Handbook of

ESSENTIAL
OILS
Science, Technology,
and Applications
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Editors

K. Hüsnü Can Başer was born on July 15, 1949 in Çankırı, Turkey. He graduated from the Eskisehir I.T.I.A. School of Pharmacy with diploma number 1 in 1972 and became a research assistant in the pharmacognosy department of the same school. He did his PhD in pharmacognosy between 1974 and 1978 at Chelsea College of the University of London.

Upon returning home, he worked as a lecturer in pharmacognosy at the school he had earlier graduated, and served as director of Eskisehir I.T.I.A. School of Chemical Engineering between 1978 and 1980. He was promoted to associate professorship in pharmacognosy in 1981.


During 1984–1994, he was appointed as the national project coordinator of Phase I and Phase II of the UNDP/UNIDO projects of the government of Turkey titled “Production of Pharmaceutical Materials from Medicinal and Aromatic Plants,” through which TBAM had been strengthened.

He was promoted to full professorship in pharmacognosy in 1987. His major areas of research include essential oils, alkaloids, and biological, chemical, pharmacological, technological, and biological activity research into natural products. He is the 1995 Recipient of the Distinguished Service Medal of IFEAT (International Federation of Essential Oils and Aroma Trades) based in London, United Kingdom and the 2005 recipient of “Science Award” (Health Sciences) of the Scientific and Technological Research Council of Turkey (TUBITAK). He has published 537 papers in international refereed journals (378 in SCI journals), 105 papers in Turkish journals, and 134 papers in conference proceedings.

For more information: http://www.khcbaser.com

Gerhard Buchbauer was born in 1943 in Vienna, Austria. He studied pharmacy at the University of Vienna, from where he received his master’s degree (Mag.pharm.) in May 1966. In September 1966, he assumed the duties of university assistant at the Institute of Pharmaceutical Chemistry and received his doctorate (PhD) in pharmacy and philosophy in October 1971 with a thesis on synthetic fragrance compounds. Further scientific education was practised as post doc in the team of Professor C.H. Eugster at the Institute of Organic Chemistry, University of Zurich (1977–1978), followed by the habilitation (post doctoral lecture qualification) in Pharmaceutical Chemistry with the inaugural dissertation entitled “Synthesis of Analogies of Drugs and Fragrance Compounds with Contributions to Structure-Activity-Relationships” (1979) and appointment to the permanent staff of the University of Vienna, and head of the first department of the Institute of Pharmaceutical Chemistry. In November 1991, he was appointed as a full
professor of Pharmaceutical Chemistry, University of Vienna; in 2002, he was elected as head of this institute. He retired in October 2008. He is married since 1973 and was a father of a son since 1974.


Based on the sound interdisciplinary education of pharmacists, it was possible to establish almost completely neglected area of fragrance and flavor chemistry as a new research discipline within the pharmaceutical sciences. Our research team is the only one that conducts fragrance research in its entirety and covers synthesis, computer-aided fragrance design, analysis, and pharmaceutical/medicinal aspects. Because of our efforts, it is possible to show and to prove that these small molecules possess more properties than merely emitting a good odor. Now, this “Viennese Centre of Flavour research” has gained a worldwide scientific reputation documented by more than 400 scientific publications, about 100 invited lectures, and about 200 contributions to symposia, meetings, and congresses, as short lectures and poster presentations.
Contributors

Timothy B. Adams  
Flavor & Extract Manufacturers Association  
Washington, DC

Yoshinori Asakawa  
Tokushima Bunri University  
Tokushima, Japan

K. Hüsnü Can Başer  
Department of Pharmacognosy  
Anadolu University  
Eskisehir, Turkey

Hugo Bovill  
Treatt PLC  
Bury St. Edmunds  
Suffolk, United Kingdom

W. S. Brud  
Warsaw University of Technology and  
Pollena-Aroma Ltd  
Warsaw, Poland

Gerhard Buchbauer  
Department of Clinical Pharmacy and  
Diagnostics  
University of Vienna  
Vienna, Austria

Jan C. R. Demyttenaere  
European Flavour and Fragrance Association  
(EFFA)  
Brussels, Belgium

Giovanni Dugo  
Department of Drug-Chemical  
University of Messina  
Messina, Italy

Paola Dugo  
Department of Food Science and the  
Environment  
University of Messina  
Messina, Italy

Elaine Elisabetsky  
Laboratory of Ethnopharmacology  
The Federal University of  
Rio Grande do Sul  
Porto Alegre, Brazil

Micheli Figueiró  
Laboratory of Ethnopharmacology  
The Federal University of  
Rio Grande do Sul  
Porto Alegre, Brazil

Chlodwig Franz  
Institute for Applied Botany and  
Pharmacognosy  
University of Veterinary Medicine Vienna  
Vienna, Austria

Bob Harris  
SARL Essential Oil Consultants  
Provence, France

Eva Heuberger  
Department of Clinical Pharmacy and  
Diagnostics  
University of Vienna  
Vienna, Austria

Walter Jäger  
Department of Clinical Pharmacy and  
Diagnostics  
University of Vienna  
Vienna, Austria
Jan Karlsen  
Department of Pharmaceutics  
University of Oslo  
Oslo, Norway

Maria M. Kettenring  
Neu-Isenburg, Germany

Karl-Heinz Kubeczka  
Untere Steigstrasse  
Germany

Viviane de Moura Linck  
Laboratory of Ethnopharmacology  
The Federal University of Rio Grande do Sul  
Porto Alegre, Brazil

Maria Lis-Balchin  
South Bank University  
London, United Kingdom

Luigi Mondello  
Department of Drug-Chemical  
University of Messina  
Messina, Italy  
and  
Campus-Biomedical  
Rome, Italy

Yoshiaki Noma  
Tokushima Bunri University  
Tokushima, Japan

Johannes Novak  
Institute for Applied Botany and Pharmacognosy  
University of Veterinary Medicine Vienna  
Vienna, Austria

Domingos Sávio Nunes  
Department of Chemistry  
State University of Ponta Grossa  
Ponta Grossa, Brazil

Alexander Pauli  
ReviewScience  
Zirndorf, Germany

Klaus-Dieter Protzen  
Paul Kaders GmbH  
Hamburg, Germany

Heinz Schilcher  
Immenstadt, Allgäu, Germany

Erich Schmidt  
Nördlingen, Germany  
and  
Kurt Kitzing GmbH  
Wallerstein, Germany

Charles Sell  
Givaudan UK Ltd.  
Ashford, Kent, England

Adriana Lourenço da Silva  
Laboratory of Ethnopharmacology  
The Federal University of Rio Grande do Sul  
Porto Alegre, Brazil

Sean V. Taylor  
Flavor & Extract Manufacturers Association  
Washington, DC

Lara M. Vucemilovic  
Neu-Isenburg, Germany

Barbara d'Acampora Zellner  
Department of Drug-Chemical  
University of Messina  
Messina, Italy
1 Introduction

K. Hüsnü Can Başer and Gerhard Buchbauer

Essential oils (EOs) are very interesting natural plant products and among other qualities they possess various biological properties. The term “biological” comprises all activities that these mixtures of volatile compounds (mainly mono- and sesquiterpenoids, benzenoids, phenylpropanoids, etc.) exert on humans, animals, and other plants. This book intends to make the reader acquainted with all aspects of EOs and their constituent aromachemicals ranging from chemistry, pharmacology, biological activity, production, and trade to uses, and regulatory aspects. After an overview of research and development activities on EOs with a historical perspective (Chapter 2), Chapter 3 “Sources of Essential Oils” gives an expert insight into vast sources of EOs. The chapter also touches upon agronomic aspects of EO-bearing plants. Traditional and modern production techniques of EOs are illustrated and discussed in Chapter 4. It is followed by two important chapters “Chemistry of Essential Oils” (Chapter 5) and “Analysis of Essential Oils” (Chapter 6) illustrating chemical diversity of EOs, and analytical techniques employed for the analyses of these highly complex mixtures of volatiles.

They are followed by a cluster of articles on the biological properties of EOs, starting with “The Toxicology and Safety of Essential Oils: A Constituent-Based Approach” (Chapter 7). On account of the complexity of these natural products, the toxicological or biochemical testing of an EO will always be the sum of its constituents which either act in a synergistic or in an antagonistic way with one another. Therefore, the chemical characterization of the EO is very important for the understanding of its biological properties. The constituents of these natural mixtures upon being absorbed into the blood stream of humans or animals get metabolized and eliminated. This metabolic biotransformation leads mostly in two steps to products of high water solubility which enables the organism to get rid of these “xenobiotics” by renal elimination. This mechanism is thoroughly explained in Chapter 8, “Metabolism of Terpenoids in Animal Models and Humans.” In Chapter 9, “Biological Activities of Essential Oils,” “uncommon” biological activities of EOs are reviewed, such as anticancer properties, antinociceptive effects, antiviral activities, antiphlogistic properties, penetration enhancement activities, and antioxidative effects. The psychoactive, particularly stimulating, and sedative effects of fragrances as well as behavioral activities, elucidated, for example, by neurophysiological methods, are the topics of Chapter 10 (“Effects of Essential Oils in the Central Nervous System”), Section 10.2. Here, the emphasis is put on the central nervous system and on psychopharmacology whereas Chapter 10, Section 10.1 mainly deals with reactions of the autonomic nervous system upon contact with EOs and/or their main constituents. The phytotherapeutic uses of EOs is another overview about scientific papers in peer-reviewed journals over the last 30 years, so to say the medical use of these natural plant products excluding aromatherapeutical treatments and single case studies (Chapter 11, “Phytotherapeutic Uses of Essential Oils”). Another contribution only deals with antimicrobial activities of those EOs that are monographed in the European Pharmacopoeia. In Chapter 12, “In Vitro Antimicrobial Activities of Essential Oils Monographed in the European Pharmacopoeia 6th Edition,” more than 81 tables show the importance of these valuable properties
of EOs. Aromatherapy with EOs covers the other side of the “classical” medical uses. “Aromatherapy with Essential Oils” (Chapter 13), is written by Maria Lis-Balchin, a known expert in this field and far from esoteric quackery. It completes the series of contributions dealing with the biological properties of EO regarded from various sides and standpoints.

Chapters 14 and 15 by the world-renown experts Y. Asakawa and Y. Noma are concise treatises on the biotransformations of EO constituents. Enzymes in microorganisms and tissues metabolize EO constituents in similar ways by adding mainly oxygen function to molecules to render them water soluble to facilitate their metabolism. This is also seen as a means of detoxification for these organisms. Many interesting and valuable novel chemicals are biosynthesized by this way. These products are also considered as natural since the substrates are natural.

Encapsulation is a technique widely utilized in pharmaceutical, chemical, food, and feed industries to render EOs more manageable in formulations. Classical and modern encapsulation techniques are explained in detail in Chapter 17, “Encapsulation and Other Programmed Release techniques for EOs and Volatile Terpenes.”

EOs and aromachemicals are low-volume high-value products used in perfumery, cosmetics, feed, food, beverages, and pharmaceutical industries. Industrial uses of EOs are covered in an informative chapter from a historical perspective.

“Aroma-Vital Cuisine” (Chapter 18) looks at the possibility to utilize EOs in the kitchen, where the pleasure of eating, the sensuality, and the enjoyment of lunching and dining of mostly processed food are stressed. Here, rather the holistic point of view and not too scientific way of understanding EOs is the topic, simply to show that these volatile natural plant products can add a lot of well-feeling to their users.

EOs are not only appealing to humans but also to animals. Applications of EOs as feed additives and for treating diseases in pets and farm animals are illustrated in Chapter 19, “Essential Oils Used in Veterinary Medicine,” that comprises a rare collection of information in this subject.

The EO industry is highly complex and fragmented and the trade of EOs is rather conservative and highly specialized. EOs are produced and utilized in industrialized as well as in developing countries worldwide. Their trade situation in the world is summarized in “Trade of Essential Oils” (Chapter 20), authored by a world-renown expert Hugo Bovill.

Storage and transport of EOs are crucial issues since they are highly sensitive to heat, moisture, and oxygen. Therefore, special precautions and strict regulations apply for their handling in storage and transport. “Storage and Transport of Essential Oils” (Chapter 21) will give the reader necessary guidelines to tackle this problem.

Finally, the regulatory affairs of EOs are dealt with in Chapter 22 in order to give a better insight to those interested in legislative aspects. “Recent EU Legislation on Flavors and Fragrances and Its Impact on Essential Oils” comprises the most up-to-date regulations and legislative procedures applied on EOs in the European Union.

This book is hoped to satisfy the needs of EO producers, traders, and users as well as researchers, academicians, and legislators who will find the most current information given by selected experts under one cover.
11 Phytotherapeutic Uses of Essential Oils

Bob Harris

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11.1 INTRODUCTION

For many, the term “aromatherapy” originally became associated with the concept of the holistic use of essential oils to promote health and well-being. As time has progressed and the psychophysiological effects of essential oils have been explored further, their uses to reduce anxiety and aid sedation have also become associated with the term. This is especially so since the therapy has moved into the field of nursing, where such activities are of obvious benefit to patients in a hospital environment. More importantly, the practice of aromatherapy (in English-speaking countries) is firmly linked to the inhalation of small doses of essential oils and their application to the skin in high dilution as part of an aromatherapy massage.

This chapter is concerned with the medical use of essential oils, given to the patient by all routes of administration to treat specific conditions and in comparably concentrated amounts. Studies that use essential oils in an aromatherapy-like manner, for example, to treat anxiety by essential oil massage, are therefore excluded here.

Of the literature published in peer-reviewed journals over the last 30 years, only a small percentage concerns the administration of essential oils or their components to humans in order to treat disease processes. These reports are listed below in alphabetical order of their activity. The exception is the section on the respiratory tract, where the many activities of the two principal components (menthol and 1,8-cineole) are discussed and related to respiratory pathologies.

All of the references cited are from peer-reviewed publications; a minority is open to debate regarding methodology and/or interpretation of results, but this is not the purpose of this compilation. Reports of individual case studies have been omitted.

11.2 ACARICIDAL ACTIVITY

A number of essential oils have been found to have effective acaricidal activity against infections in the animal world. Recent examples include *Origanum onites* against cattle ticks (Coskun et al.,
2008) and *Cinnamomum zeylanicum* against rabbit mange mites (Fichi et al., 2007). In comparison to veterinary research, there have been few investigations into human acaricidal infections.

The scabies mite, *Sarcoptes scabiei* var. *hominis*, is becoming increasingly resistant to existing acaricidal compounds such as lindane, benzyl benzoate, permethrin, and oral ivermectin. The potential use of a 5% *Melaleuca alternifolia* essential oil solution to treat scabies infections was investigated *in vitro*. It was found to be highly effective at reducing mite survival times and the main active component was terpinen-4-ol. However, the *in vivo* effectiveness was only tested on one individual, in combination with benzyl benzoate and ivermectin (Walton et al., 2004).

A double-blind, randomized, parallel group study was used to compare the effects of 25% w/w benzyl benzoate emulsion with 20% w/w *Lippia multiflora* essential oil emulsion in the treatment of scabies infection in 105 patients. Applied daily, the cure rates for the oil emulsion were 50%, 80%, and 80% for 3, 5, and 7 days, respectively, compared to 30%, 60%, and 70% for the benzyl benzoate emulsion. There were also less adverse reactions to the oil emulsion, leading it to be considered as an additional formulation for the treatment of scabies (Oladimeji et al., 2005).

Although not an infection, the lethal activity of essential oils toward the house dust mite (*Dermatophagoides farina* and *Dermatophagoides pteronyssinus*) is important as these mites are a major cause of respiratory allergies and an etiologic agent in the sensitization and triggering of asthma in children. Numerous studies have been conducted, including the successful inclusion of *Eucalyptus globulus* in blanket washing solutions (Tovey and McDonald, 1997), the high acaricidal activity of clove, rosemary, eucalyptus, and caraway (El-Zemity et al., 2006), and of tea tree and lavender (Williamson et al., 2007).

### 11.3 ANTICARCINOGENIC

Despite the popularity of *in vitro* experimentation concerning the cellular mechanisms of carcinogenic prevention by essential oil components (mainly by inducing apoptosis), there is no evidence that the direct administration of essential oils can cure cancer. There is evidence to suggest that the mevalonate pathway of cancer cells is sensitive to the inhibitory actions of dietary plant isoprenoids (e.g., Elson and Yu, 1994; Duncan et al., 2005). Animal testing has shown that some components can cause a significant reduction in the incidence of chemically induced cancers when administered before and during induction (e.g., Reddy et al., 1997; Uedo et al., 1999).

Phase II clinical trials have all involved perillyl alcohol. Results demonstrated that despite preclinical evidence, there appeared to be no anticarcinogenic activity in cases of advanced ovarian cancer (Bailey et al., 2002), metastatic colorectal cancer (Meadows et al., 2002), and metastatic breast cancer (Bailey et al., 2008). Only one trial has demonstrated antitumor activity as evidenced by a reduction of tumor size in patients with recurrent malignant gliomas (Orlando da Fonseca et al., 2008).

### 11.4 ANTIMICROBIAL

Considering that the majority of essential oil research is directed toward antimicrobial activity, there is a surprising lack of corresponding *in vivo* human trials. This is disappointing since the topical and systemic application of essential oils to treat infection is a widespread practice among therapists with (apparently) good results.

#### 11.4.1 ANTIBACTERIAL

Antibiotics that affect *Propionibacterium acnes* are a standard treatment for acne but antibiotic resistance is becoming prevalent. A preliminary study of 126 patients showed that topical 2% essential oil of *Ocimum gratissimum* (thymol chemotype) in a hydrophilic cream base was more effective than 10% benzyl peroxide lotion at reducing the number of lesions when applied twice daily for 4 weeks (Orafi diya et al., 2002).
In a randomized, single-blind, parallel-group-controlled trial, the same group examined the effects of the addition of aloe vera gel at varying concentrations to the *Ocimum gratissimum* cream and compared its activity with 1% clindamycin phosphate. In the 84 patients with significant acne, it was found that increasing the aloe gel content improved efficacy; the essential oil preparations formulated with undiluted or 50% aloe gels were more effective at reducing lesions than the reference product. The aloe vera gels alone had minimal activity (Orafidiya et al., 2004).

A later report judged the efficacy of a 5% *Melaleuca alternifolia* gel in the amelioration of mild to moderate acne, since a previous study (Raman et al., 1995) had demonstrated the effectiveness of tea tree oil components against *Propionibacterium acnes*. The randomized, double-blind, placebo-controlled trial used 60 patients who were given the tea tree oil gel or the gel alone twice daily for 45 days. The total acne lesion count was significantly reduced by 43.64% and the acne severity index was significantly reduced by 40.49% after the tea tree oil treatment, as compared to the placebo scores of 12.03% and 7.04%, respectively (Enshaieh et al., 2007).

