clove stem	Linaloe wood	Rose absolute
Armoise (*A. alba*)	Coriander leaf/herb	*Litsea cubebe*	Rose, Bulg
Armoise (*A. vulgar.*)	Coriander seed	Mace	Rose, China
Artemisia	Deertongue leaf abs	Melissa (lem. balm)	Rose, Maroc
Basil, linal. type	Euc. Citriodora	Mentha citrata	Rose, Turkey
Bay	Flouve	Myrtle	Rosewood
Cabreuva	Geranium, Bourbon	Neroli	Sandalwood Aus. (*S. spicatum*)
Cananga	Geranium, Chin.	Nutmeg	Snakeroot
Cardamon	Geranium, N. Afr.	Orange flower abs	Styrax
Carnation abs	Hay abs	Osmanthus abs	Thyme, wild (*T. serpyllum*)
Carrot seed	Hyacinth absolute	Palmarosa	Tolu abs
Cassia	Labdanum	Peru balsam oil	Tonka abs
Cassie abs (Acacia)	Laurel (Sweet bay)	Peru balsam resinoid	Tuberose abs
Cinnamon bark	Lavandin abs	Petit grain, bergamot	Turmeric
Cinnamon leaf	Lavender	Petit grain, lemon	Verbena abs
Citronella Ceylon	Lavender abs	Petit grain, orange	Verbena oil
Citronella Java	Lemon	Petit grain, Paraguay	Ylang extra sup.
Clary sage	Lemongrass	Pimento berry	Ylang extra, Com. (Mad.)
Clove bud	Lime, dist	Pimento leaf	Ylang, Com., (Mad.)
Clove leaf	Lime, expr.	Rhodinol	

Source: EFFA-CoP.

ANNEX 22.3
Maximum Limits for Certain Substances Obtained from Flavorings and other Food Ingredients with Flavoring Properties Present in Foodstuffs as Consumed in which Flavorings have been Used (Annex II of 88/388/EC)

Substances	Foodstuffs (mg/kg)	Beverages (mg/kg)	Exceptions and/or Special Restrictions
Agaric acid (1)	20	20	100 mg/kg in alcoholic beverages and foodstuffs containing mushrooms
Aloin (1)	0.1	0.1	50 mg/kg in alcoholic beverages
β-Asarone (1)	0.1	0.1	1 mg/kg in alcoholic beverages and seasonings used in snack foods
Berberine (1)	0.1	0.1	10 mg/kg in alcoholic beverages
Coumarin (1)	2	2	10 mg/kg in certain types of caramel confectionery 50 mg/kg in chewing gum 10 mg/kg in alcoholic beverages
Hydrocyanic acid (1)	1	1	50 mg/kg in nougat, marzipan or its substitutes or similar products 1 mg/% volume of alcohol in alcoholic beverages 5 mg/kg in canned stone fruit
Hypericine (1)	0.1	0.1	10 mg/kg in alcoholic beverages 1 mg/kg in confectionery

continued

ANNEX 22.3 (continued)
Maximum Limits for Certain Substances Obtained from Flavorings and other Food Ingredients with Flavoring Properties Present in Foodstuffs as Consumed in which Flavorings have been Used (Annex II of 88/388/EC)

Substances	Foodstuffs (mg/kg)	Beverages (mg/kg)	Exceptions and/or Special Restrictions
Pulegone (1)	25	100	250 mg/kg in mint or peppermint-flavored beverages 350 mg/kg in mint confectionery
Quassine (1)	5	5	10 mg/kg in confectionery in pastille form 50 mg/kg in alcoholic beverages
Safrole and isosafrole (1)	1	1	2 mg/kg in alcoholic beverages with not more than 25% volume of alcohol 5 mg/kg in alcoholic beverages with more than 25% volume of alcohol 15 mg/kg in foodstuffs containing mace and nutmeg
Santonin (1)	0.1	0.1	1 mg/kg in alcoholic beverages with more than 25% volume of alcohol
Thuyone (α and β) (1)	0.5	0.5	5 mg/kg in alcoholic beverages with not more than 25% volume of alcohol 10 mg/kg in alcoholic beverages with more than 25% volume of alcohol 25 mg/kg in foodstuffs containing preparations based on sage 35 mg/kg in bitters