### 11.4.1.1 Methicillin-Resistant *Staphylococcus aureus*

A number of papers have demonstrated the *in vitro* effects of various essential oils against methicillin-resistant *Staphylococcus aureus* (MRSA); for example, *Lippia origanoides* (Dos Santos et al., 2004), *Backhousia citriodora* (Hayes and Markovic, 2002), *Mentha piperita*, *Mentha arvensis*, and *Mentha spicata* (Imai et al., 2001), and *Melaleuca alternifolia* (Carson et al., 1995). There have been no trials involving the use of essential oils to combat active MRSA infections, although there have been two studies involving the use of tea tree oil as a topical decolonization agent for MRSA carriers.

A pilot study compared the use of 2% mupirocin nasal ointment and triclosan body wash (routine care) with 4% *Melaleuca alternifolia* essential oil nasal ointment and 5% tea tree oil body wash in 30 MRSA patients. The interventions lasted for a minimum of 3 days and screening for MRSA was undertaken at 48 and 96 h post-treatment from sites previously colonized by the bacteria. There was no correlation between length of treatment and outcome in either group. Of the tea tree oil group, 33% were initially cleared of MRSA carriage while 20% remained chronically infected at the end of the treatment; this was in comparison with routine care group of 13% and 53%, respectively. The trial was too small to provide significant results (Caelli et al., 2000).

A randomized, controlled trial compared the use of a standard regime for MRSA decolonization with *Melaleuca alternifolia* essential oil. The 5-day study involved 236 patients. The standard treatment group was given 2% mupirocin nasal ointment thrice daily, 4% chlorhexidine gluconate soap as a body wash once daily, and 1% silver sulfadiazine cream for skin lesions, wounds, and leg ulcers once daily. The tea tree oil group received 10% essential oil cream thrice daily to the nostrils and to specific skin sites and 5% essential oil body wash at least once daily. In the tea tree oil group, 41% were cleared of MRSA as compared to 49% using the standard regime; this was not a significant difference. Tea tree oil cream was significantly less effective at clearing nasal carriage than mupirocin (47% compared to 78%), but was more effective at clearing superficial sites than chlorhexidine or silver sulfadiazine (Dryden et al., 2004).

### 11.4.2 Antifungal

The essential oil of *Citrus aurantium* var. *amara* was used to treat 60 patients with tinea corporis, cruris, or pedis. One group received a 25% bitter orange (BO) oil emulsion thrice daily, a second group was treated with 20% bitter orange oil in alcohol (BOa) thrice daily, and a third group used undiluted BO oil once daily. The trial lasted for 4 weeks and clinical and mycological examinations were performed every week until cure, which was defined as an elimination of signs and symptoms. In the BO group, 80% of patients were cured in 1–2 weeks and the rest within 2–3 weeks. By using BOa, 50% of patients were cured in 1–2 weeks, 30% in 2–3 weeks, and 20% in 3–4 weeks. With the undiluted essential oil, 25% of patients did not continue treatment, 33.3% were cured in 1 week, 60% in 1–2 weeks, and 6.7% in 2–3 weeks (Ramadan et al., 1996).
A double-blind, randomized, placebo-controlled trial investigated the efficacy of 2% butenafine hydrochloride cream with added 5% *Melaleuca alternifolia* essential oil in 60 patients with toenail onychomycosis. After 16 weeks, 80% of patients in the treatment group were cured, as opposed to none in the control group (Syed et al., 1999). However, butenafine hydrochloride is a potent antimycotic in itself and the results were not compared with this product when used alone.

After an initial *in vitro* study, which showed that the essential oil of *Eucalyptus pauciflora* had a strong fungicidal activity against *Epidermophyton floccosum, Microsporum canis, Microsporum nanum, Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton tonsurans,* and *Trichophyton violaceum,* an *in vivo* trial was commenced. Fifty patients with confirmed dermatophytosis were treated with 1% v/v essential oil twice daily for 3 weeks. At the end of the treatment, a cure was demonstrated in 60% of patients with the remaining 40% showing significant improvement (Shahi et al., 2000).

On the surmise that infection with *Pityrosporum ovale* is a major contributing factor to dandruff and that anti-*Pityrosporum* drugs such as nystatin were proven effective treatments, the use of 5% *Melaleuca alternifolia* essential oil was investigated. In this randomized, single-blind, parallel-group study tea tree oil shampoo or placebo shampoo was used daily for 4 weeks by 126 patients with mild to moderate dandruff. In the treatment group, the dandruff severity score showed an improvement of 41%, as compared to 11% in the placebo group. The area involvement and total severity scores also demonstrated a statistically significant improvement, as did itchiness and greasiness. Scaliness was not greatly affected. The condition resolved for one patient in each group and so ongoing application of tea tree oil shampoo was recommended for dandruff control (Satchell et al., 2002a).

For inclusion in a randomized, double-blind, controlled trial, 158 patients with the clinical features of intertriginous tinea pedis and confirmed dermatophyte infection were recruited. They were administered 25% or 50% *Melaleuca alternifolia* essential oil (in an ethanol and polyethylene glycol vehicle) or the vehicle alone, twice daily for 4 weeks. There was an improvement in the clinical severity score, falling by 68% and 66% in the 25% and 50% tea tree oil groups, in comparison with 41% for the placebo. There was an effective cure in the 25% and 50% tea tree oil and placebo groups of 48%, 50%, and 13%, respectively. The essential oil was less effective than standard topical treatments (Satchell et al., 2002b).

The anticandida properties of *Zataria multiflora* essential oil and its active components (thymol, carvacrol, and eugenol) were demonstrated *in vitro* by Mahmoudabadi et al. (2006). A randomized, clinical trial was conducted with 86 patients with acute vaginal candidiasis. They were treated with a cream containing 0.1% *Zataria multiflora* essential oil or 1% clotrimazole once daily for 7 days. Statistically significant decreases in vulvar pruritis (80.9%), vaginal pruritis (65.5%), vaginal burning (73.95), urinary burning (100%), and vaginal secretions (90%) were obtained by the essential oil treatment as compared to the clotrimazole treatment of 73.91%, 56.7%, 82.1%, 100%, and 70%, respectively. In addition, the *Zataria multiflora* cream reduced erythema and satellite vulvar lesions in 100% of patients, vaginal edema in 100%, vaginal edema in 83.3%, and vulvo-vaginal excoriation and fissures in 92%. The corresponding results for clotrimazole were 100%, 100%, 76%, and 88%. In terms of overall efficacy, the rates of improvement were 90% and 74.8% for the *Zataria multiflora* and clotrimazole groups, respectively. Use of the cream alone provided no significant changes (Khosravi et al., 2008).

11.4.3 Antiviral

The *in vitro* studies that have been conducted so far indicate that many essential oils possess antiviral properties, but they affect only enveloped viruses and only when they are in the free state, that is, before the virus is attached to, or has entered the host cell (e.g., Schnitzler et al., 2008). This is in contrast to the majority of synthetic antiviral agents, which either bar the complete penetration of viral particles into the host cell or interfere with viral replication once the virus is inside the cell.
A randomized, investigator-blinded, placebo-controlled trial used 6% *Melaleuca alternifolia* essential oil gel to treat recurrent herpes labialis. It was applied five times daily and continued until re-epithelialization occurred and the polymerase chain reaction (PCR) for Herpes simplex virus was negative for two consecutive days. The median time to re-epithelialization after treatment with tea tree oil was 9 days as compared to 12.5 days with the placebo, which is similar to reductions caused by other topical therapies. The median duration of PCR positivity was the same for both groups (6 days) although the viral titers appeared slightly lower in the oil group on days 3 and 4. None of the differences reached statistical significance, probably due to the small group size (Carson et al., 2001).

Children below 5 years were enrolled in a randomized trial to test a 10% v/v solution of the essential oil of *Backhousia citriodora* against molluscum contagiosum (caused by Molluscipox-virus). Of the 31 patients, 16 were assigned to the treatment group and the rest to the control of olive oil. The solutions were applied directly to the papules once daily at bedtime for 21 days or until the lesions had resolved. In the essential oil group, five children had a total resolution of lesions and four had reductions of greater than 90% at the end of 21 days. In contrast, none of the control group had any resolution or reduction of lesions by the end of the study period (Burke et al., 2004).

A study was conducted on 60 patients who were chronic carriers of hepatitis B or C. The essential oils of *Cinnamomum camphora* ct 1,8-cineole, *Daucus carota*, *Ledum groelandicum*, *Laurus nobilis*, *Helichrysum italicum*, *Thymus vulgaris* ct thujanol, and *Melaleuca quinquenervia* were used orally in various combinations. They were used as a monotherapy or as a complement to allopathic treatment. The objectives of treatment were normalization of transaminase levels, reduction of viral load, and stabilization or regression of fibrosis. There was an improvement of 100%, when patients with hepatitis C were given bitherapy with essential oils. With essential oil monotherapy, improvements were noted in 64% of patients with hepatitis C and there were two cures of hepatitis B (Giraud-Robert, 2005).

### 11.4.4 **MICROBES OF THE ORAL CAVITY**

The activities of essential oils against disease-producing microbes in the oral cavity have been documented separately because there are numerous reports of relevance. The easy administration of essential oils in mouthrinses, gargles, and toothpastes, and the success of such commercial preparations, has no doubt contributed to the popularity of this research.

The *in vitro* activities of essential oils against the oral microflora are well documented and these include effects on cariogenic and periodontopathic bacteria. One example is the *in vitro* activity of *Leptospermum scoparium*, *Melaleuca alternifolia*, *Eucalyptus radiata*, *Lavandula officinalis*, and *Rosmarinus officinalis* against *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Streptococcus mutans*. The essential oils inhibited all of the test bacteria, acting bactericidally except for *Lavandula officinalis*. In addition, significant adhesion-inhibiting activity was shown against *Streptococcus mutans* by all essential oils and against *Porphyromonas gingivalis* by tea tree and manuka (Takarada et al., 2004).

There have been at least six *in vivo* studies concerning the activity of individual essential oils against the microflora of the oral cavity. In addition, a review of the literature finds a surprising number of *in vivo* papers that detail the activities of “an essential oil mouthrinse.” Closer examination reveals that the essential oil mouthrinse is the commercial product, Listerine. Although Listerine contains 21% or 26% alcohol (depending on the exact product), a 6-month study has shown that it contributes nothing to the efficaciousness of the mouthrinse (Lamster et al., 1983). The active ingredients are 1,8-cineole (0.092%), menthol (0.042%), methyl salicylate (0.06%), and thymol (0.64%). For this reason, a small random selection of such papers is included below.
11.4.4.1 Activity of Listerine against Plaque and/or Gingivitis

An observer-blind, 4-day plaque regrowth, crossover study compared the use of Listerine® with a triclosan mouthrinse and two placebo controls in 32 volunteers. All normal hygiene procedures were suspended except for the rinses. The triclosan product produced a 45% reduction in plaque area and a 12% reduction in plaque index against its placebo, in comparison with 52% and 17%, respectively, for the essential oil rinse. The latter was thus deemed more effective (Moran et al., 1997).

A similar protocol was used to compare the effects of Listerine against an amine fluoride/stannous fluoride-containing mouthrinse (Meridol®) and a 0.1% chlorhexidine mouthrinse (Chlorhexamed®) in inhibiting the development of supragingival plaque. On day 5 of each treatment, the results from 23 volunteers were evaluated. In comparison with their placebos, the median plaque reductions were 12.2%, 23%, and 38.2% for the fluoride, essential oil, and chlorhexidine rinses, respectively. The latter two results were statistically significant (Riep et al., 1999).

After the assessment for the presence of gingivitis and target pathogens (*Porphyromonas gingivalis, Fusobacterium nucleatum, and Veillonella sp.*) and total anaerobes, 37 patients undertook a twice daily mouthrinse with Listerine for 14 days. After a washout period, the study was conducted again using a flavored hydroalcoholic placebo. The results of this randomized, double-blind, crossover study showed that the essential oil rinse significantly lowered the number of all target pathogens by 66.3–79.2%, as compared to the control (Fine et al., 2007).

The effect of adding Listerine mouthrinse to a standard oral hygiene regime in 50 orthodontic patients was examined. The control group brushed and flossed twice daily, whereas the test group also used the mouthrinse twice daily. Measurements of bleeding, gingival, and plaque indices were conducted at 3 and 6 months. All three indices were significantly lowered in the test group as compared to the control at both time intervals (Tufekci et al., 2008).

The same fixed combination of essential oils that is found in Listerine mouthrinse has been incorporated into a dentifrice. Such a dentifrice was used in a 6-month double-blind study to determine its effect on the microbial composition of dental plaque as compared to an identical dentifrice without essential oils. Supragingival plaque and saliva samples were collected at baseline and their microbial content characterized, after which the study was conducted for 6 months. The essential oil dentifrice did not significantly alter the microbial flora and opportunistic pathogens did not emerge, nor was there any sign of developing resistance to the essential oils in tested bacterial species (Charles et al., 2000).

The same dentifrice was examined for antiplaque and antigingivitis properties in a blinded, randomized, controlled trial. Before treatment, 200 patients were assessed using a plaque index, a modified gingival index (GI), and a bleeding index. The dentifrice was used for 6 months, after which another assessment was made. It was found that the essential oil dentifrice had a statistically significant lower whole-mouth and interproximal plaque index (18.3% and 18.1%), mean GI (16.2% and 15.5%), and mean bleeding index (40.5% and 46.9%), as compared to the control. It was therefore proven to be an effective antiplaque and antigingivitis agent (Coelho et al., 2000).

11.4.4.2 Antiviral Listerine

A trial was conducted to examine whether a mouthrinse could decrease the risk of viral cross-contamination from oral fluids during dental procedures. Forty patients with a perioral outbreak of recurrent herpes labialis were given a 30-s mouthrinse with either water or Listerine. Salivary samples were taken at baseline, immediately following the rinse and 30 min after the rinse and evaluated for the viral titer. Infectious virions were reduced immediately to zero postrinse and there was a continued significant reduction 30 min postrinse. The reduction by the control was not significant (Meiller et al., 2005).

11.4.4.3 Activity of Essential Oils

The antibacterial activity of the essential oil of *Lippia multiflora* was first examined *in vitro* for antimicrobial activity against ATCC strains and clinical isolates of the buccal flora. A significant
activity was found, with an MBC of 1/1400 for streptococci and staphylococci, 1/800 for enterobacteria and neisseria, and 1/600 for candida. A mouthwash was prepared with the essential oil at a 1/500 dilution and this was used in two clinical trials.

The buccodental conditions of 26 French children were documented by measuring the percentage of dental surface free of plaque, gum inflammation, and the papillary bleeding index (PBI). After 7 days of rinsing with the mouthwash for 2 min, the test group was found to have a reduction of dental plaque in 69% of cases and a drop in PBI with a clear improvement of gum inflammation in all cases. The second trial was conducted in the Cote d’Ivoire with 60 adult patients with a variety of conditions. After using the mouthwash after every meal for 5 days, it was found that candidiasis had disappeared in most cases, gingivitis was resolved in all patients, and 77% of dental abscesses had resorbed (Pélissier et al., 1994).

Fluconazole-refractory oropharyngeal candidiasis is a common condition in HIV patients. Twelve such patients were treated with 15 mL of a Melaleuca alternifolia oral solution (Breath-Away) four times daily for 2 weeks, in a single center, open-label clinical trial. The solution was swished in the mouth for 30–60 s and then expelled, with no rinsing for at least 30 min. Clinical assessment was carried out on days 7 and 14 and also on days 28 and 42 of the follow-up. Two patients were clinically cured and six were improved after the therapy; four remained unchanged and one deteriorated. The overall clinical response rate was thus 67% and was considered as a possible alternative antifungal treatment in such cases (Jandourek et al., 1998).

A clinical pilot study compared the effect of 0.34% Melaleuca alternifolia essential oil solution with 0.1% chlorhexidine on supragingival plaque formation and vitality. Eight subjects participated, with a 10-day washout period between each treatment regime of 1 week. The plaque area was calculated using a stain and plaque vitality was estimated using a fluorescence technique. Neither of these parameters was reduced by the tea tree oil treatment (Arweiler et al., 2000).

A gel containing 2.5% Melaleuca alternifolia essential oil was used in a double-blind, longitudinal noncrossover trial and compared with a chlorhexidine gel positive control and a placebo gel in the treatment of plaque and chronic gingivitis. The gels were applied as a dentifrice twice daily by 49 subjects for 8 weeks and the treatment was assessed using a gingival index (GI), a PBI, and a plaque staining score. The tea tree group showed a significant reduction in PBI and GI scores, although plaque scores were not reduced. It was apparent that the tea tree gel decreased the level of gingival inflammation more than the positive or negative controls (Soukoulis and Hirsch, 2004).

A mouthcare solution consisting of an essential oil mixture of Melaleuca alternifolia, Mentha piperita, and Citrus limon in a 2:1:2 ratio diluted in water to a 0.125% solution was used to treat oral malodor in 32 intensive care unit patients, 13 of whom were ventilated. The solution was used to clean the teeth, tongue, and oral cavity twice daily. The level of malodor was assessed by a nurse using a visual analogue scale, and volatile sulfur compounds (VSC) were measured via a probe in the mouth, before, 5 and 60 min after treatment. On the second day, the procedure was repeated using benzydamine hydrochloride (BH), which is normally used for oral hygiene, instead of essential oil solution. The perception of oral malodor was significantly lowered after the essential oil treatment but not after the BH treatment. There was a decrease in VSC levels at 60 min for both treatment groups, but not after 5 min for the oil mixture. The results suggested that just one session with the essential oil mixture could improve oral malodor and VSC in intensive care patients (Hur et al., 2007).

The essential oil of Lippia sidoides (rich in thymol and carvacrol) was used in a double-blind, randomized, parallel-armed study against gingival inflammation and bacterial plaque. Fifty-five patients used a 1% essential oil solution as a mouthrinse twice daily for 7 days and the results were compared with a positive control, 0.12% chlorhexidine. Clinical assessment demonstrated decreased plaque index and gingival bleeding scores as compared to the baseline, with no significant difference between test and control. The essential oil of Lippia sidoides was considered a safe and effective treatment (Botelho et al., 2007).
11.4.5 **CONTROLLING MICROFLORA IN ATOPIC DERMATITIS**

Rarely found on healthy skin, *Staphylococcus aureus* is usually present in dry skin and is one of the factors that can worsen atopic dermatitis. Toxins and enzymes deriving from this bacteria cause skin damage and form a biofilm from fibrin and glyocalyx, which aids adhesion to the skin and resistance to antibiotics. An initial *in vitro* study found that a mixture of xylitol (a sugar alcohol) and farnesol was an effective agent against *Staphylococcus aureus*; xylitol inhibited the formation of glyocalyx whereas farnesol dissolved fibrin and suppressed *Staphylococcus aureus* growth without affecting *Staphylococcus epidermidis* (Masako et al., 2005a).