(1) May not be added as such to foodstuffs or to flavorings. May be present in a foodstuff either naturally or following the addition of flavorings prepared from natural raw materials

ANNEX 22.4
List of Source Materials to which Restrictions Apply for Their Use in the Production of Flavorings and Food Ingredients with Flavoring Properties (Annex IV of Draft Flavouring Regulation, According to Council Proposal, December 10, 2007)

Part A: Source materials which shall not be used for the production of flavorings and food ingredients with flavoring properties

Source Material	
Latin Name	Common Name
Tetraploid form of *Acorus calamus*	Tetraploid form of Calamus

Part B: Conditions of use for flavorings and food ingredients with flavoring properties produced from certain source materials

Source Material		
Latin Name	**Common Name**	**Conditions of Use**
Quassia amara L. and *Picrasma excelsa* (Sw)	Quassia	Flavorings and food ingredients with flavoring properties produced from the source material may only be used for the production of beverages and bakery wares
Laricifomes officinales (Vill.: Fr) Kotl. et Pouz or *Fomes officinalis*	White agaric mushroom	Flavorings and food ingredients with flavoring properties produced from the source material may only be used for the production of alcoholic beverages
Hypericum perforatum	St Johns wort	
Teucrium chamaedrys	Wall germander	

ANNEX 22.5
List of Traditional Food Preparation Processes (Annex II of Draft Flavouring Regulation, According to Council Proposal)

Chopping	Coating
Heating, cooking, baking, frying (up to 240°C at atmospheric pressure) and pressure cooking (up to 120°C)	Cooling
Cutting	Distillation/rectification
Drying	Emulsification
Evaporation	Extraction, including solvent extraction in accordance with Directive 88/344/EEC
Fermentation	Filtration
Grinding	
Infusion	Maceration
Microbiological processes	Mixing
Peeling	Percolation
Pressing	Refrigeration/freezing
Roasting/grilling	Squeezing
Steeping	

REFERENCES

7th Amendment of the Cosmetic Directive: Directive 2003/15/EC of the European Parliament and of the Council of February 27, 2003 (OJ L 66/26, 11.3.2003).

18th ATP of DSD: Commission Directive 93/21/EEC of April 27, 1993 adapting to technical progress for the 18th time Council Directive 67/548/EEC on the Approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (OJ L 110, 4.5.1993, p. 20).

88/389/EEC: Council Decision of June 22, 1988 on the establishment, by the Commission, of an inventory of the source materials and substances used in the preparation of flavourings (OJ L 184, 15.7.1988, p. 67).

Becker, K., E. Temesvari, and I. Nemeth, 1994. Patch testing with fragrance mix and its constituents in a Hungarian population. *Cont. Dermat.*, 30: 185–186.

Chaintreau, A. et al., 2003. GC-MS quantification of fragrance compounds suspected to cause skin reactions. 1. *J. Agric. Food Chem.*, 51: 6398–6403.

Chaintreau, A. et al., 2007. GC-MS quantification of suspected volatile allergens in fragrances. 2. Data treatment strategies and method performances. *J. Agric. Food Chem.*, 55: 25–31.

Commission Directive 2006/8/EC of January 23, 2006 (OJ L 19/12, 24.1.2006).

Commission Directive 91/71/EEC of January 16, 1991 completing Council Directive 88/388/EEC on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production (OJ L 42, 15.2.1991, p. 25).

Cosmetic Directive: Council Directive 76/768/EEC of July 27, 1976 on the approximation of the laws of the Member States relating to cosmetic products (OJ L 262, 27.7.1976, p. 169).

Council Proposal, July 15, 2008. Proposal for a Regulation of the European Parliament and of the Council on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulations (EEC) No. 1576/89 and (EEC) No. 1601/91, Regulation (EC) No. 2232/96 and Directive 2000/13/EC – Outcome of the European Parliament's second reading (Strasbourg, 7 to 10 July 2008), Brussels, 15 July 2008. 11479/08, CODEC 922, DENLEG 86.