The same mixture of xylitol and farnesol was used in a double-blind, randomized, placebo-controlled study of 17 patients with mild to moderate atopic dermatitis on their arms. A skin-care cream containing 0.02% farnesol and 5% xylitol or the cream alone was applied to either the left or the right arms for 7 days. The ratio of *Staphylococcus aureus* to other aerobic skin microflora was significantly decreased in the test group compared to placebo, from 74% to 41%, while the numbers of coagulase-negative staphylococci increased. In addition, skin conductance (indicating hydration of skin surface) significantly increased at the test cream sites compared to before application and to the placebo (Masako et al., 2005b).

11.4.6 **ODOR MANAGEMENT FOR FUNGATING WOUNDS**

Fungating wounds may be caused by primary skin carcinomas, underlying tumors or via spread from other tissues. The malodor associated with such necrosis is caused by the presence of aerobic and anaerobic bacteria. The wounds rarely heal and require constant palliative treatment, leading to social isolation of the patients and poor quality of life.

Smell reduction with essential oils was first reported by Warnke et al. (2004) in 25 malodorous patients with inoperable squamous cell carcinoma of the head and neck. A commercial product containing eucalyptus, grapefruit, and tea tree essential oils (Megabac®) was applied topically to the wounds twice daily. Normal medication apart from Betadine disinfection was continued. The smell disappeared completely within 2–3 days and signs of superinfection and pus secretion were reduced in the necrotic areas.

Megabac has also been used in a small pilot study (10 patients) to treat gangrenous areas, being applied via spray thrice daily until granulation tissue formed. The treatment was then continued onto newly formed split skin grafts. All wounds healed within 8 weeks and no concurrent antibiotics were used (Sherry et al., 2003).

Use of essential oils to reduce the smell of fungating wounds in 13 palliative care patients was detailed by another group the following year. *Lavandula angustifolia*, *Melaleuca alternifolia*, and *Pogostemon cablin* essential oils were used alone or in combinations at 2.5–5% concentrations in a cream base. The treatments were effective (Mercier and Knevitt, 2005).

A further study was conducted with 30 patients suffering incurable head and neck cancers with malodorous necrotic ulcers. A custom-made product (Klonemax®) containing eucalyptus, tea tree, lemongrass, lemon, clove, and thyme essential oils was applied topically (5 mL) twice daily. All patients had a complete resolution of the malodor; in addition to the antibacterial activity, an anti-inflammatory effect was also noted (Warnke et al., 2006).

The use of essential oils to treat malodorous wounds in cancer patients is becoming widespread in many palliative care units although no formal clinical trials have been conducted as yet.

11.5 **DISSOLUTION OF HEPATIC AND RENAL STONES**

11.5.1 **GALL AND BILIARY TRACT STONES**

Rowachol and Rowatinex are two commercial products that have been marketed for many years and are based on essential oil components. They are sometimes thought of as being the same product but
in fact they are different. The compositions have changed slightly over the years and the most recently disclosed are shown in Table 11.1.

Rowachol has been in use for over 50 years for the dissolution of gallstones and biliary tract stones. There have been many published works on its effects and at least one double-blind trial (Lamy, 1967). It has been stated that although the dissolution rate of Rowachol is not impressive, it is still much greater than Rowatinex and could occur spontaneously (Doran and Bell, 1979). It has been employed alone as a useful therapy for common duct stones (Ellis and Bell, 1981) although improved results were demonstrated when Rowachol was used in conjunction with bile acid therapy (Ellis et al., 1981).

Rowachol has been shown to inhibit hepatic cholesterol synthesis mediated by a decreased hepatic \( S\)-3-hydroxy-3-methylgutaryl-CoA reductase activity (Middleton and Hui, 1982); the components mostly responsible for this activity were menthol and 1,8-cineole, with pinene and camphene having no significant effect (Clegg et al., 1980). A reduction in cholesterol crystal formation in the bile of gallstone patients has been demonstrated in a small trial using Rowachol (von Bergmann, 1987).

Two early uncontrolled trials reported that Rowachol significantly increased plasma high-density lipoprotein (HDL) cholesterol when administered to patients with low HDL cholesterol; a twofold increase was found in 10 subjects after 6 weeks of treatment (Hordinsky and Hordinsky, 1979), while a progressive increase in HDL of 14 subjects was noted, >100% after 6 months (Bell et al., 1980). This was interesting as low plasma concentrations of HDLs are associated with an elevated risk of coronary heart disease. However, a double-blind, placebo-controlled trial that administered six capsules of Rowachol daily for 24 weeks to 19 men found that there were no significant HDL-elevating effects of the treatment (Cooke et al., 1998). It is currently thought that monoterpenes have no HDL-elevating potential that is useful for disease prevention.

In vitro, a solution of 97% \( d\)-limonene was found to be 100-fold better at solubilizing cholesterol than sodium cholate. A small trial followed with 15 patients, whereby 20 ml of the \( d\)-limonene preparation was introduced into the gallbladder via a catheter on alternate days for up to 48 days. The treatment was successful in 13 patients with gallstone dissolution sometimes occurring after three infusions. Side effects included vomiting and diarrhea (Igimi et al., 1976).

A further study was conducted by Igimi et al. (1991) using the same technique with 200 patients. Treatments lasted from 3 weeks to 4 months. Complete or partial dissolution of gallstones was achieved in 141 patients, with complete disappearance of stones in 48% of cases. Epigastric pain was experienced by 168 patients and 121 suffered nausea and vomiting. Further trials have not been conducted.

<table>
<thead>
<tr>
<th>Component</th>
<th>Rowachol</th>
<th>Rowatinex</th>
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<tbody>
<tr>
<td>( \alpha)-pinene</td>
<td>20.0</td>
<td>37.0</td>
</tr>
<tr>
<td>( \beta)-pinene</td>
<td>5.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Camphene</td>
<td>8.0</td>
<td>22.0</td>
</tr>
<tr>
<td>1,8-cineole</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Fenchone</td>
<td>—</td>
<td>6.0</td>
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<tr>
<td>Menthol</td>
<td>9.0</td>
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<tr>
<td>Borneol</td>
<td>8.0</td>
<td>15.0</td>
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<tr>
<td>Menthol</td>
<td>48.0</td>
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<tr>
<td>anethole</td>
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11.5.2 Renal Stones

While Rowachol is used as a measure against gallstones and biliary tract stones, Rowatinex is used in the treatment of renal stones. The first double-blind, randomized trial was conducted by Mukamel et al. (1987) on 40 patients with acute renal colic. In the Rowatinex group, there was a significantly higher expulsion rate of stones ≥3 mm in diameter in comparison with the placebo (61% and 28%, respectively). There was also a higher overall success rate in terms of spontaneous stone expulsion and/or disappearance of ureteral dilatation in the treatment group compared to placebo (78–52%), but the difference was not statistically significant.

A second double-blind, randomized trial was conducted on 87 patients with ureterolithiasis. Four Rowatinex capsules were prescribed four times a day, the average treatment time being two weeks. The overall stone expulsion rate was significantly higher in the Rowatinex group as compared to placebo; 81% and 51%, respectively. Mild to moderate gastrointestinal disturbances were noted in seven patients. It was concluded that the early treatment of ureteral stones with Rowatinex was preferable before more aggressive measures were considered (Engelstein et al., 1992).

Rowatinex has also been used with success in the removal of residual stone fragments after extracorporeal shock wave lithotripsy, a situation that occurs in up to 72% of patients when given this therapy. With 50 patients, it was found that Rowatinex decreased the number of calculi debris, reducing the number of late complications and further interventions. By day 28, 82% of patients were free of calculi whereas this situation is normally reached after 3 months without Rowatinex treatment (Siller et al., 1998).

A minor study examined the use of Rowatinex in the management of childhood urolithiasis. Six children aged from 4 months to 5 years were administered varying doses of the preparation from 10 days to 12 weeks. All patients became stone-free with no side effects, although a definite conclusion as to the efficacy of treatment could not be established due to the small patient number involved (Al-Mosawi, 2005).

A comparison of the effects of an α-blocker (tamsulosin) and Rowatinex for the spontaneous expulsion of ureter stones and pain control was undertaken using 192 patients. They were divided into three groups: analgesics only, Rowatinex with analgesics, and tamsulosin with analgesics. For ureter stones less than 4 mm in diameter, their excretion was accelerated by both Rowatinex and tamsulosin. The use of these two treatments also decreased the amount of analgesics required and it was concluded that they should be considered as adjuvant regimes (Bak et al., 2007).

11.6 Functional Dyspepsia

Several essential oils have been used in the treatment of functional (nonulcer) dyspepsia. All of the published trials have concerned the commercial preparation known as Enteroplant®, an enteric-coated capsule containing 90 mg of Mentha × piperita, and 50 mg of Carum carvi essential oils.

The combination of peppermint and caraway essential oils has been shown to act locally in the gut as an antispasmodic (Micklefield et al., 2000, 2003) and to have a relaxing effect on the gallbladder (Goerg and Spilker, 2003). The antispasmodic effect of peppermint is well documented and that of caraway essential oil has also been demonstrated (Reiter and Brandt, 1985). The latter alone has also been shown to inhibit gallbladder contractions in healthy volunteers, increasing gallbladder volume by 90% (Goerg and Spilker, 1996).

One of the first studies involved 45 patients in a double-blind, placebo-controlled multicenter trial with the administration of Enteroplant thrice daily for 4 weeks. It was found to be superior to placebo with regard to pain frequency, severity, efficacy, and medical prognosis. Clinical Global Impressions were improved for 94.5% of patients using the essential oil combination (May et al., 1996).

The activity of Enteroplant (twice daily) was compared with that of cisapride (30 mg daily), a serotonin 5-HT₄ agonist that stimulates upper gastrointestinal tract motility, over a 4-week period.
This double-blind, randomized trial found that both products had comparable efficacy in terms of pain severity and frequency, Dyspeptic Discomfort Score, and Clinical Global Impressions (Madisch et al., 1999).

Another double-blind, randomized trial administered either Enteroplant or placebo twice daily for 28 days. Pain intensity and pressure, heaviness, and fullness were reduced in the test group by 40% and 43% as compared to 22% for both in the placebo group, respectively. In addition, Clinical Global Impressions were improved by 67% for the peppermint/caraway combination whereas the placebo scored 21% (May et al., 2000).

Holtmann et al. (2001) were the first to investigate the effect of Enteroplant (twice daily) on disease-specific quality of life as measured by the Nepean Dyspepsia Index. All scores were significantly improved compared to the placebo. In 2002, the same team also demonstrated that patients suffering with severe pain or severe discomfort both responded significantly better in comparison with the placebo.

Approximately 50% of patients suffering from functional dyspepsia are infected with *Helicobacter pylori* (Freidman, 1998). The *Helicobacter* status of 96 patients and the efficacy of Enteroplant were compared by May et al. (2003). They found that patients with *Helicobacter pylori* infection demonstrated a substantially better treatment response than those who were not infected. However, a previous study found no efficacy differences between infected and noninfected functional dyspepsia patients (Madisch et al., 2000) and so the effect of the presence of the bacterium on Enteroplant treatment has yet to be elucidated.

A short review of the literature concluded that treatment with the fixed peppermint/caraway essential oil combination had demonstrated significant efficacy in placebo-controlled trials, had good tolerability and safety, and could thus be considered for the long-term management of functional dyspepsia patients (Holtmann et al., 2003).

### 11.7 GASTROESOPHAGEAL REFLUX

*d*-Limonene has been found to be effective in the treatment of gastroesophageal reflux disorder. Nineteen patients took one capsule of 1000 mg *d*-limonene every day and rated their symptoms using a severity/frequency index. After 2 days, 32% of patients had significant relief and by day 14, 89% of patients had complete relief of symptoms (Wilkins, 2002).

A double-blind, placebo-controlled trial was conducted with 13 patients who were administered one 1000 mg capsule of *d*-limonene daily or on alternate days. By day 14, 86% of patients were asymptomatic compared to 29% in the placebo group (Wilkins, 2002).

The mechanism of action of *d*-limonene has not been fully elucidated in this regard but it is thought that it may coat the mucosal lining and offer protection against gastric acid and/or promote healthy peristalsis.

### 11.8 HYPERLIPOPROTEINEMIA

Girosital is a Bulgarian encapsulated product consisting of rose essential oil (68 mg) and vitamin A in sunflower vegetable oil. Initial animal studies found that rose oil administered at 0.01 and 0.05 mL/kg had a hepatoprotective effect against ethanol. Dystrophy and lipid infiltration were lowered and glycogen tended to complete recovery, suggesting a beneficial effect of rose oil on lipid metabolism (Kirov et al., 1988a).

Girosital was administered to 33 men with long-standing alcohol abuse, twice daily for 3 months. It significantly reduced serum triglycerides and low-density lipoprotein and increased the level of HDL-cholesterol; it was particularly effective for the treatment of hyperlipoproteinemia types IIb and IV. Liver lesions relating to alcohol intoxication improved and subjective complaints such as dyspeptic symptoms and pain were reduced (Konstantinova et al., 1988).
The hypolipidemic effect of Girosital was again studied by giving a capsule once daily for 20 days in 35 patients with hyperlipoproteinemia. In type IIa hyperlipoproteinemia cases, the total lipids were reduced by 23.91% and the total cholesterol by 10.64%. For type IIb patients, the total lipid reduction was 15.93%, triglycerides fell by 25.45%, and cholesterol by 14.06%; in type IV cases the reductions were 33.83%, 25.33%, and 36%, respectively. Girosital was more effective in comparison with the treatment with bezalipe and clofibrate (Stankusheva, 1988).

Thirty-two patients with hyperlipoproteinemia and arterial hypertension were administered one Girosital capsule twice daily for 110 days. A marked reduction in hyperlipoproteinemia was demonstrated in all patients. The hypocholesterolemic effect manifested first in type IIa patients after 20 days, and later in type IIb cases. Reduction of serum triglycerides in type IIb began 50 days after the commencement of treatment (Kirov et al., 1988b).

A further study (Mechkov et al., 1988) examined the effect of Girosital capsules twice daily for 110 days in 30 patients with cholelithiasis, liver steatosis, and hyperlipoproteinemia. Total cholesterol decreased after 20 days of treatment although it tended to rise slightly later in the test period. The triglycerides were most affected in hyperlipoproteinemia types IIb and IV. The \( \beta \)-lipoprotein values were not altered by the treatment.

### 11.9 IRRITABLE BOWEL SYNDROME

The essential oil of *Mentha × piperita* has been used for many years as a natural carminative of the gastrointestinal tract. This effect is principally due to the antispasmodic activity of menthol, which acts as a calcium channel antagonist of the intestinal smooth muscle (Taylor et al., 1984, 1985). Secondary effects include a reduction of gastrointestinal foam by peppermint oil (Harries et al., 1978) and a choleretic activity that is attributed to menthol (Rangelov et al., 1988). The reduction of intestinal hydrogen production caused by bacterial overgrowth has also been demonstrated in patients by enteric-coated peppermint oil (Logan and Beaulne, 2002).

The first clinical trial of peppermint for the treatment of irritable bowel syndrome was conducted by Rees et al. (1979). They prescribed 0.2 mL of peppermint oil in enteric-coated capsules (1–2 capsules depending on symptom severity) thrice daily. Patient assessment considered the oil to be superior to the placebo in relieving abdominal symptoms.

Since then, a further 15 double-blind and two open trials have been conducted; examples of these can be seen in Table 11.2.

Eight studies used the commercial preparation known as Colpermin® and two used Mintoil®, the capsules of which contain 187 and 225 mg of peppermint oil, respectively. The other studies used enteric-coated capsules usually containing 0.2 mL of the essential oil.

The latest trial (Cappello et al., 2007) used a randomized, double-blind, placebo-controlled design to test the efficacy of two capsules of Mintoil twice daily for 4 weeks. The symptoms evaluated before the treatment and at 4 and 8 weeks post-treatment were abdominal bloating, pain or discomfort, diarrhea, constipation, incomplete or urgency of defecation, and the passage of gas or mucus. The frequency and intensity of these symptoms was used to calculate the total irritable bowel syndrome symptoms score. At 4 weeks, 75% of patients in the peppermint oil group demonstrated a >50% reduction of the symptoms score as compared to 38% in the placebo group. At 4 and 8 weeks in the peppermint oil group compared to that before the treatment, there was a statistically significant reduction of the total irritable bowel syndrome symptoms score whereas there was no change with the placebo.

A critical review and meta-analysis of the use of peppermint oil for irritable bowel syndrome was published by Pittler and Ernst (1998). They examined five double-blind, placebo-controlled trials; there was a significant difference between peppermint oil and placebo in three cases and no significant difference in two cases. It was concluded that although a beneficial effect of peppermint oil was demonstrated, its role in treatment was not established.
A review of 16 trials was conducted by Grigoleit and Grigoleit (2005). They concluded that there was reasonable evidence that the administration of enteric-coated peppermint oil (180–200 mg) thrice daily was an effective treatment for irritable bowel syndrome when compared to placebo or the antispasmodic drugs investigated (mebeverine, hyoscyamine, and alverine citrate).

A comparison between two commercial delayed release peppermint oil preparations found that there were differences in the pharmacokinetics in relation to bioavailability times and release site. A capsule that is more effective in delivering the peppermint oil to the distal small intestine and ascending colon would be more beneficial in the treatment of irritable bowel syndrome (White et al., 1987). It has also been suggested that the conflicting results in some trials may be due to the inclusion of patients suffering from lactose intolerance, syndrome of small intestinal bacterial overgrowth, and celiac disease, all of which have symptoms similar to irritable bowel disease (Cappello et al., 2007).

### 11.10 MEDICAL EXAMINATIONS

Although not employed in a treatment context, the antispasmodic activity of peppermint essential oil has been used to facilitate examinations of the upper and lower gastrointestinal tract. A few examples are highlighted below.

Peppermint oil has also been used during double-contrast barium enemas. The study comprised 383 patients in four groups, two being no-treatment and Buscopan groups. The preparation, consisting of 8 mL of essential oil, 0.2 mL of Tween 80 in 100 mL water, was administered in 30 mL quantities via the enema tube or mixed in with the barium meal. Peppermint oil had the same spasmylic effect as systemic Buscopan in the transverse and descending colon and a stronger effect in the cecum and ascending colon. Both methods of peppermint oil administration were equally effective (Asao et al., 2003).
Orally administered peppermint oil was used in a randomized trial in 430 patients undergoing a double-contrast barium meal examination, without other antispasmodics. A reduction in spasms of the esophagus, lower stomach, and duodenal bulb was found, along with an inhibition of barium flow to the distal duodenum and an improvement of diagnostic quality (Shigeaki et al., 2006).