Dangerous Preparations Directive: Directive 1999/45/EC of the European Parliament and of the Council of May 31, 1999 concerning the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labeling of dangerous preparations (OJ L 200, 30.7.1999, p. 1).

Dangerous Substances Directive: Council Directive 67/548/EEC of June 27, 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (OJ P 196, 16.8.1967, p. 1).

EFFA Code of Practice (EFFA CoP): Home page of the European Flavour & Fragrance Association (EFFA): www.effa.be

First amendment of the Detergent Regulation: Commission Regulation (EC) No. 907/2006 of June 20, 2006 (OJ L 168/5, 21.6.2006).

Flavouring Regulation of December 31, 2008. Regulation (EC) No. 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No. 1601/91, Regulations (EC) No. 2232/96 and (EC) No. 110/2008 and Directive 2000/13/EC – OJ L 354, 31.12.2008, p. 34.

Frosch, P.J., B. Pilz, K.E. Andersen, et al., 1995. Patch testing with fragrances : Results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Cont. Dermat.*, 33: 333–342.

Hagvall, L. and A.-T. Karlberg, 2006. Air exposure turns common fragrance terpenes into strong allegens. Book of Abstracts, *37th Int. Symp. on Essential Oils (ISEO 2006)*, Grasse, September 10–13, PL-2.

IFRA Code of Practice (IFRA CoP): Home page of the International Fragrance Association (IFRA): www.ifraorg.org

Johansen, J.D., K.E. Andersen, C. Svedman, et al., 2003. Chloroatranol, an extremely potent allergen hidden in perfumes: A doseresponse elicitation study. *Cont. Dermat.*, 49: 180–184.

Johansen, J.D., G. Bernard, E. Gimenez-Arnau, J.P. Lepoittevin, M. Bruze, and K.E. Andersen, 2006. Comparison of elicitation potential of chloroatranol and atranol—2 allergens in oak moss absolute. *Cont. Dermat.*, 54: 192–195.

Karlberg, A.-T. et al., 1992. Air oxidation of *d*-limonene (the citrus solvent) creates potent allergens. *Cont. Dermat.*, 26: 332–340.

Karlberg, A.-T. and A. Dooms-Goossens, 1992. Contact allergy to oxidized *d*-limonene among dermatitis patients. *Cont. Dermat.*, 36: 201–206.

Larsen, W., H. Nakayama, M. Lindberg, et al., 1996. Fragrance contact dermatitis. A worldwide multicentre investigation (Part I). *Am. J. Cont. Dermat.*, 7: 77–83.

Memorandum on the SCCNFP Opinion concerning Fragrance Allergy in Consumers adopted by the SCCNFP during the 16th Plenary Meeting of March 13, 2001.

Proposal for a Regulation of the European Parliament and of the Council on flavourings and certain food ingredients with flavouring properties for use in and on foods (COM(2006) 427 final. DG Sanco, Brussels, 28.7.2006). Current Flavouring Directive: Council Directive 88/388/EC of June 22, 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production (OJ L 184, 15.7.1988, p. 61).

Regulation (EC) No. 1336/2008 of the European Parliament and of the Council of 16 December 2008 amending Regulation (EC) No. 648/2004 in order to adapt it to Regulation (EC) 1272/2008 on classification, labelling and packaging of substances and mixtures. OJ L 354, 31.12.2008, p. 60.

Regulation (EC) No. 648/2004 of the European Parliament and of the Council of March 31, 2004 on detergents (OJ L 104, 8.4.2004, p. 1).

SCCNFP Opinion concerning Oakmoss/Treemoss Extracts and Appropriate Consumer Information adopted by the SCCNFP during the 14th plenary meeting of October 24, 2000.

SCCNFP/0017/98 Final of December 1999: SCCNFP opinion concerning: Fragrance allergy in consumers—a review of the problem: Analysis of the need for appropriate consumer information and identification of consumer allergens.

SCCNFP/0202/99: SCCNFP Opinion concerning the interim position on fragrance allergy—adopted by the Scientific Committee on Cosmetic Products and Non-food Products intended for Consumers during the plenary session of June 23, 1999.