During endoscopic retrograde cholangiopancreatography, Buscopan or glucagon is used to inhibit duodenal motility but produce adverse effects. Various concentrations of peppermint oil were introduced into the upper gastrointestinal tract of 40 patients undergoing the procedure. Duodenal relaxation was obtained with 20 mL of 1.6% peppermint oil solution and the procedure was performed successfully in 91.4% of patients. The inhibitory effect of peppermint oil appeared to be identical to that of glucagon, but without side effects (Yamamoto et al., 2006).

11.11 NAUSEA

A small study examined a variety of aromatherapy treatments to 25 patients suffering from nausea in a hospice and palliative care facility. Patients were offered the essential oils of *Foeniculum vulgare* var. *dulce*, *Chaemomelum nobile*, and *Mentha × piperita*, either singly or in blends, depending on individual preferences. Delivery methods included abdominal compress or massage, personal air spritzer or scentball diffuser. Only 32% of patients reported no response to the treatments and they had all just finished heavy courses of chemotherapy. Using a visual-numeric analogue scale, the remainder of patients experienced an improvement in their nausea symptoms when using the aromatherapy interventions. All patients were also taking antiemetic drugs and so the essential oils were regarded as successful complements to standard medications (Gilligan, 2005).

A 6-month trial investigated the effect of inhaled 5% *Zingiber officinale* essential oil in the prevention of postoperative nausea and vomiting (PONV). All patients were at a high risk for PONV and all used similar combinations of prophylactic intravenous antiemetics. The test group had the essential oil applied to the volar aspects of both wrists via a rollerball immediately prior to surgery. In the recovery room, patients were questioned as to their feelings of nausea. Any patient who felt that they required further medication was considered a “failure.” Prevention of PONV by ginger essential oil was effective in 80% of cases, as measured by no complaint of nausea during the recovery period. In those patients who did not receive the essential oil, 50% experienced nausea (Geiger, 2005).

Another experiment used essential oils to prevent PONV, but they were applied after surgery if the patient complained of nausea. An undiluted mixture of *Zingiber officinale*, *Elettaria cardamomum*, and *Artemisia dracunculus* essential oils in equal parts was applied with light friction to the sternocleidomastoid area and carotid-jugular axis of the neck. Of the 73 cases treated, 50 had a positive response, that is, a complete block of nausea and vomiting within 30 min. It was found that the best response (75%) was with patients who had received a single analgesic/anesthetic (de Pradier, 2006).

The use of essential oils to alleviate motion sickness has also been investigated. A blend of *Zingiber officinale*, *Lavandula angustifolia*, *Mentha spicata*, and *Mentha × piperita* essential oils in an inhalation dispenser (QueaseEase™) was given to 55 ocean boat passengers with a history of motion sickness. The oil blend was inhaled as needed during the trip and queasiness was assessed using a linear analogue scale. The product was more effective than the placebo in lowering sensations of nausea when the seas were roughest, but was not significant at other times (Post-White and Nichols, 2007).

11.12 PAIN RELIEF

There follows a number of differing conditions that have been treated with essential oils with varying biological activities, such as antispasmodic, anti-inflammatory, and so on. They all share a common effect, that of pain relief.
11.12.1 DYSMENORRHEA

The seeds of *Foeniculum vulgare* have been used in traditional remedies for the treatment of dysmenorrhea, an action attributed to the antispasmodic effect of the essential oil. An *in vitro* experiment demonstrated that fennel essential oil inhibited oxytocin- and prostaglandin E$_2$ (PGE$_2$)-induced contractions of isolated uterus; the former was considered to have a similar activity to diclofenac, a nonsteroidal anti-inflammatory drug. The overall mechanism of action is still unknown (Ostad et al., 2001).

A randomized, double-blind crossover study examined the effect of oral fennel essential oil at 1% or 2% concentration as compared to placebo for the treatment of 60 women with mild to moderate dysmenorrhea. Up to 1 mL of the solution was taken as required for the pain at intervals of not less than 4 h. In the treatment groups, the severity of the pain was significantly decreased; the efficacy of the 2% fennel oil was 67.4%, which was comparable to the efficacy of nonsteroidal anti-inflammatory drugs (Khorshidi et al., 2003).

Thirty patients with moderate to severe dysmenorrhea took part in a study to compare the activity of mefenamic acid with the essential oil of *Foeniculum vulgare* var. dulce. The evaluation was carried out during the first 5 days of three consecutive menstrual cycles. In the first cycle, no intervention was given (control); during the second cycle, 250 mg of mefenamic acid 6 hourly was prescribed; and in the third, 25 drops of a 2% solution of fennel essential oil were given 4 hourly. A self-scoring linear analogue technique was used to determine effect and potency. Both interventions effectively relieved menstrual pain as compared to the control. Mefenamic acid was more potent on the second and third days, but the result was not statistically significant. It was concluded that fennel essential oil was a safe and effective remedy but was probably less effective than mefenamic acid at the dosage used (Jahromi et al., 2003).

A third study used aromatherapy massage for the relief of the symptoms of dysmenorrhea in 67 students. The essential oils of *Lavandula officinalis*, *Salvia sclarea*, and *Rosa centifolia* (2:1:1 ratio) were diluted to 3% in 5 mL of almond oil and applied in a 15-min abdominal massage daily, 1 week before the start of menstruation, and stopping on the first day of menstruation. The control group received no treatment and the placebo group received massage with almond oil only. The results showed a significant improvement of dysmenorrhea as assessed by a verbal multidimensional scoring system for the essential oil group compared to the other two groups (Han et al., 2006).

11.12.2 HEADACHE

The effect of peppermint and eucalyptus essential oils on the neurophysiological, psychological, and experimental algesimetric parameters of headache mechanisms were investigated using a double-blind, placebo-controlled trial with 32 healthy subjects. Measurements included sensitivity to mechanical, thermal, and ischemically induced pain. Four preparations consisting of varying amounts of peppermint and/or eucalyptus oils in ethanol were applied to the forehead and temples. Eucalyptus alone had no effect on the parameters studied. A combination of both oils (10% peppermint and 5% eucalyptus) increased cognitive performance and had a muscle-relaxing and mentally relaxing effect, but did not influence pain sensitivity. Peppermint alone (10%) had a significant analgesic effect with reduction in sensitivity to headache. It was shown to exert significant effects on the pathophysiological mechanisms of clinical headache syndromes (Göbel et al., 1995a).

A second study used the same essential oils when investigating the skin perfusion of the head in healthy subjects and migraine patients. In the former, capillary flow was increased by 225% in comparison with baseline by peppermint oil, while eucalyptus decreased the flow by 16%. In migraine patients, neither essential oil had any effect. It was suggested that the absence of capillary vasodilation (normally caused by menthol) was due to impaired calcium channel function in migraine patients (Göbel et al., 1995b).
11.12.3 INFANTILE COLIC
Since animal studies had demonstrated that the essential oil of Foeniculum vulgare reduced intestinal spasm and increased the motility of the small intestine, it was used in a double-blind, randomized, placebo-controlled trial in the treatment of infantile colic. The 125 infants were all 2–12 weeks of age and those in the treatment group received a water emulsion of 0.1% fennel essential oil and 0.4% polysorbate (5–20 mL) up to four times a day. The dose was estimated to provide about 12 mg/kg/day of fennel essential oil. The control group received the polysorbate only. The treatment provided a significant improvement of colic, eliminating symptoms in 65% of infants as compared to 23.7% for the control. No side effects were noted (Alexandrovich et al., 2003).

11.12.4 JOINT PHYSIOTHERAPY
Six sports physiotherapists treated 30 patients suffering from knee or ankle pathologies of traumatic or surgical origin. Two commercial products were used simultaneously, Dermasport® and Solution Cryo®. The former was a gel consisting of the essential oils of Betula alba, Melaleuca leucadendron, Cinnamomum camphora, Syzygium aromaticum, Eucalyptus globulus, and Gaultheria procumbens. Solution Cryo contained the same essential oils minus Gaultheria procumbens but with the addition of Chamaemelum nobile, Citrus limon, and Cupressus sempervirens. Both products were at an overall concentration of 6%. Thirty minutes after application a net reduction in movement pain and joint circumference was demonstrated, along with an increase in articular flexion and extension of both joints in all patients (Le Faou et al., 2005).

11.12.5 NIPPLE PAIN
Nipple cracks and pain are a common cause of breastfeeding cessation. In a randomized trial 196 primiparous women were studied during the first 2 weeks postpartum. The test group applied peppermint water (essential oil in water, concentration not given) to the nipple and areola after each breastfeed while the control group applied expressed breast milk. The overall nipple crack rate at the end of the period in the peppermint group was 7% as compared to 23% for the control. Only 2% of peppermint group experienced severe nipple pain in contrast to 23% of the control, with 93% and 71% experiencing no pain, respectively (Melli et al., 2007).

11.12.6 OSTEOARTHRITIS
A blend of Zingiber officinale (1%) and Citrus sinensis (0.5%) essential oils was used in an experimental double-blind study using 59 patients with moderate to severe knee pain caused by osteoarthritis. The treatment group received six massage sessions over a 3-week period; the placebo received the same massage sessions but without the essential oils and the control had no intervention. Assessment of pain intensity, stiffness, and physical functioning was carried out at baseline and at post 1 and 4 weeks. There were improvements in pain and function for the intervention group in comparison with the placebo and control at post 1 week but this was not sustained to week 4. The treatment was suggested for the relief of short-term knee pain (Yip et al., 2008).

11.12.7 POSTHERPETIC NEURALGIA
A double-blind crossover study examined the effect of the essential oil of Pelargonium spp. on moderate to severe postherpetic pain in 30 subjects. They were assigned to groups receiving 100, 50, or 10% geranium essential oil (in mineral oil), mineral oil placebo, or capsaicin control. Pain relief was measured using a visual analogue scale from 0 to 60 min after treatment. Mean values for the time integral of spontaneous pain reduction was 21.3, 12.7, and 8.0 for the 100%, 50%, and 10% geranium
oils and evoked pain-reduction values were 15.8, 7.7, and 5.9, respectively. Both evoked and spontaneous pains were thus significantly reduced in a dose-dependent manner (Greenway et al., 2003).

The result is interesting because topical capsaicin cream (one of the standard treatments for this condition) relieves pain gradually over 2 weeks, while the essential oil acted within minutes. Geranium essential oil applied cutaneously in animal studies has suppressed cellular inflammation and neutrophil accumulation in inflammatory sites (Maruyama et al., 2006) but postherpetic neuralgia normally occurs after the inflammation has subsided. One of the main components of the essential oil, geraniol, and the minor components of geranial, nerol, and neral, have been shown to interact with the transient receptor potential channel, TRPV1, as does capsaicin (Stotz et al., 2008). This sensory inhibition may explain the efficacy of topical geranium oil.

11.12.8 Postoperative Pain
A randomized, placebo-controlled clinical trial was conducted to determine whether the inhalation of lavender essential oil could reduce opioid requirements after laparoscopic adjustable gastric banding. In the postanesthesia care unit, 54 patients were given either lavender (two drops of a 2% dilution) or nonscented oil in a face mask. It was found that patients in the lavender group required significantly less morphine postoperatively than the placebo group (2.38 and 4.26 mg, respectively). Moreover, significantly more patients in the placebo group required analgesics in comparison with the lavender group; 82% compared to 46% (Kim et al., 2007).

A similar study in the previous year with 50 patients who had undergone breast biopsy surgery had found that lavender essential oil had no significant effect on postoperative pain or analgesic requirements. However, a significantly higher satisfaction with pain control was noted by patients in the lavender group (Kim et al., 2006).

11.12.9 Prostatitis
One study has evaluated the use of Rowatinex for the treatment of chronic prostatitis/chronic pelvic pain syndrome, the rationale being based on the known anti-inflammatory properties of the product. A 6-week, randomized single-blind trial compared the use of Rowatinex 200 mg thrice daily with ibuprofen 600 mg thrice daily in 50 patients. Efficacy was measured by the National Institutes of Health (NIH)-Chronic Prostatitis Symptom Index (NIH-CPSI) that was completed by the patients on four occasions. The decrease in the NIH-CPSI was significant in both groups at the end of treatment and a 25% improvement in the total score was superior in the Rowatinex group (68%) compared to the ibuprofen group (40%). Although the symptomatic response was significant, no patients became asymptomatic (Lee et al., 2006).

11.12.10 Pruritis
Pruritis is one of the most common complications of patients undergoing hemodialysis. Thirteen such patients were given an arm massage with lavender and tea tree essential oils (5% dilution in sweet almond and jojoba oil) thrice a week for 4 weeks. A control group received no intervention. Pruritis score, pruritis-related biochemical markers, skin pH, and skin hydration were measured before and after the study. There was a significant decrease in the pruritis score and blood urea nitrogen level for the test group. The control group showed a decreased skin hydration between pre- and post-test whereas for the essential oil group it was significantly increased (Ro et al., 2002). The lack of a massage only group in the study meant that the effects could not be definitely associated with the essential oils.

11.13 Pediculicidal Activity
The activity of essential oils against the human head louse, *Pediculus humanus capitis*, has been investigated in a number of reports. Numerous essential oils have been found to exhibit
pediculicidal activity in vitro, with common oils such as *Eucalyptus globulus*, *Origanum marjorana*, *Rosmarinus officinalis*, and *Elettaria* cardamomum being comparable to, or more effective than *d*-phenothrin and pyrethrum (Yang et al., 2004). *Melaleuca alternifolia* and *Lavandula angustifolia* have also been found to be highly effective pediculicidal agents (Williamson et al., 2007).

Despite the availability of positive in vitro results, only one trial involving application to humans has been documented; a mixture of anise and ylang ylang essential oils in coconut extract (Paranix®) was applied once to five children. Viable lice were not found after 7 days (Scanni and Bonifazi, 2006).

### 11.14 RECURRENT APHTHOUS STOMATITIS

Recurrent aphthous stomatitis (RAS), also known as canker sores, are the most common oral mucosal lesions and although the process is sometimes self-limiting, the ulcer activity is mostly continuous and some forms may last for 20 years. Predisposing agents include bacteria and fungi, stress, mouth trauma, certain medications, and food allergies. Two essential oils both endemic to Iran have been investigated for treatment of this condition: *Zataria multiflora*, a thyme-like plant containing thymol, carvacrol, and linalool as major components, and *Satureja khuzistanica* containing predominantly carvacrol.

In a double-blind, randomized study, 60 patients with RAS received either 30 mL of an oral mouthwash composed of 60 mg of *Zataria multiflora* essential oil in an aqueous-alcoholic solution or placebo thrice daily for 4 weeks. In the treatment group, 83% of patients responded well while 17% reported a deterioration of their condition. This was compared with 13% and 87% for the placebo group, respectively. A significant clinical improvement with regard to less pain and shorter duration of the condition was found in the essential oil group (Mansoori et al., 2002).

*Satureja khuzistanica* essential oil 0.2% v/v was prepared in a hydroalcoholic solution and used in double-blind, randomized trial with 60 RAS patients. Its activity was compared with a 25% hydroalcoholic extract of the same plant and a hydroalcoholic placebo. A cotton pad was impregnated with 5 drops of preparation and placed on the ulcers for 1 min (fasting for 30 min afterwards) four times a day. The results of the extract and the essential oil groups were similar, with a significantly lower time for both pain elimination and complete healing of the ulcers in comparison with the placebo (Amanlou et al., 2007). The reported antibacterial, analgesic, antioxidant, and anti-inflammatory activities of this essential oil (Abdollahi et al., 2003; Amanlou et al., 2004, 2005) were thought responsible for the result.

### 11.15 RESPIRATORY TRACT

Given the volatile nature of essential oils, it should come as no surprise that their ability to directly reach the site of intended activity via inhalation therapy has led to their use in the treatment of a range of respiratory conditions. Moreover, a number of components are effective when taken internally, since they are bioactive at the level of bronchial secretions during their excretion. With the exception of one report, all of the research has used the individual components of either 1,8-cineole or menthol, or has employed them in combination with several other isolated essential oil components within commercial preparations.

### 11.15.1 MENTHOL

Menthol-containing essential oils have been used in the therapy of respiratory conditions for many years and the individual component is present in a wide range of over-the-counter medications. Of the eight optical isomers of menthol, *l*-(−)-menthol is the most abundant in nature and imparts a cooling sensation to the skin and mucous membranes.
Menthol is known to react with a temperature-sensitive (8–28°C range) transient receptor potential channel, leading to an increase in intracellular calcium, depolarization and initiation of an action potential (Jordt et al., 2003). This channel, known as TRPM8, is expressed in distinct populations of afferent neurons; primarily thinly myelinated Aδ cool fibers and to a lesser extent, unmyelinated C-fiber nociceptors (Thut et al., 2003). It is the interaction with the TRPM8 thermoreceptor that is responsible for the cooling effect of menthol when it is applied to the skin. This activity is not confined to the dermis, since the presence of TRPM8 has been demonstrated by animal experimentation in the squamous epithelium of the nasal vestibule (Clarke et al., 1992), the larynx (Sant’Ambrogio et al., 1991), and lung tissue (Wright et al., 1998). Thus the activation of cold receptors via inhaled menthol leads to a number of beneficial effects.

### 11.15.1.1 Antitussive

Despite being used as a component in cough remedies since the introduction of a “vaporub” in 1890, there are few human trials of menthol used alone as being effective. In a citric acid-induced cough model in healthy subjects, Packman and London (1980) found that menthol was effective, although 1,8-cineole was more efficacious. The use of an aromatic unction rather than direct inhalation may have affected the results, since the inhalation of menthol has been shown in animal models to be significantly more effective at cough frequency reduction (28% and 56% at 10 and 30 g/l, respectively) compared to 1,8-cineole (Laude et al., 1994).

A single-blind pseudorandomized crossover trial in 42 healthy children was used to compare the effect of an inhalation of either menthol or placebo on citric acid-induced cough. It was found that cough frequency was reduced in comparison with the baseline but not to that of the placebo (Kenia et al., 2008). However, the placebo chosen was eucalyptus oil, whose main component is 1,8-cineole and known to have similar antitussive properties to menthol.

Along with other ion channel modulators, menthol is recognized as a potential “novel therapy” for the treatment of chronic cough (Morice et al., 2004, p. 489). It is not clear whether the antitussive activity of menthol is due solely to its stimulation of airway cold receptors; it may also involve pulmonary C-fibers (a percentage of which also express TRPM8) or there may be a specific interaction with the neuronal cough reflex.

### 11.15.1.2 Nasal Decongestant

Menthol is often thought of as a decongestant, but this effect is a sensory illusion. Burrow et al. (1983) and Eccles et al. (1988) showed that there was no change in nasal resistance to airflow during inhalation of menthol, although the sensation of nasal airflow was enhanced. In the former experiment, 1,8-cineole and camphor were also shown to enhance the sensation of airflow, but to a lesser extent than menthol.