SCCP/00847/04: SCCP Opinion on atranol and chloroatranol present in natural extracts (e.g., oak moss and tree moss extract), adopted by the SCCP during the 2nd plenary meeting of December 7, 2004.

Santussi, B., A. Cristaudo, C. Cannistraci, and M. Picardo, 1987. Contact dermatitis to fragrances. *Cont. Dermat.*, 16: 93–95.

Schnuch, A., J. Geier, W. Uter, et al., 1997. National rates and regional differences in sensitization to allergens of the standard series. Population adjusted frequencies of sensitization (PAFS) in 40,000 patients from a multicenter study (IVDK). *Cont. Dermat.*, 37: 200–209.

Schnuch, A., J. Geier, W. Uter, and P.J. Frosch, 2002. Another look at allergies to fragrances: Frequencies of sensitization to the fragrance mix and its constituents. Results from the IVDK. *Exogenous Dermatol.*, 1: 231–237.

Schnuch, A., H. Lessmann, J. Geier, P.J. Frosch, and W. Uter, 2004. Contact allergy to fragrances: Frequencies of sensitization from 1996 to 2002. Results of the IVDK. *Cont. Dermat.*, 50: 65–76.

Schnuch, A., W. Uter, J. Geire, H. Lessmann, and P.J. Frosch, 2007. Sensitization to 26 fragrances to be labelled according to current European regulation. *Cont. Dermat.*, 57: 1–10.

Index

Note: (Alphanumeric/numeric character) indicates figure subset serial number.

B

C

D

E

F

G

H

I

J

K

M

N

O

P

Q

R

T

FIGURE 4.3 Lavender drying on the field.

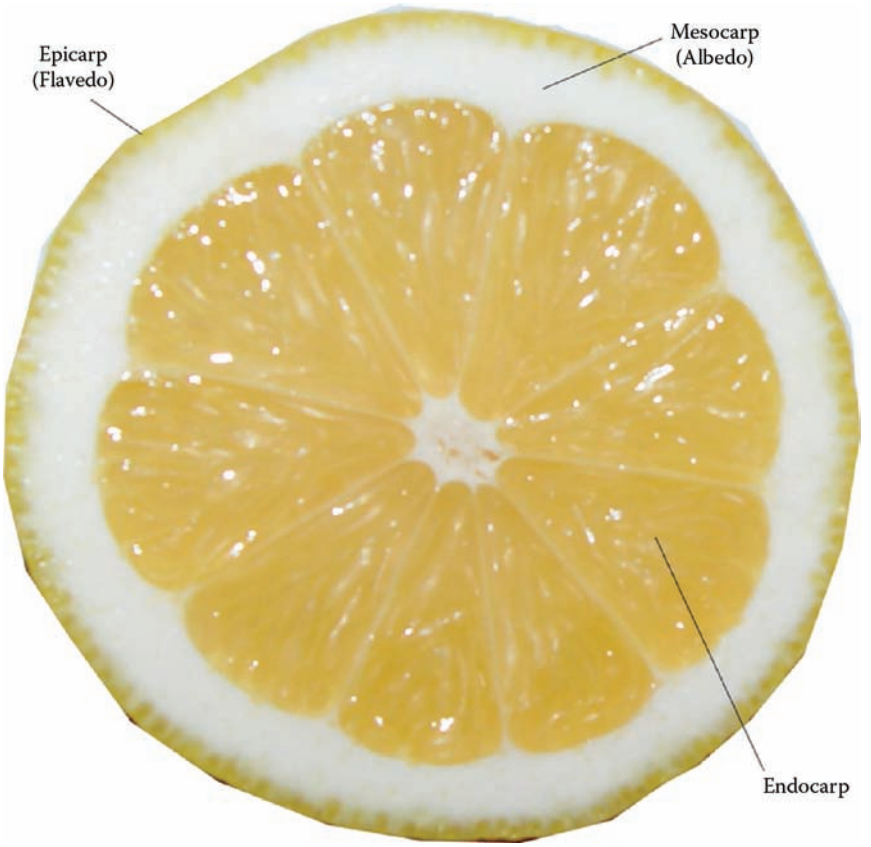


FIGURE 4.4 Parts of a citrus fruit.