In a double-blind, randomized trial subjects suffering from the common cold were given lozenges containing 11 mg of menthol. Posterior rhinomanometry could detect no change in nasal resistance to airflow after 10 min; however, there were significant changes in the nasal sensation of airflow (Eccles et al., 1990).

A single-blind pseudorandomized crossover trial compared the effect of an inhalation of either menthol or placebo. The main outcome measures were nasal expiratory and inspiratory flows and volumes, as measured by a spirometer and the perception of nasal patency, assessed with a visual analogue scale. It was found that there was no effect of menthol on any of the spirometric measurements although there was a significant increase in the perception of nasal patency (Kenia et al., 2008).

Thus it has been demonstrated that menthol is not a nasal decongestant. However, it is useful in therapy since stimulation of the cold receptors causes a subjective sensation of nasal decongestion and so relieves the feeling of a blocked nose. In commercial preparations that include menthol, a true decongestant such as oxymetazoline hydrochloride is often present.
11.15.1.3 Inhibition of Respiratory Drive and Respiratory Comfort
When cold air was circulated through the nose in human breath-hold experiments, subjects were able to hold their breath longer (McBride and Whitelaw, 1981) and inhaling cold air was shown to inhibit normal breathing patterns (Burgess and Whitelaw, 1988). This indicated that cold receptors could be one source of monitoring inspiratory flow rate and volume. Several animal experiments demonstrated that the inhalation of cold air, warm air, plus menthol, or menthol alone (390 ng/mL) significantly enhanced ventilator inhibition (Orani et al., 1991; Sant'Ambrogio et al., 1992).

Sloan et al. (1993) conducted breath-hold experiments with 20 healthy volunteers. The ingestion of a lozenge containing 11 mg of menthol significantly increased the hold time, indicating a depression of the ventilatory drive. It was later postulated by Eccles (2000) that in addition to chemoreceptors detecting oxygen and carbon dioxide in the blood, cold receptors in the respiratory tract may also modulate the drive to breathe.

Eleven healthy subjects breathed through a device that had either an elastic load or a flow-resistive load. Sensations of respiratory discomfort were compared using a visual analogue scale before, during, and after inhalation of menthol. It was found that the discomfort associated with loaded breathing was significantly reduced and was more effective during flow-resistive loading than elastic loading. Inhalation of another fragrance had no effect and so the result was attributed to a direct stimulation of cold receptors by menthol, a reduction in respiratory drive being perhaps responsible (Nishino et al., 1997).

During an investigation of dyspnea, the effect of menthol inhalation on respiratory discomfort during loaded breathing was found to be inconsistent. Further tests found that the effect of menthol was most important during the first few minutes of inhalation and in the presence of high loads (Peiffer et al., 2001). The therapeutic application of menthol in the alleviation of dyspnea has yet to be described.

11.15.1.4 Bronchodilation and Airway Hyperresponsiveness
The spasmolytic activity of menthol on airway smooth muscle has been demonstrated in vitro (Taddei et al., 1988). To examine the bronchodilatory effects of menthol, a small trial was conducted on six patients with mild to moderate asthma. A poultice-containing menthol was applied daily for 4 weeks and it was found that bronchoconstriction was decreased and airway hyperresponsiveness improved (Chiyotani et al., 1994b).

A randomized, placebo-controlled trial examined the effects of menthol (10 mg nebulized twice daily for 4 weeks) on airway hyperresponsiveness in 23 patients with mild to moderate asthma. The diurnal variation in the peak expiratory flow rate (a value reflecting airway hyperexcitability) was decreased but the forced expiratory volume was not significantly altered. This indicated an improvement of airway hyperresponsiveness without affecting airflow limitation (Tamaoki et al., 1995). Later in vivo research examined the effect of menthol on airway resistance caused by capsaicin- and neurokinin-induced bronchoconstriction; there was a significant decrease in both cases by inhalation of menthol at 7.5 μg/L air concentration. The in vitro effect of menthol on bronchial rings was also studied. It was concluded that menthol attenuated bronchoconstriction by a direct action on bronchial smooth muscle (Wright et al., 1997).

In cases of asthma, the beneficial effects of menthol seem to be mainly due to its bronchodilatory activity on smooth muscle; interaction with cold receptors and the respiratory drive may also play an important role.

Recent in vitro studies have shown that a subpopulation of airway vagal afferent nerves expresses TRPM8 receptors and that activation of these receptors by cold and menthol excite these airway autonomic nerves. Thus, activation of TRPM8 receptors may provoke an autonomic nerve reflex to increase airway resistance. It was postulated that this autonomic response could provoke menthol- or cold-induced exacerbation of asthma and other pulmonary disorders (Xing et al., 2008). Direct
cold stimulation or inhalation of menthol can cause immediate airway constriction and asthma in some people; perhaps the TRPM8 receptor expression is upregulated in these subjects. The situation is far from clear.

11.15.1.5 Summary
The respiratory effects of menthol that have been demonstrated are as follows:

1. Antitussive at low concentration.
2. Increases the sensation of nasal airflow giving the impression of decongestion.
3. No physical decongestant activity.
4. Depresses ventilation and the respiratory drive at comparatively higher concentration.
5. Reduces respiratory discomfort and sensations of dyspnea.

A number of in vitro and animal experiments have demonstrated the bronchomucotropic activity of menthol (Boyd and Sheppard, 1969; Welsh et al., 1980; Chiyotani et al., 1994a), whereas there have been conflicting reports as to whether menthol is a mucociliary stimulant (Das et al., 1970) or is ciliotoxic (Su et al., 1993). Apart from the inclusion of relatively small quantities of menthol in commercial preparations that have known beneficial mucociliary effects, there are no documented human trials to support the presence of these activities.

11.15.2 1,8-Cineole
This oxide has a number of biological activities that make it particularly useful in the treatment of the respiratory tract. 1,8-Cineole has been registered as a licensed medication in Germany for over 20 years and is available as enteric-coated capsules (Soledum®). It is therefore not surprising that the majority of the trials originate from this country and use oral dosing of 1,8-cineole instead of inhalation. Rather than discuss specific pathologies, the individual activities will be examined and their relevance (alone or in combination) in treatment regimes should become apparent.

11.15.2.1 Antimicrobial
The anti-infectious properties of essential oils high in 1,8-cineole content may warrant their inclusion into a treatment regime but other components are more effective in this regard. 1,8-Cineole is often considered to have marginal or no antibacterial activity (Kotan et al., 2007), although it is very effective at causing leakage of bacterial cell membranes (Carson et al., 2002). It may thus allow more active components to enter the bacteria by permeabilizing their membranes.

1,8-Cineole does possess noted antiviral properties compared to the common essential oil components of borneol, citral, geraniol, limonene, linalool, menthol, and thymol; only that of eugenol was greater (Bourne et al., 1999). However, in comparison with the potent thujone, the antiviral potential of 1,8-cineole was considered relatively low (Sivropoulou et al., 1997).

A placebo-controlled, double-blind, randomized parallel-group trial examined the long-term treatment of 246 chronic bronchitics during winter with myrtol standardized Gelomyrtol® forte. This established German preparation consists mainly of 15% α-pinene, 35% limonene, and 47% 1,8-cineole and was administered thrice daily in 300 mg capsules. It was found to reduce the requirement for antibiotics during acute exacerbations; 51.6% compared to 61.2% under placebo. Of those patients needing antibiotics, 62.5% in the test group required them for ≤7 days whereas 76.7% of patients in the placebo group needed antibiotics for more than 7 days. Moreover, 72% of patients remained without acute exacerbations in the test group compared to 53% in the placebo group (Meister et al., 1999).

Although emphasis was given to antibiotic reduction, a significant antimicrobial effect by the preparation is unlikely to have paid an important contribution. Indeed, Meister et al. refer to reduced health impairment due to sputum expectoration and cough, and note other beneficial properties of 1,8-cineole that will be discussed in Sections 11.15.2.2 through 11.15.2.6.
11.15.2.2 Antitussive

The antitussive effects of 1,8-cineole were first proven by Packman and London in 1980, who induced coughing in 32 healthy human subjects via the use of an aerosol spray containing citric acid. This single-blind crossover study examined the effect of a commercially available chest rub containing, among others, eucalyptus essential oil. The rub was applied to the chest in a 7.5 mg dose and massaged for 10–15 s after which the frequency of the induced coughing was noted. It was found that the chest rub produced a significant decrease in the induced cough counts and that eucalyptus oil was the most active component of the rub.

1,8-Cineole interacts with TRPM8, the cool-sensitive thermoreceptor that is primarily affected by menthol. In comparison with menthol, the effect of 1,8-cineole on TRPM8 (as measured by Ca\(^{2+}\) influx kinetics) is much slower and declines more rapidly (Behrendt et al., 2004). In a similar manner to menthol, the antitussive activity of 1,8-cineole may be due in part to its stimulation of airway cold receptors.

11.15.2.3 Bronchodilation

*In vitro* tests using guinea pig trachea determined that the essential oil of *Eucalyptus tereticornis* had a myorelaxant, dose-dependent effect (10–1000 µg/mL) on airway smooth muscle, reducing tracheal basal tone and K\(^+\)-induced contractions, as well as attenuating acetylcholine-induced contractions at higher concentrations (Coelho-de-Souza et al., 2005). This activity was found to be mainly due to 1,8-cineole, although the overall effect was thought due to a synergistic relationship between the oxide and α- and β-pinene. Similar results were obtained using the essential oil of *Croton nepetaefolius*, whose major component was also 1,8-cineole (Magalhães et al., 2003).

A double-blind, randomized clinical trial over 7 days compared oral pure 1,8-cineole (3 ¥ 200 mg/day) to Ambroxol (3 ¥ 30 mg/day) in 29 patients with chronic obstructive pulmonary disease (COPD). Vital capacity, airway resistance, and specific airway conductance improved significantly for both drugs, whereas the intrathoracic gas volume was reduced by 1,8-cineole but not by Ambroxol. All parameters of lung function, peak flow, and symptoms of dyspnea were improved by 1,8-cineole therapy, but were not statistically significant in comparison with Ambroxol due to the small number of patients. In addition to other properties, it was noted that the oxide seemed to have bronchodilatory effects (Wittman et al., 1998).

11.15.2.4 Mucolytic and Mucociliary Effects

Mucolytics break down or dissolve mucus and thus facilitate the easier removal of these secretions from the respiratory tract by the ciliated epithelium, a process known as mucociliary clearance. Some mucolytics also have a direct action on the mucociliary apparatus itself.

Administered via steam inhalation to rabbits, 1,8-cineole in concentrations that produced a barely detectable scent (1–9 mg/kg) augmented the volume output of respiratory tract fluid from 9.5% to 45.3% (Boyd and Sheppard, 1971), an effect that they described as “mucotropic.” Interestingly, in the same experiment fenchone at 9 mg/kg increased the output by 186.2%, thus confirming the strong effects of some ketones in this regard. Also using rabbits, Zanker (1983) found that oxygenated monoterpenoids reduced mucus deposition and partially recovered the activity of ciliated epithelium.

Because of these early animal experiments, the beneficial effects of 1,8-cineole on mucociliary clearance have been clearly demonstrated in a number of human trials. Dorow et al. (1987) examined the effects of a 7-day course of either Gelomyrtol forte (4 ¥ 300 mg/day) or Ambroxol (3 ¥ 30 mg/day) in 20 patients with chronic obstructive bronchitis. Improved mucociliary clearance was observed in both groups, although improvement in lung function was not detected.

Twelve patients with chronic obstructive bronchitis were given a 4-day treatment with 1,8-cineole (4 ¥ 200 mg/day). By measuring the reduction in percentage radioactivity of an applied radioaerosol, significant improvements in mucociliary clearance were demonstrated at the 60 and 120 min after each administration (Dorow, 1989).

In a small double-blind study, the expectorant effect of Gelomyrtol forte (1 ¥ 300 mg/day, 14 days) was examined in 20 patients with chronic obstructive bronchitis. The ability to expectorate,
frequency of coughing attacks, and shortness of breath were all improved by the therapy, as was sputum volume and color. Both patients and physicians rated the effects of Gelomyrtol forte as better than the placebo, but due to the small group size statistically significant differences could not be demonstrated (Ulmer and Schött, 1991).

A randomized, double-blind, placebo-controlled trial was used to investigate the use of mucolytics to alleviate acute bronchitis (Mattys et al., 2000). They compared Gelomyrtol forte (4 x 300 mg, days 1–14), with Ambroxol (3 x 30 mg, days 1–3; 2 x 30 mg, days 4–14) and Cefuroxime (2 x 250 mg, days 1–6) in 676 patients. By monitoring cough frequency data, regression of the frequency of abnormal auscultation, hoarseness, headache, joint pain, and fatigue, it was shown that Gelomyrtol forte was very efficacious and comparable to the other active treatments. Overall, it scored slightly more than Ambroxol and Cefuroxime and was therefore considered to be a well-evidenced alternative to antibiotics for acute bronchitis.

Several studies have demonstrated a direct effect of 1,8-cineole on the ciliated epithelium itself. Kaspar et al. (1994) conducted a randomized, double-blind three-way crossover 4-day study of the effects of 1,8-cineole (3 x 200 mg/day) or Ambroxol (3 x 30 mg/day) on mucociliary clearance in 30 patients with COPD. Treatment with the oxide resulted in a statistically significant increase in the ciliary beat frequency of nasal cilia, a phenomenon that did not occur with the use of Ambroxol (an increase of 8.2% and 1.1%, respectively). A decrease of “saccharine-time” was clinically relevant and significant after 1,8-cineole therapy (241 s) but not after Ambroxol (48 s). Lung function parameters were significantly improved equally by both drugs.

After the ingestion of Gelomyrtol forte (3 x 1 capsule/day for 4 days) by four healthy persons and one person after sinus surgery, there was a strong increase in mucociliary transport velocity, as detected by movement of a radiolabeled component (Behrbohm et al., 1995).

In sinusitis, the ciliated beat frequency is reduced and 30% of ciliated cells convert to mucus-secreting goblet cells. The impaired mucociliary transport, excessive secretion of mucus, and edema block drainage sites leading to congestion, pain, and pressure.

To demonstrate the importance of drainage and ventilation of sinuses as a therapeutic concept, Federspil et al. (1997) conducted a double-blind, randomized, placebo-controlled trial using 331 patients with acute sinusitis. The secretolytic effects of Gelomyrtol forte (300 mg) over a 6-day period proved to be significantly better than the placebo.

Kehrl et al. (2004) used the known stimulatory effects of 1,8-cineole on ciliated epithelium and its mucolytic effect as a rationale for treating 152 acute rhinosinusitis patients in a randomized, double-blind, placebo-controlled study. The treatment group received 3 x 200 mg 1,8-cineole daily for 7 days. There was a clinically relevant and significant improvement in frontal headache, headache on bending, pressure point sensitivity of the trigeminal nerve, nasal obstruction, and rhinological secretions in the test group, as compared to the control group. It was concluded that 1,8-cineole was a safe and effective treatment for acute nonpurulent rhinosinusitis before antibiotics are indicated.

### 11.15.2.5 Anti-Inflammatory Activity

The effects of 1,8-cineole on stimulated human monocyte mediator production were studied in vitro and compared with that of budesonide, a corticosteroid agent with anti-inflammatory and immuno-suppressive effects (Juergens et al., 1998a). At therapeutic levels, both substances demonstrated a similar inhibition of the inflammatory mediators leukotriene B4 (LTB4), PGE2, and interleukin-1β (IL-1β). This was the first evidence of a steroid-like inhibition of arachidonic acid metabolism and IL-1β production by 1,8-cineole.

Later that year, the same team (Juergens et al., 1998b) reported a dose-dependent and highly significant inhibition of tumor necrosis factor-α (TNF-α), IL-1β, thromboxane B2, and LTB4 production by 1,8-cineole from stimulated human monocytes in vitro.

A third experiment combined ex vivo and in vivo testing; 10 patients with bronchial asthma were given 3 x 200 mg of 1,8-cineole daily for 3 days. Lung function was measured before the first dose, at the end of the third dose and 4 days after discontinuation of the therapy. At the same time, blood
samples were taken from which monocytes were collected and stimulated ex vivo for LTB4 and PGE2 production. Twelve healthy volunteers also underwent the treatment and their blood was taken for testing. It was found that by the end of the treatment and 4 days after, the production of LTB4 and PGE2 from the monocytes of both asthmatics and healthy individuals was significantly inhibited. Lung function parameters of asthmatic patients were significantly improved (Juergens et al., 1998c).

These three reports suggested a strong anti-inflammatory activity of 1,8-cineole via both the cyclooxygenase and 5-lipoxygenase pathways, and the possibility of a new, well-tolerated treatment of airway inflammation in obstructive airway disease.

Juergens et al. (2003) conducted a double-blind, placebo-controlled clinical trial involving 32 patients with steroid-dependent severe bronchial asthma. The subjects were randomly assigned to receive either a placebo or a 3 × 200 mg 1,8-cineole daily for 12 weeks. Oral glucocorticosteroids were reduced by 2.5 mg increments every 3 weeks with the aim of establishing the glucocorticosteroid-sparing capacity of 1,8-cineole. The majority of asthma patients receiving oral 1,8-cineole remained clinically stable despite a mean reduction of oral prednisolone dosage of 36%, equivalent to 3.8 mg/day. In the placebo group, where only four patients could tolerate a steroid decrease, the mean reduction was 7%, equivalent to 0.9 mg/day. Compared with the placebo group, 1,8-cineole recipients maintained their lung function four times longer despite receiving lower doses of prednisolone.

Increased mucus secretion often appears as an initial symptom in exacerbated COPD and asthma, where stimulated mediator cells migrate to the lungs to produce cytokines; of particular importance are TNF-α, IL-1β, IL-6, and IL-8 and those known to induce immunoglobulin E (IgE) antibody synthesis and maintain allergic eosinophilic inflammation (IL-4 and IL-5). Therefore, a study was conducted to investigate the role of 1,8-cineole in inhibiting cytokine production in stimulated human monocytes and lymphocytes in vitro (Juergens et al., 2004). It was shown that 1,8-cineole is a strong inhibitor of TNF-α and IL-1β in both cell types. At known therapeutic blood levels, it also had an inhibitory effect on the production of the chemotactic cytokines IL-8 and IL-5 and may possess additional antiallergic activity by blocking IL-4 production.

A clinically relevant anti-inflammatory activity of 1,8-cineole has thus been proven for therapeutic use in airway diseases.

11.15.2.6 Pulmonary Function

An inhaler was used to apply 1,8-cineole (Soledum Balm) to 24 patients with asthma or chronic bronchitis in an 8-day-controlled trial. In all but one patient, an objective rise in expiratory peak flow values was demonstrated. The subjective experience of their illness was significantly improved for all subjects (Grimm, 1987).

In an open trial of 100 chronic bronchitics using both inhaled (4 × 200 mg) and oral (3 × 200 mg) 1,8-cineole over 7 days, the clinical parameters of forced vital capacity, forced expiratory volume, peak expiratory flow, and residual volume were all significantly improved when compared to initial values before treatment (Mahlo, 1990).

In a randomized, double-blind, placebo-controlled study of 51 patients with COPD, 1,8-cineole (3 × 200 mg/day) was given for 8 weeks. For the objective lung functions of “airway resistance” and “specific airway resistance,” there was a clinically significant reduction of 21% and 26%, respectively. The improvement was attributed to a positive influence on disturbed breathing patterns, mucociliary clearance, and anti-inflammatory effects (Habich and Repges, 1994).

The majority of the in vivo trials involving 1,8-cineole report good, if not significant, changes in lung function parameters, whether the investigation concerns the common cold or COPD. This is not a convenient, accidental side effect of treatment but is a direct result of one or more of the factors already discussed that have direct effects on the pathophysiology of the airways. The ability to breathe more effectively and easily is an important consequence of the therapy that is sometimes minimized when dealing with the specific complexities of infection, inflammation, and so on. A compilation of human trials with 1,8-cineole is given in Table 11.3.
### Table 11.3
Summary of Human Trials Demonstrating the Beneficial Effects of 1,8-Cineole in Various Respiratory Conditions

<table>
<thead>
<tr>
<th>Patients</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asthma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Cineole inhalation, 8 days</td>
<td>Objective rise in expiratory peak flow found. Subjective experiences of illness significantly improved</td>
<td>Grimm (1987)</td>
</tr>
<tr>
<td>10</td>
<td>Cineole $3 \times 200$ mg daily, 3 days</td>
<td>LTB$_4$ and PGE$_2$ production by monocytes was significantly inhibited. Lung functions were significantly improved</td>
<td>Juergens et al. (1998c)</td>
</tr>
<tr>
<td>32</td>
<td>Cineole $3 \times 200$ mg daily, 12 weeks</td>
<td>Twelve of 16 patients in cineole group remained stable despite a 36% reduction in oral steroid dosage</td>
<td>Juergens et al. (2003)</td>
</tr>
<tr>
<td><strong>Acute bronchitis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Vaporub 3 min</td>
<td>Decreased breathing frequency, suggesting “easier breathing”</td>
<td>Berger et al. (1978a)</td>
</tr>
<tr>
<td>676</td>
<td>Gelomyrtol $4 \times 300$ mg daily, 14 days</td>
<td>Coughing, sputum consistency, well-being, bronchial hyperreactivity, and associated symptoms all improved similarly by Gelomyrtol, Ambroxol, and Cefuroxime</td>
<td>Mattys et al. (2000)</td>
</tr>
<tr>
<td><strong>Chronic bronchitis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Cineole inhalation, 8 days</td>
<td>Objective rise in expiratory peak flow found. Subjective experiences of illness significantly improved</td>
<td>Grimm (1987)</td>
</tr>
<tr>
<td>100</td>
<td>Cineole $3 \times 200$ mg daily, 7 days</td>
<td>All lung function parameters significantly improved</td>
<td>Mahlo (1990)</td>
</tr>
<tr>
<td>246</td>
<td>Gelomyrtol $3 \times 300$ mg daily, 6 months</td>
<td>Reduced acute exacerbations, reduced requirement for antibiotics, reduced treatment times when antibiotics taken. Well-being significantly improved</td>
<td>Meister et al. (1999)</td>
</tr>
<tr>
<td><strong>COPD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Gelomyrtol $4 \times 0.3$ g daily, 7 days</td>
<td>Improved mucociliary clearance</td>
<td>Dorow et al. (1987)</td>
</tr>
<tr>
<td>20</td>
<td>Gelomyrtol $1 \times 0.3$ g daily, 14 days</td>
<td>All parameters relating to coughing improved. Sputum volume increased</td>
<td>Ulmer and Schött (1991)</td>
</tr>
<tr>
<td>12</td>
<td>Cineole $4 \times 200$ mg, 4 days</td>
<td>Significant improvement of mucociliary clearance</td>
<td>Dorow (1989)</td>
</tr>
<tr>
<td>51 including 16 asthmatics</td>
<td>Cineole $3 \times 200$ mg daily, 8 weeks</td>
<td>Significant improvement in airway resistance (21%), positive effects on sputum output and dyspnea</td>
<td>Habich and Repges (1994)</td>
</tr>
<tr>
<td>30</td>
<td>Cineole $1 \times 200$ mg daily, 4 days</td>
<td>Significant improvements in lung functions of FVC and FEV$_1$ (Ambroxol and cineole equieffective), significant increase in ciliary beat frequency</td>
<td>Kaspar et al. (1994)</td>
</tr>
<tr>
<td>29</td>
<td>Cineole $3 \times 200$ mg daily, 7 days</td>
<td>All lung function parameters, peak flow and dyspnea improved from day 1 onward</td>
<td>Wittmann et al. (1998)</td>
</tr>
<tr>
<td><strong>Common cold</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Eucalyptus oil (9% of a mixture)</td>
<td>Reversed lung function abnormalities in small and large airways</td>
<td>Cohen and Dressler (1982)</td>
</tr>
<tr>
<td><strong>Sinusitis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>331</td>
<td>Gelomyrtol $300$ mg, 6 days</td>
<td>Effective treatment instead of antibiotics</td>
<td>Federspil et al. (1997)</td>
</tr>
<tr>
<td>152</td>
<td>Cineole $3 \times 200$ mg daily, 7 days</td>
<td>Effective reduction of symptoms without the need for antibiotics</td>
<td>Kehrl et al. (2004)</td>
</tr>
</tbody>
</table>
11.15.2.7  **Summary**

Although discussed separately, the multifaceted activities of 1,8-cineole perform together in harmony to provide an effective intervention that can inherently adapt to the needs of the individual patient. As already described, 1,8-cineole is known to possess the following properties:

1. Antimicrobial
2. Antitussive
3. Bronchodilatory
4. Mucolytic
5. Ciliary transport promotion
6. Anti-inflammatory
7. Lung function improvement.

Therefore, it may be seen that a diverse range of respiratory conditions of varying complexities will benefit from the use of pure 1,8-cineole or from essential oils containing this oxide as a major component.

11.15.3  **Treatment with Blends Containing Both Menthol and 1,8-Cineole**

A study measured transthoracic impedance pneumographs of 60 young children (2–40 months) with acute bronchitis before and after a 3-min application of Vaporub® to the back and chest. The data showed an early increase in amplitude up to 33%, which slowly descended during the 70-min post-treatment period to slightly above the control. Breathing frequency progressively decreased during the same period by 19.4%. Clinical observations combined with these results suggested a condition of “easier breathing” (Berger et al., 1978a). Currently, the active ingredients of Vaporub are camphor 4.8%, 1,8-cineole 1.2%, and menthol 2.6, but these components and percentages may have changed over the years.

The same team employed a similar experiment but used the pneumographic data to examine the quiet periods, that is, parts of the pneumogram where changes in the baseline were at least half of the average amplitude in more than five consecutive breathing excursions. It was found that the application of Vaporub increased quiet periods by up to 213.8%, whereas the controls (petroleum jelly application or rubbing only) never exceeded 62.4%. Thus the breathing restlessness of children with bronchitis was diminished and this was confirmed by clinical observations (Berger et al., 1978b).

By the measurement of lung and forced expiratory volumes, nasal, lower, and total airway resistances, closing volume data, the phase III slope of the alveolar plateau, and the maximum expiratory flow volume, peripheral airway dysfunction was confirmed in 24 adults with common colds. In a randomized, controlled trial, an aromatic mixture of menthol, eucalyptus oil, and camphor (56%, 9%, and 35% w/w, respectively) were vaporized in a room where the subjects were seated. Respiratory function measurements were made at baseline, 20 and 60 min after exposure. After the last measurement, phenylephrine was sprayed into the nostrils and the measurements taken again 5–10 min later to determine potential airway responsiveness. The control consisted of tap water. The results showed significant changes in forced vital capacity, forced expiratory volume, closing capacity, and the phase III slope after aromatic therapy as compared to the control. It was concluded that the aromatic inhalation favorably modified the peripheral airway dysfunction (Cohen and Dressler, 1982).

In a randomized, placebo-controlled trial of citric acid-induced cough in 20 healthy subjects, the inhalation of a combination of menthol and eucalyptus oil (75% and 25%, respectively) significantly decreased the cough frequency (Morice et al., 1994).

The effect of an aromatic inunction (Vaporub) was studied by the inhalation of a radioaerosol in a randomized, single-blinded, placebo-controlled crossover trial with 12 chronic bronchitics. It was found that after the application of 7.5 g of the product to the chest, removal of the tracheobronchial
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deposit was significantly enhanced at 30 and 60 min postinhalation, although further effects could not be demonstrated during the following 5 h, despite further application of the rub. During the first hour, mucociliary clearance was correlated with the concentration level of the aromatics (Hasani et al., 2003).

Another commercial preparation Pinimenthol®, a mixture of eucalyptus and pine needle oils plus menthol, reduced bronchospasm and demonstrated significant secretolytic effects when insufflated through the respiratory tract and when applied to the epilated skin of animals (Schäfer and Schäfer, 1981). In addition to the known effects of menthol and 1,8-cineole, pine needle oil is considered to be weakly antiseptic and secretolytic (Approved Herbs, 1998).

In a randomized, double-blind 14-day trial, 100 patients with chronic obstructive bronchitis received a combination of theophylline with β-adrenergica 2–3 times daily. The test group also received Pinimenthol. The parameters were investigated were objective (measurement of lung function and sputum) and subjective (cough, respiratory insufficiency, and pulmonary murmur). All differences in the subjective evaluations were statistically significant and of clinical importance; secretolysis was clearly shown. The addition of Pinimenthol showed a clear superiority to the basic combination therapy alone (Linsenmann and Swoboda, 1986).

A postmarketing survey was conducted of 3060 patients prescribed Pinimenthol suffering from cold, acute or chronic bronchitis, bronchial catarrh, or hoarseness. The product was given by inunction (29.6%), inhalation (17.3%), or inunction and inhalation (53.1%). Only 22 patients reported adverse effects and the efficacy of the product was judged as excellent or good by 88.3% of physicians and 88.1% of patients (Kamin and Kieser, 2007).

11.16 ALLERGIC RHINITIS

In a proof of concept study, a nasal spray was made from the essential oil of Artemisia abrotanum L. (4 mg/mL) and flavonoid extracts (2.5 μg/mL) from the same plant. The essential oil consisted primarily of 1,8-cineole and davanone at approximately 40% and 50%, respectively. Apart from a spasmolytic activity (Perfumi et al., 1995), little is known about the biological activity of davanone. The flavonoids present were thought to inhibit histamine release and interfere with arachidonic acid metabolism. The nasal spray was self-administered by 12 patients with allergic rhinitis, allergic conjunctivitis, and/or bronchial obstructive disease. They were instructed to use 1–2 puffs in each nostril at the first sign of symptoms, to a maximum of six treatments per day. All patients experienced rapid and significant relief of nasal symptoms and for those with allergic conjunctivitis, a significant relief of subjective eye symptoms was also experienced. Three of six patients with bronchial obstructive disease experienced rapid and clinically significant bronchial relief (Remberg et al., 2004).

11.17 SNORING

A blend of 15 essential oils was developed into a commercial product called “Helps stop snoring” and 140 adult snorers were recruited into a randomized trial using the product as a spray or gargle. Visual analogue scales were completed by the snorers’ partners relating to sleep disturbance each night. The treatment lasted for 14 days and results were compared to a pretrial period of the same length. The partners of 82% of the patients using the spray and 71% of patients using the gargle reported a reduction in snoring. This was compared to 44% of placebo users. The mode of action was postulated as being antispasmodic to the soft palate and pharynx (Pritchard, 2004).

11.18 SWALLOWING DYSFUNCTION

A delayed triggering of the swallowing reflex, mainly in elderly people, predisposes to aspiration pneumonia. To improve dysphagia, two different approaches using essential oils have been tried with success.
As black pepper is a strong appetite stimulant, it was postulated that nasal inhalation of the essential oil may stimulate cerebral blood flow in the insular cortex, the dysfunction of which has been reported to play a role in dysphagia. A randomized, controlled study of 105 elderly patients found that the inhalation of black pepper oil for 1 min significantly shortened the delayed swallowing time and increased the number of swallowing movements. Emission computed tomography demonstrated activation of the anterior cingulate cortex by the treatment. The inhalation of lavender essential oil or water had no effects (Ebihara et al., 2006a).

A second study used the established stimulating effects of menthol on cold receptors, since cold stimulation was known to restore sensitivity to trigger the swallowing reflex in dysphagic patients. Menthol was introduced into the pharynx of patients with mild to moderate dysphagia via a nasal catheter. The latent time of swallowing reflex was reduced significantly by menthol in a concentration-dependent manner; $10^{-2}$ menthol reduced the time to 9.4 s as compared to 13.8 s for distilled water. The use of a menthol lozenge before meals was thought appropriate (Ebihara et al., 2006b).

### 11.19 CONCLUSION

It is apparent from the diverse range of conditions that have benefitted from the administration of essential oils that their therapeutic potential is vast and yet underdeveloped. Moreover, since they are not composed of a single “magic bullet” with one target, they often have multiple effects that have additive or synergistic properties within a treatment regime.

A great many research papers investigating the bioactivity of essential oils conclude that the results are very encouraging and that clinical trials are the next step. For the majority, this step is never taken. The expense is one limiting factor and it is not surprising that clinical trials are mostly conducted once the essential oils have been formulated into a commercial product that has financial backing. It is evident that many of the claims made for essential oils in therapeutic applications have not been substantiated and an evidence base is clearly lacking. However, there is similarly a lack of research to demonstrate that essential oils are not effective interventions.

With the continuing search for new medicaments from natural sources, especially in the realm of antimicrobial therapy, it is hoped that future research into the efficacy of essential oils will be both stimulated and funded.

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Handbook of Essential Oils


# Aromatherapy with Essential Oils

*Maria Lis-Balchin*

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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 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 (*Opopanax 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, fennel, fennel-greek, 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 cassulette 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 \textit{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, benzoïn, 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, benzoïn, 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 benzoïn, 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, rolling, 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 novaehollandiae), 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,
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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.
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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 sensizers. 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

Berloque 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 Vilaplana, 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 acetylene tetramethyltetralin (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).

\textit{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 (\textit{Octeoa 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 (\textit{Cymbopogon flexuosa}), also \textit{Tagetes} oil (Basnyet, 1999). \textit{Melaleuca rosalina} (\textit{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.

\textbf{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, cetaryl 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,
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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 pre-operative 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).
Aromatherapy with Essential Oils

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.

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14 Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals

Yoshiaki Noma and Yoshinori Asakawa

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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](image-url)
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 (3R)-3,7-dimethyl-6-octene-1,2-diol (310) and (3S)-3,7-dimethyl-6-octene-1,2-diol (310′), respectively (Takeuchi and Miyazawa, 2005) (Figure 14.3).

### 14.2.2 A CYCLIC 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
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).
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 citronelic 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-butenolic 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](modified_from_Joglekar_S.S_and_R.S._Dhavlikar_1969._Appl._Microbiol._18_1084_1087.)
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...
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 (275) and neral (276) = 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

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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).

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 monoterpenes is the biotransformation of citral by *Botrytis cinerea*. *Botrytis cinerea* is a fungus of high interest in winemaking (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 (2E,5E)-3,7-dimethyl-2,5-octadiene-1,7-diol (318), (E)-3,7-dimethyl-2,7-octadiene-1,6-diol (319), and (2E,6E)-2,6-dimethyl-2,6-octadiene-1,8-diol (300); nerol (272) to (2Z,5E)-3,7-dimethyl-2,5-octadiene-1,7-diol (314), (Z)-3,7-dimethyl-2,7-octadiene-1,6-diol (315), and (2E,6Z)-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 *Penicillium 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
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
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-(2E,6E)-octadienal (322), and 1-hydroxy-3,7-dimethyl-(2E,6E)-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.
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...
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-vinyltetrahydrofuran-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

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\text{FIGURE 14.17 Four stereoisomers of furanoid linalool oxides. (Modified from Noma, Y. et al., Proc. 30th TEAC, pp. 204–206.)}
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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 cinnamomnensis 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 14.18](image1.png) Four stereoisomers of pyranoid linalool oxides.

![Figure 14.19](image2.png) Biotransformation of linalool (206) by Pseudomonas incognita (Madyastha et al., 1977) and Streptomyces albus NRRL B1865. (Modified from David, L. and H. Veschambre, 1984. Tetrahedron Lett., 25: 543–546.)
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 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.


Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals

*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, (+)-(3R,6R)-cis-pyranoid linalool oxide (217a and a′) was converted to (3R,6R)-pyranoid-cis-linalool oxide-3-malonate (217a′-1), (−)-(3S,6S)-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′), (−)-(3R,6S)-trans-linalool oxide (217b′) was transformed to (3R,6S)-trans-linalool oxide-3-malonate (217b′-1) (Figure 14.23). (+)-(3S,6S)-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).

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- 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, (±)-dihydromyrcenyl acetate (469-Ac) was not hydrolyzed by the above microorganisms (Oritani and Yamashita, 1973a).

14.2.2.3 Dihydromycenol

Dihydromycenol (329) was fed by Spodptera litura to give 1,2-epoxydihydro-2-mycenol (330) as a main product and 3β-hydroxydihydro-2-mycenol (331) as a minor product. Dihydromycenyl acetate (332) was converted to 1,2-dihydroxidihydromycenyl acetate (333) (Murata and Miyazawa, 1999) (Figure 14.26).
Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals

14.3 METABOLIC PATHWAYS OF CYCLIC MONOTERPENOIDS

14.3.1 MONOCYCLIC MONOTERPENE HYDROCARBON

14.3.1.1 Limonene

Limonene is the most widely distributed terpene in nature after $\alpha$-pinene (4) (Krasnobajew, 1984). (4R)-(+)-Limonene (68) is present in Citrus peel oils at a concentration of over 90%; a low concentration of the (4S)-(−)-limonene (68') is found in oils from the Mentha species and conifers.
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 (pathway 1), 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

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-\(\text{oxo-8-}\)\(p\)-menthen-7-oic acid (85), \(\beta\)-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 1\(\alpha\)-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 (+)-\(\alpha\)-terpineol (34) and (+)-perillic acid (82). This was the first report of the microbial conversion of limonene to (+)-\(\alpha\)-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.
species. One of these strains, designated as *Cladosporium* sp. T7 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 T12, 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 *Penicillium digitatum* and *Pencillium italicum*. (+)-(S)-Carvone (93) is a natural potato sprout inhibiting, fungicidal, 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, *Pencillium 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%), perilyl 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.