FIGURE 4.5 "Pellatrici method." The spiked Archimedes screw with lemons, washed with water.

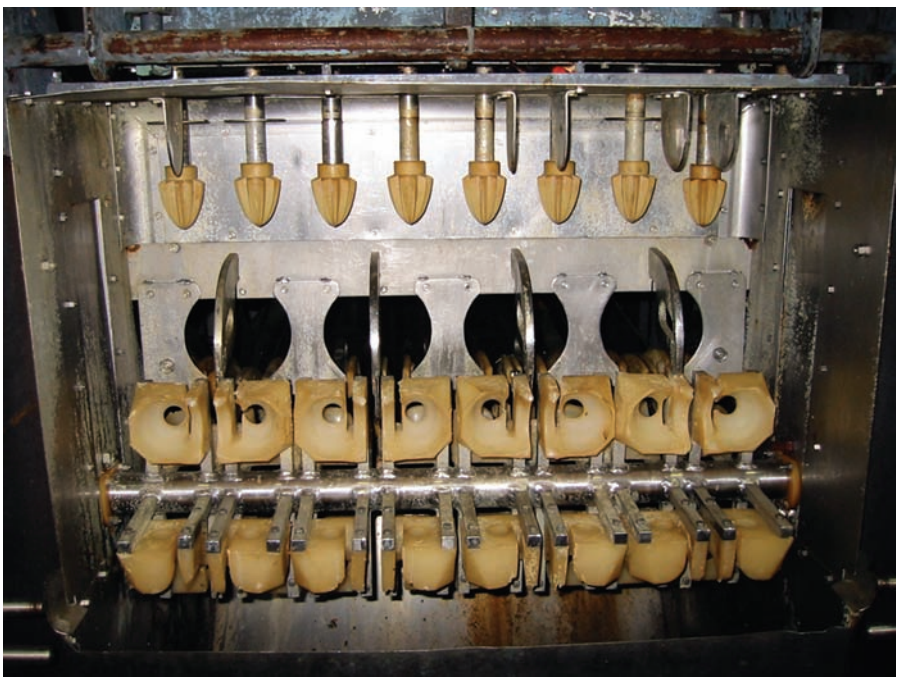


FIGURE 4.6 "Brown" process. A battery of eight juice squeezers waiting for fruits.

FIGURE 4.7 FMC extractor.

FIGURE 4.18 Oil and muddy water in the Florentine flask.

GENERAL CHEMISTRY

Handbook of ESSENTIAL OILS

Science, Technology, and Applications

Egyptian hieroglyphs, Chinese scrolls, and Ayurvedic literature record physicians administering aromatic oils to their patients. Today society looks to science to document their health choices and the oils do not disappoint. The growing body of evidence of their efficacy for more than just scenting a room underscores the need for production standards, quality control parameters for raw materials and finished products, and well-defined Good Manufacturing Practices. Edited by two renowned experts, the ***Handbook of Essential Oils*** covers all aspects of essential oils from chemistry, pharmacology, and biological activity, to production and trade, to uses and regulation.

Bringing together significant research and market profiles, this comprehensive handbook provides a much-needed compilation of information related to the development, use, and marketing of essential oils, including their chemistry and biochemistry. A select group of authoritative experts explores the historical, biological, regulatory, and microbial aspects. This reference also covers sources, production, analysis, storage, and transport of oils as well as aromatherapy, pharmacology, toxicology, and metabolism. It includes discussions of biological activity testing, results of antimicrobial and antioxidant tests, and penetration-enhancing activities useful in drug delivery.

New information on essential oils may lead to an increased understanding of their multidimensional uses and better, more ecologically friendly production methods. Reflecting the immense developments in scientific knowledge available on essential oils, this book brings multidisciplinary coverage of essential oils into one all-inclusive resource.

63154

ISBN: 978-1-4200-6315-8
90000
9 781420 063158

CRC Press
Taylor & Francis Group
an **informa** business
www.crcpress.com

6000 Broken Sound Parkway, NW
Suite 300, Boca Raton, FL 33487
270 Madison Avenue
New York, NY 10016
2 Park Square, Milton Park
Abingdon, Oxon OX14 4RN, UK

www.crcpress.com