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

![Chemical Structures](image)

**FIGURE 14.31** (+)- and (−)-limonenes (68 and 68') and related compounds.
converted to (+)-isopiperitenone (111) (19%) as new metabolite, (1S,2S,4R) (+)-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-menthan-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. T7 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 14.32](image-url1)  **FIGURE 14.32**  Biotransformation of (+)-limonene (68) by *Aspergillus cellulosae* IFO4040. (Modified from Noma, Y. et al., 1992b. *Phytochemistry*, 31: 2725–2727.)

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

(4S)-(−)-limonene (68) was fed by Spodoptera litura to give (−)-limonene-7-oic acid (82), (−)-limonene-9-oic acid (70), and (−)-limonene-8,9-diol (79); (−)-limonene (68¢) was converted to (−)-limonene-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, [α]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).

(4S)- and (4R)-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).


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 (1R,2R,4R)-(−)-trans- (71a) and (15,2S,4S)-(−)-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).
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 peroxide (H$_2$O$_2$).


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**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.)
peroxide and \( p \)-cresol by a radical mechanism with the peroxidase. \((R)\)-limonene (68), \((S)\)-limonene (68'), \((1S,5R)\)-\( \alpha \)-pinene (4), \((1R,5R)\)-\( \alpha \)-pinene (4), and \((1R,6R)\)-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

*S. litura* converted \((1R)\)-trans-

### 14.3.1.3 \( p \)-Menthane

Hydroxylation of *trans*-

### 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 \( \beta \)-isopropyl pimelic acid (248) and methylisopropyl ketone (251) (Hungund et al., 1970) (Figure 14.48).

![FIGURE 14.45](image-url)

**FIGURE 14.45**  Epoxidation of limonene (68), \( \alpha \)-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.)

![FIGURE 14.46](image-url)

As shown in Figure 14.49, *Spodoptera litura* converted (4R)-p-menth-1-ene (62) at C-7 position to (4R)-phellandric acid (65) (Miyazawa et al., 1996b). On the other hand, when *Cladosporium* sp. T₁ 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. T₈ 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).
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 (1R)-8-hydroxy-3-*p*-menthen-2-one (347), (1R)-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), cuminic acid (194), 3-hydroxycuminic acid (196), 2,3-dihydroxycuminic acid (197),


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 cuminic 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 14.57** 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-shisooic acid (360a) and cis-shisooic 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-shisoo (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-shisoo (75a) as the major product with cis-shisoo (75b) and 8-hydroxy-cis-shisoo (361b). Furthermore, trans-shisoo (75a) and cis-shisoo (75b) are hydroxylated to 8-hydroxy-trans-shisoo (361a) and 8-hydroxy-cis-shisoo (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-shisooic acid (360a) and cis-shisooic 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.
A soil Pseudomonad has been found to grow with \((-\)-perillaldehyde) as the sole carbon source and to produce \((-\)-perillic acid) in a mineral salt medium (Kayahara et al., 1973). On the other hand, rabbit metabolized \((-\)-perillaldehyde) to \((-\)-perillic acid) along with minor shisool (Ishida et al., 1981a).

14.3.2.2 Phellandral and 1,2-Dihydrophellandral

Biotransformation of \((-\)-phellandral), \(\text{trans}\)-tetrahydroperillaldehyde, and \(\text{cis}\)-tetrahydroperillaldehyde was carried out by microorganisms (Noma et al., 1986, 1991a, 1991b, 1997). \((-\)-Phellandral) was metabolized mainly via \((-\)-phellandrol) to \(\text{trans}\)-tetrahydroperillyl alcohol (66a). \(\text{trans}\)-Tetrahydroperillaldehyde and \(\text{cis}\)-tetrahydroperillaldehyde were also transformed to \(\text{trans}\)-tetrahydroperillyl alcohol (66a) and \(\text{cis}\)-tetrahydroperillyl alcohol (66b).
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
Menthol (137) is one of the rare naturally occurring monocyclic monoterpenic 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 (+)-menthal (137b¢) by Aspergillus niger and Aspergillus cellulosa was described

![Figure 14.60](image-url)  

![Figure 14.61](image-url)  
Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals

(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), 7β- (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).


O-COCH2CH2COOH OH
Rodotrla
137b′-succinate

O-COCH2CH2COOH OH
Rodotrla
137b-succinate

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)

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>138b</th>
<th>142b</th>
<th>139bb</th>
<th>143b</th>
<th>139bb</th>
<th>144ba</th>
<th>141b</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. awamori</em> IFO 4033</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td><em>A. fumigatus</em> IFO 4400</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>A. sojae</em> IFO 4389</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>++++</td>
<td>−</td>
</tr>
<tr>
<td><em>A. usami</em> IFO 4338</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td><em>A. cellulosae</em> M-77</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td><em>A. cellulosae</em> IFO 4040</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><em>A. terreus</em> IFO 6123</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>A. niger</em> IFO 4049</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td><em>A. niger</em> IFO 4040</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+++</td>
<td>−</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td><em>A. niger</em> TBUYN-2</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

* Symbols +, ++, +++ etc. are relative concentrations estimated by GC-MS.
Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals

(−)-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)

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>138b¢</th>
<th>142b¢</th>
<th>140ba¢</th>
<th>143b¢</th>
<th>139ba¢</th>
<th>144ba¢</th>
<th>141b¢</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. awamori IFO 4033</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>+++</td>
<td>−</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>A. fumigatus IFO 4400</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>A. sojae IFO 4389</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>A. usami IFO 4338</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>A. cellulosae M-77</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>A. cellulosae IFO 4040</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. terreus IFO 6123</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>A. niger IFO 4049</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>A. niger IFO 4040</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>A. niger TBUYN-2</td>
<td>++</td>
<td>+</td>
<td>−</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

* Symbols +, ++, +++ etc. are relative concentrations estimated by GC-MS.
Handbook of Essential Oils

(-)-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 \( K \) and \( V_{\text{max}} \) values for the oxidation of (-)- (137b) and (+)-menthol (137b') recombinant CYP2A6 and CYP2B1 were determined to be 28 \( \mu \text{M} \) and 10.33 nmol/min/nmol P450 and 27 \( \mu \text{M} \), 5.29 nmol/min/nmol P450, 28 \( \mu \text{M} \) and 3.58 nmol/min/nmol P450, and 33 \( \mu \text{M} \) and 5.3 nmol/min/nmol P450, respectively (Nakanishi and Miyazawa, 2005) (Figure 14.68).

![Figure 14.66](image1.png)  
**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](image2.png)  
**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](image3.png)  
**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).

(±)-Isomenthenyl 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.)](image-url)
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-\(\beta\)-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 \(\beta\)-menthane-1-\(\beta\),2\(\alpha\),8-triol (334), 2\(\alpha\)-hydroxy-1,8-cineole (125b), and \(+\)-trans-sobrerol (95a) (Noma and Asakawa 2006a, 2007a) (Figure 14.76). *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).

\(-\)-α-Terpineol (34′) was biotransformed to give \(\beta\)-menthane-1-\(\beta\),2\(\alpha\),8-triol (334′), 2\(\alpha\)-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 also biotransformed 34′ to give 95a′ and \(-\)-oleuropeyl alcohol (204′), 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).

14.3.3.6 (–)-Terpinen-4-ol

*Gibberella cyanea* DSM 62719 biotransformed (S)-(–)-terpinen-4-ol (342) (1-\(p\)-menthen-4-ol) to give 2\(\alpha\)-hydroxy-1,4-cineole (132b), 1-\(p\)-menthene-4\(\alpha\),6-diol (372), and \(p\)-menthane-1\(\beta\),2\(\alpha\),4\(\alpha\)-triol (371) (Abraham et al., 1986). On the other hand, *Aspergillus niger* TBUYN-2 also biotransformed (–)-terpinen-4-ol (342) to give 2\(\alpha\)-hydroxy-1,4-cineole (132b) and (4\(R\))\(-\)\(p\)-menthane-1\(\beta\),2\(\alpha\),4\(\alpha\)-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).
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 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-Me) and

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), (+)-isodihydrocarveol (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') was reduced to give mainly (+)-trans-carveol (81a') and (+)-cis-carveol (81b') (60:40 in GC ratio). However, 41 strains of actinomycetes converted (+)-cis-carveol (81b') to give (4R,6R)-(+)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 mCPBA to (+)-carvone-8,9-epoxide (96'), followed by stereoselective reduction with NaBH4 to alcohol, which was immediately cyclized with 0.1 N H2SO4 to give diastereom mixture of bottrospicatol (92a' and b') (Nishimura et al., 1983a) (Figure 14.84).

![Figure 14.81](image-url) 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.)
Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals


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).


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

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

\textit{Streptomyces bottropensis} SY-2-1 has also different metabolic pathways for \((\pm)-\textit{trans}\)-carveol (81a) and \((\pm)-\textit{cis}\)-carveol (81b) (Noma and Iwami, 1994). Namely, \textit{Streptomyces bottropensis} SY-2-1 converted \((\pm)-\textit{trans}\)-carveol (81a) to \((\pm)-\text{carvone} (93), (\pm)-\text{carvone-8,9-epoxide} (96),\) and \((\pm)-5\alpha\)-hydroyxycarvone (98a) (Noma and Nishimura, 1982, 1984) (Figure 14.87). On the other hand, \textit{Streptomyces bottropensis} SY-2-1 converted \((\pm)-\text{cis}\)-carveol (81b) to give \((\pm)-\text{isobottrospicatol} (92b)\) and \((\pm)-\text{5-hydroxy-\textit{cis}}\)-carveol (94b) as the main products and \((\pm)-\text{bottrospicatol} (92a)\) 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 \((\pm)-\text{bottrospicatol} (92a)\) 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).

\begin{table}
\centering
\caption{Effects of \((\pm)-\textit{cis}\) (81b) and \((\pm)-\textit{trans}\)-Carveol (81a) Conversion Products by \textit{Streptomyces bottropensis} SY-2-1 on the Germination of Lettuce Seeds}
\begin{tabular}{|c|c|c|}
\hline
Compounds & Germination Rate (%) & \\
\hline
\((\pm)-\text{Carvone} (93)\) & 47 & 89 \\
\((\pm)-\text{Bottrospicatol} (92)\) & 3 & 48 \\
\((\pm)-\text{Carvone-8,9-epoxide} (96)\) & 2 & 77 \\
5\beta\text{-Hydroxyneodihydrocarveol} (102aa) & 86 & 96 \\
5\beta\text{-Hydroxycarveol} (98a) & 91 & 96 \\
Control & 95 & 96 \\
\hline
\end{tabular}
\end{table}

\textit{Note:} Concentration of each compound was adjusted at 200 ppm.

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 germination inhibitory activity of (+)-bottrospicatol (92a') was the highest of isomers. Interestingly, (−)-isobottrospicatol (92b) was not effective even in a concentration of 500 ppm. (+)-Bottrospicatal 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 (+)-bottrospicatal (92a'). Furthermore, an oxidized compound, (+)-bottrospical (374a'), exhibited higher activity than (+)-bottrospicatol (92a'). So, the germination inhibitory activity of (+)-bottrospical (374a') against several plant seeds, lettuce, green foxtail, radish, garden cress, wheat, and

![Diagram of chemical structures](image-url)
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 enantioselectivity 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).

(4R)-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-menthene-2,8-diol (50a*), 8-p-menthene-2, 8-diol (107a*), and p-menthene-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-menthene-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-menthene-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](image-url)  
(+)\-(102b) and (–)-Dihydrocarveol (102b′) were converted by 10 kinds of Aspergillus spp. to give mainly (+)- (106b) and (–)-10-hydroxydihydrocarveol (106b, 8-p-menthene-2,10-diol) and (+)- (105b) and (–)-8-hydroxydihydrocarveol (105b, 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).

![Chemical structure of eight kinds of dihydrocarveols.](image)
Euglena gracilis Z. converted (−)- and (+)-neodihydrocarveol (102a and a') to give the corresponding 8-hydroxyneoisodihydrocarveols (50a and 50a'), 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).


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.

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

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.)
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-perillyl-β-glucopyranoside (376) was converted into the corresponding oligosaccharides (377–381) using a cyclodextrin glucanotransferase (Yonemoto et al., 2005) (Figure 14.102).
14.3.3.14 Carvomenthol

(+) Iso- (49c) and (+)-neoisocarvomenthol (49d) were formed from (+)-carvotanacetone (47) via (−)-isorcarvomenthone (48b) by Pseudomonas ovalis, strain 6-1, whereas (+)-neocarvomenthol (49a′) and (−)-carvomenthol (49b′) were formed from (−)-carvotanacetone (47′) via (+)-isocarvomenthone (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 carvomenthol acetate, racemic isocarvomenthol acetate, and racemic neoisocarvomenthol acetate were asymmetrically hydrolyzed to (−)-carvomenthol (49b′) with (+)-carvomenthol acetate, (−)-isocarvomenthol (49c) with (+)-isocarvomenthol acetate, and (+)-neoisocarvomenthol (49d′) with (−)-neoisocarvomenthol acetate, respectively; racemic neocarvomenthol 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.)

14.3.4 MONOCYCLIC MONOTERPENE KETONE

14.3.4.1 α, β-Unsaturated Ketone

14.3.4.1.1 Carvone

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*

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Number of Microorganisms Used</th>
<th>Numbers of Carvone Convertible Microorganisms</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>40</td>
<td>16 (Ethanol, 93)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 (Ethanol, 93′)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26 (Glucose, 93)</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26 (Glucose, 93′)</td>
<td>65</td>
</tr>
<tr>
<td>Yeasts</td>
<td>68</td>
<td>51 (93)</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51 (93′)</td>
<td>75</td>
</tr>
<tr>
<td>Fungi</td>
<td>40</td>
<td>34 (93)</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36 (93′)</td>
<td>90</td>
</tr>
<tr>
<td>Actinomycetes</td>
<td>48</td>
<td>27 (93)</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43 (93′)</td>
<td>90</td>
</tr>
</tbody>
</table>

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¢ → 102a¢

**TABLE 14.6**

<table>
<thead>
<tr>
<th>Microorganisms that Carried Out the Hydrogenation of C=C Double Bond of Carvone by Si Plane Attack toward Microorganisms that Converted Carvone</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>100a</td>
</tr>
<tr>
<td></td>
<td>96b</td>
</tr>
<tr>
<td>Yeasts</td>
<td>74</td>
</tr>
<tr>
<td>Fungi</td>
<td>80</td>
</tr>
<tr>
<td>Actinomycetes</td>
<td>39</td>
</tr>
</tbody>
</table>

*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 metabolizing (+)-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 (1R,2S,4R)-(−)-p-menthane-2,8-diol (50a′), (1R,2S,4R)-(−)-8-p-menthene-2,10-diol (107a′), and the mixture of (1R,2S,4R,8S/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 (1R,2S,4R)-(−)-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 repellent 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 belonging to Schizosaccharomyces, Endomycopsis, Saccharomyces, Schwanniomyces, Debaryomyces, Pichia, Hansenula, Lipomyces, Torulopsis, Saccharomyces, 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 Phytophthora spp., and 48 strains of actinomycetes belonging to Streptomyces, Actinoplanes, Nocardia, Micromonospora, Microbispora, Micropolyspora, Amorphosphorangium, Thermopolspora, Planomonospora, and Streptosporangium spp.
As a result, 65% of bacteria, 75% of yeasts, 90% of fungi, and 90% of actinomycetes converted \((-\text{carvone} \ 93')\) to \((+\text{-dihydrocarvone} \ 101a')\) or \((+\text{-neodihydrocarveol} \ 102a')\) (Figure 14.105). Many microorganisms are capable of converting \((-\text{carvone} \ 93')\) to \((+\text{-neodihydrocarveol} \ 102a')\) stereospecifically. Some of the useful microorganisms are listed in Tables 14.7 and 14.8. There is no good chemical method to obtain \((+\text{-neodihydrocarveol} \ 102a')\) 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 \((+\text{-neodihydrocarveol} \ 102a')\).

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

In case of the plant pathogenic fungus \textit{Absidia glauca} \((-\text{carvone} \ 93')\) was metabolized to give the diol, 10-hydroxy- \((+\text{-neodihydrocarveol} \ 107a')\) (Nishimura et al., 1983b).

\((-\text{carvone} \ 93')\) was converted by five bacteria and one fungus (Verstegen-Haaksma et al., 1995) to give \((-\text{-dihydrocarvone} \ 101a)\), \((-\text{-isodihydrocarvone} \ 101b)\), and \((-\text{-neoisodihydrocarveol} \ 102d)\).
TABLE 14.7
Summary of Microbial and Chemical Hydrogenation of (−)-Carvone (93¢) for the Formation of (+)-Dihydrocarvone (101a¢) and (+)-Isodihydrocarvone (101b¢)

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

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).

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

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>101a'</th>
<th>101b'</th>
<th>102a'</th>
<th>102b'</th>
<th>102c'</th>
<th>102d'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torulopsis xylinus IFO 454</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Monascus anka var. rubellus IFO 5965</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fusarium anguoides Sherbakoff IFO 4467</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phytophthora infestans IFO4872</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kloeckera magna IFO 0868</td>
<td>0</td>
<td>0</td>
<td>98</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kloeckera antillarum IFO 0669</td>
<td>19</td>
<td>4</td>
<td>72</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptomyces rimosus</td>
<td>+</td>
<td>0</td>
<td>98</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Penicillium notatum Westling IFO 464</td>
<td>6</td>
<td>2</td>
<td>92</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Candida pseudotropicalis IFO 0882</td>
<td>17</td>
<td>4</td>
<td>79</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Candida parapsilosis IFO 0585</td>
<td>16</td>
<td>4</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LiAlH₄</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>67</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Meerwein–Ponndorf–Verley reduction</td>
<td>0</td>
<td>0</td>
<td>29</td>
<td>55</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>


14.3.4.1.1 **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-carvole (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 (+)-bottropicatol (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-dihydrocarveol (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.
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(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 14.109 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).

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Group 2. Carvone (93)–carveols (89)–bottrospicatols (92)–5-hydroxy-cis-carveols (12)


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, 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 \((-\text{carvone})\) to give \((-\text{-carvone-8,9-epoxide})\) via pathway 4 and \((+\text{-5\beta-hydroxycarvone})\), \((+\text{-5\alpha-hydroxycarvone})\) via pathway 3 (Noma and Sakai, 1984).

On the other hand, in case of \((+\text{-carvone})\) conversion, 44% of rare actinomycetes were capable of converting \((+\text{-carvone})\) to give \((+\text{-carvone-8,9-epoxide})\) via pathway 4 and \((-\text{-5\alpha-hydroxycarvone})\), \((-\text{-5\beta-hydroxycarvone})\), and \((-\text{-5\alpha-hydroxyneodihydrocarveol})\) via pathway 3 (Noma and Asakawa, 2008) (Figure 14.114).

14.3.4.1.1.2 Biotransformation of Carvone by Citrus Pathogenic Fungi, Aspergillus niger Tiegh

Citrus pathogenic Aspergillus niger Tiegh (CBAYN) and Aspergillus niger TBUYN-2 hydrogenated C=C double bond at C-1, 2 position of \((+\text{-carvone})\) to give \((-\text{-isodihydrocarvone})\) as the major product together with a small amount of \((-\text{-dihydrocarvone})\), of which \((-\text{-isodihydrocarvone})\) was further metabolized through two kinds of pathways as follows; namely one is the pathway to give \((-\text{-1\alpha-hydroxyisodihydrocarveol})\) via \((-\text{-1\alpha-hydroxyisodihydrocarvone})\) and the other one is the pathway to give \((+\text{-4\alpha-hydroxy-isodihydrocarvone})\) (Noma and Asakawa, 2008) (Figure 14.114).

The biotransformation of enones such as \((-\text{-carvone})\) by the cultured cells of Chlorella miniata was examined. It was found that the cells reduced stereoselectively the enones from \(\text{si-face}\) at \(\alpha\)-position of the carbonyl group and then the carbonyl group from \(\text{re-face}\) (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 \(\text{R configuration}\). \([2-2\text{H}]-(-\text{-carvone})\) was synthesized in order to clear up the hydrogenation mechanism at C-2 by microorganisms. (Compound \([2-2\text{H}]-93s\) was also easily biotransformed to \([2-2\text{H}]-8\text{-hydroxy-} (+\text{-neodihydro-carveol})\) via \([2-2\text{H}]-(+\text{-neodihydrocarveol})\). On the basis of \(1\text{H-NMR}\) spectral data of compounds \(102a\) and \(50a\), the hydrogen addition of the carbon–carbon double bond at the \(\text{C}_1\) and \(\text{C}_2\) position by Aspergillus niger TBUYN-2, Euglena gracilis Z., and Dunaliella tertiolecta occurs from the \(\text{si face}\) and \(\text{re face}\), respectively, namely, \text{anti} addition (Noma et al., 1995) (Figure 14.115) (Table 14.9).

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, (−)-(81b') 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).

![Figure 14.115](image_url)

**TABLE 14.9**

The Summary for the Stereospecificity of the Reduction of the C=C Double Bond of [2-2H]-(−)-Carvone ([2-2H]-93) by Various Kinds of Microorganisms

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Stereochemistry at C-2H of Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger TBUYN-2</td>
<td>102a</td>
</tr>
<tr>
<td><em>Euglena gracilis</em> Z</td>
<td>102a</td>
</tr>
<tr>
<td>Dunaliella tertiolecta</td>
<td>102a</td>
</tr>
<tr>
<td>The cultured cells of <em>Nicotiana tabacum</em> (Suga et al., 1986)</td>
<td>102a</td>
</tr>
</tbody>
</table>
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

### Table 14.10

<table>
<thead>
<tr>
<th>Purification of the Reductase from <em>Euglena gracilis</em> Z.</th>
<th>Total Protein (mg)</th>
<th>Total Activity Unit × 10^4</th>
<th>Sp. Act Units per Gram Protein</th>
<th>Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>125</td>
<td>2.2</td>
<td>1.7</td>
<td>1</td>
</tr>
<tr>
<td>DEAE Toyopearl</td>
<td>7</td>
<td>1.5</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>AF-Blue Toyopearl</td>
<td>0.1</td>
<td>0.03</td>
<td>30</td>
<td>18</td>
</tr>
</tbody>
</table>

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).
Cyclohexenone derivatives (379 and 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).

(101a'). Cyclohexenone derivatives (379 and 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

<table>
<thead>
<tr>
<th>Microsomal Enzyme</th>
<th>Substrate</th>
<th>Product</th>
<th>ee</th>
<th>Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>379</td>
<td>382a</td>
<td>&gt;99</td>
<td>R</td>
</tr>
<tr>
<td>-</td>
<td>385</td>
<td>386a</td>
<td>&gt;99</td>
<td>R</td>
</tr>
<tr>
<td>+</td>
<td>379</td>
<td>382b</td>
<td>85</td>
<td>S</td>
</tr>
<tr>
<td>+</td>
<td>385</td>
<td>386b</td>
<td>80</td>
<td>S</td>
</tr>
</tbody>
</table>

* Preferred configuration at α-position to the carbonyl group of the products.
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') and an the latter compound is further converted to \((+\)-neoisocarvomenthol (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 \(5\beta\)-hydroxy-\((+\)-neoisocarvomenthol (139db), \(5\alpha\)-hydroxy-\((+\)-carvotanacetone (51a), \(5\beta\)-hydroxy- \((-\)-carvomenthone (52ab), 8-hydroxy-\((+\)-carvotanacetone (44), and 8-hydroxy- \((-\)-carvomenthone (45a), whereas \((-\)-carvotanacetone (47') was converted to give \(5\beta\)-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 \((55aa'\) \((8R)\) and \(55ab'\) \((8S)\) in the
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-(−)-isosacarvomenthone (53) and 8-hydroxy-(−)-isosacarvomenthone (45b) as shown in Figure 14.123 (Noma et al., 1988b).

14.3.4.1.3 Piperitone
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

![Diagram 1](attachment:Diagram1.png)

**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.)

![Diagram 2](attachment:Diagram2.png)

Axula, 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 1-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 (−)-(1R)-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).

Biotransformation 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 exocyclic C=C double bond of pulegone (119) to give menthone derivatives (Watanabe et al., 2007) (Tables 14.12 and 14.13).

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 (4S)-isopiperitenone (111) to (3R,4S)-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

<table>
<thead>
<tr>
<th>Entry No. (Reaction Time)</th>
<th>Substrates</th>
<th>Products</th>
<th>Conversions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (3 h)</td>
<td>(R)-(+)Pulegone (119)</td>
<td>(1R, 4R)-Isomenthone (149b)</td>
<td>4.4</td>
</tr>
<tr>
<td>2 (12 h)</td>
<td>(R)-Pulegone (119)</td>
<td>(1S, 4R)-Menthone (149a)</td>
<td>6.8</td>
</tr>
<tr>
<td>3 (3 h)</td>
<td>(S)-(−)Pulegone (119')</td>
<td>(1R, 4R)-Isomenthone (149b)</td>
<td>14.3</td>
</tr>
<tr>
<td>4 (12 h)</td>
<td>(S)-Pulegone (119')</td>
<td>(1S, 4R)-Menthone (149a)</td>
<td>15.7</td>
</tr>
<tr>
<td>5 (12 h)</td>
<td>(R)-(−)-Carvone (93')</td>
<td>(1S, 4S)-Isomenthone (149b')</td>
<td>0.3</td>
</tr>
<tr>
<td>6 (12 h)</td>
<td>(S)-(−)-Carvone (93)</td>
<td>(1R, 4S)-Menthone (149a')</td>
<td>0.5</td>
</tr>
<tr>
<td>7 (12 h)</td>
<td>(1S, 5S)-Verbenone (24)</td>
<td>(1S, 4S)-Isomenthone (149b')</td>
<td>1.6</td>
</tr>
<tr>
<td>8 (12 h)</td>
<td>(1R, 5R)-Verbenone (24')</td>
<td>(1R, 4S)-Menthone (149a')</td>
<td>2.1</td>
</tr>
</tbody>
</table>

N.d.—denotes not detected.
### TABLE 14.13

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Products</th>
<th>Conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-(+)Pulegone (119)</td>
<td>(1R,4R)-Isomethone (149b)</td>
<td>26.8</td>
</tr>
<tr>
<td>(S)-(−)Pulegone (119')</td>
<td>(1S,4R)-Menthone (149a)</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>(1S,4S)-Isomethone (149b')</td>
<td>32.3</td>
</tr>
<tr>
<td></td>
<td>(1R,4S)-Menthone (149a')</td>
<td>7.1</td>
</tr>
</tbody>
</table>

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
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).

However, *Pseudomonas fragi*, IFO 3458, *Pseudomonas fluorescens*, IFO 3081, and *Aerobacter aerogenes*, 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 KH₂PO₄–Na₂HPO₄ 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 KH₂PO₄–Na₂HPO₄ 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, N₂ 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 (1S, 2S, 4S)-(−)-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

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
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-cyclohexene carboxylic acid (164b), 3-oxo-4-isopropyl-1-cyclohexene carboxylic acid (164a), and (±)-3-hydroxy-4-isopropyl-1-cyclohexene carboxylic acid (165ab) (Figure 14.131).

Soil plant pathogenic fungi, *Rhizoctonia solani* 189 converted (−)-menthone (149a) to 4β-hydroxy-(−)-menthone (392, 29%) and 1α, 4β-dihydroxy-(−)-menthone (393, 71%) (Nonoyama et al., 1999) (Figure 14.131). (−)-Menthone (149a) was transformed by *Spodoptera litura* to give 7-hydroxymenthone (151a), 7-hydroxyisomenthol (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).
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 (1S,4R,6S)-(+)-2α-hydroxy-1,8-cineole (225a), (1S,4R,6R)-(−)-2β-hydroxy-1,8-cineole (125a), (1S,4R)-(+)−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 14.131 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.)
Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals


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 Et2O 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 ikutanensis*, Ya-2-1. (Modified from Noma, Y. and H. Nishimura, 1981. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 196.)

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, [α]D + 9.3, 65.3% ee) and 3β-hydroxy-1,8-cineole (123a, [α]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...
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 (1S,2S,4R)-2α-hydroxy-1,8-cineole-2-yl-malonate (130b′) in 45% yield (ee 100%). The recovered alcohol showed 100% ee of the (1S,2S,4R)-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 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), Streptomycetes 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).
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 (1R,2R,4S)-2α-hydroxy-1,4-cineole-2-yl malonate (136') in 45% yield (ee 100%). The recovered alcohol showed an ee of 100% of the (1S,2S,4R)-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

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aspergillus niger TBUYN-2</strong></td>
<td>125a and a', 125b and b', 123b and b', 123a and a'</td>
</tr>
<tr>
<td><strong>Botryosphaeria dothidea</strong></td>
<td>2:43:49:6</td>
</tr>
<tr>
<td></td>
<td>50:50 41:59</td>
</tr>
<tr>
<td><strong>Pseudomonas flava</strong></td>
<td>4:59:34:3</td>
</tr>
<tr>
<td></td>
<td>100:0 53:47</td>
</tr>
<tr>
<td></td>
<td>29:71:0:0</td>
</tr>
<tr>
<td></td>
<td>100:0</td>
</tr>
</tbody>
</table>

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](https://example.com/figure14.142.png)

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

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-Pine (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). Isovaleral (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 degraded α-pinene (4) by the pathway proposed in Figure 14.149 to give two hydrocarbon, limonene (68) and terpinolene (346), and neutral metabolite,
borneol (36). A probable pathway has been proposed for the terminal oxidation of \( \beta \)-isopropylpimelic acid (248) in the PL strain and PIN 18 (Shukla and Bhattacharyya, 1968).

Pseudomonas PX 1 biotransformed \((+)-\alpha\)-pinene (4) to give \((+)-\alpha\)-thujone (29) and \((+)-\alpha\)-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 \((-)-\alpha\)-pinene (4¢) to give \((-)-\alpha\)-terpineol (34¢) and \((-)-\alpha\)-sobrerol (43a¢) (Noma et al., 2001). The mosquitocidal \((+)-1(R,2S,4R)-1\)-menthane-2,8-diol (50a¢) was also obtained as a crystal in the biotransformation of \((-)-\alpha\)-pinene (4¢) by Aspergillus niger TBUYN-2 (Noma et al., 2001; Noma, 2007) (Figure 14.151).

\((1R)-(+)\)-\(\alpha\)-Pinene (4) and its enantiomer (4¢) were fed to Spodoptera litura to give the corresponding \((+)-\) and \((-)-\)verbenones (24 and 24¢) and \((+)-\) and \((-)-\)myrtenols (5 and 5¢) (Miyazawa et al., 1996c) (Figure 14.152).

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

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

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

### 14.4.1.2 \(\beta\)-Pinene

\[\begin{align*}
1 & \quad (+)-\beta\text{-Pinene} \\
1' & \quad (-)-\beta\text{-Pinene}
\end{align*}\]
(+)-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 putidarvilla* (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
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 Spodoptera litura. (Modified from Miyazawa, M. et al., 1996c. *Proc. 40th TEAC*, pp. 84–85.)

\[
\begin{align*}
\text{S. litura} \quad &\quad \text{4} \quad\rightarrow\quad \text{24} + \text{5} \\
\text{S. litura} \quad &\quad \text{4′} \quad\rightarrow\quad \text{24′} + \text{5′}
\end{align*}
\]


\[
\begin{align*}
\text{P450 2B6} \quad &\quad \text{4′} \quad\rightarrow\quad \text{23′} + \text{5′}
\end{align*}
\]


\[
\begin{align*}
\text{Rabbit} \quad &\quad \text{4} \quad\rightarrow\quad \text{23a} + \text{5} + \text{7} \\
\text{Rabbit} \quad &\quad \text{4′} \quad\rightarrow\quad \text{23a′}
\end{align*}
\]


\[
\begin{align*}
\text{B. cinerea} \quad &\quad \text{4′} \quad\rightarrow\quad \text{24′} + \text{2a′} + \text{434′} + \text{468′}
\end{align*}
\]
As shown in Figure 14.159, (+)-I 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).


(−)-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-hydroxymenthone (45a′) to (−)-carvone (93′) to give 6β-hydroxyfenchol (349ba′) and 6β,7-dihydroxyfenchol (412ba′). Furthermore, (−)-trans-pinocarveol (2a′) was metabolized by rearrangement 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.
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Rearrangement reaction (Noma and Asakawa, 2005a) (Figure 14.162). 6β-Hydroxyfenchol (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 metabolized 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-hydroxycurcumenone (44'), (−)-8-hydroxycurvomenthone (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 (myrtnanol) (8ba') (39%) and (−)-1-p-methene-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 parafficussus and Dendroctonus brevicomis, respectively (Ishida et al., 1981b) (Figure 14.164).

14.4.1.3 (+-)Camphene
Racemate camphene (437 and 437') is a bicyclic monoterpenic hydrocarbon found in Liquidamar species, Chrysanthemum, Zingiber officinale, Rosmarinus officinalis, and among other plants. It

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.
**14.4.1.4 3-Carene and Carane**


Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals

(+)-3-Carene (439) was biotransformed by rabbits to give \(\text{m-mentha-4,6-dien-8-ol} \) (71.6\%) as the main metabolite together with its aromatized \(\text{m-cymen-8-ol} \). 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 (\(-\))\text{-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 \textit{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**

\textit{Euglena gracilis} Z biotransformed (\(-\))-myrtenal (6\') to give (\(-\))-myrtenol (5\') as the major product and (\(+\))-mytenoic 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)-\textit{Trans} and (R)-\textit{cis}-myrtanal (435a\' and 435b\') were also transformed to trans- and cis-myrtanol (8a\' and 8b\') as the major products and (S)-\textit{trans}- and (R)-\textit{cis}-myrtnoic acid (436a\' and 436b\') as the minor products, respectively (Noma et al., 1991a) (Figure 14.168).
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
Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals

(--)-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'),

\[
\begin{align*}
\text{2a} & : 15,3R,5S \\
\text{2b} & : 15,3S,5R \\
\text{2a'} & : 1R,3S,5R \\
\text{2b'} & : 1R,3R,5S
\end{align*}
\]

Pinocarveol

\[
\begin{align*}
\text{8a} & : S. litura \\
\text{8a'} & : S. litura
\end{align*}
\]
(-)-3-isopinanone (413'), and (+)-2α-hydroxy-3-pinanone (414') to (+)-2α,5-dihydroxy-3-pinanone (415') (pathway 1). Furthermore, (+)-trans-pinocarveol (2a') was metabolized by 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).

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 (−)-\(\text{420ba}^-\) and (+)-isopinocampheol (\(\text{420ba}'\)) was observed. The phylogenetic position of the individual strains could be seen in their ability to form the products from (+)-isopinocampheol (\(\text{420ba}'\)). The formation of 1,3-dihydroxypinane (\(\text{421ba}'\)) is a domain of bacteria, while 3,5-(\(\text{415ba}'\)) or 3,6-dihydroxypinane (\(\text{428baa}'\)) 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 (\(\text{420ba}'\)) was metabolized to \(4\beta\)-hydroxy-(+)-isopino-campheol (\(\text{424}'\)), \(2\beta\)-hydroxy-(+)-isopinocampherol acetate (\(\text{425ba}'\)-Ac), and \(2\alpha\)-methyl,3-(2-methyl-2-hydroxy-propyl)-cyclopenta-1\(\beta\)-ol (\(\text{432}'\)) (Wolf-Rainer, 1994) (Figure 14.174).
(-)-Isopinocampheol (420ba) was converted by Spodoptera litura to give (1R,2S,3R,5S)-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 (+)-(1S,2S,3S,5R)-pinane-3,5-diol (422ba, 6.6%), (-)-(1R,2R,3R,5S)-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 (+)-(1S,2S,3S,5R)-pinane-3,5-diol (422ba', 6.3%) and (-)-(1R,2R,3R,5S)-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 (1R,2R,3S,4S,5R)-3,4-pinanediol (484ba) and (1S,2S,3S,5R,6R)-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 14.175  Biotransformation of (-)- (420ba) and (+)-isopinocampheol (420ba') by Spodoptera litura. (Modified from Miyazawa, M. et al., 1997c. Phytochemistry, 45: 945–950.)

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).


(+) (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

(1R,2R,4S)-(+) - Fenchol (11a) was converted by *Aspergillus niger* TBUNY-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).
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 14.182** 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 Aspergillus niger 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).


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 hydroxyanisol (BHA) (Noma and Asakawa, 2006b,c).

Citrus pathogenic fungi, Aspergillus niger Tiegh (CBAYN) also transformed (−)-nopol (455') 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

![Verbenone](image)
Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals


(−)-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
(+)-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 C1 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 monoxygenases in ring cleavage strategies were established (Trudgill, 1990). A degradation pathway of (+)-camphor (37) by Pseudomonas putida ATCC 17453 and Mycobacterium rhodochrous T1 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-pyrene (231), isohydroxy-camphoric acid (232), isoketocamphoric acid (233), and 3,4,4-trimethyl-5-o xo-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-pyrene (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-oxocamphor (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-endo-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

(+)-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 14.190  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
14.4.4.2.3 3-Pinanone (Pinocamphone and Isopinocamphone)

(+)- (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
(-)-2α-Hydroxy-3-pinaneone (414) was incubated with *Aspergillus niger* TBUYN-2 to give (-)-2α, 5-dihydroxy-3-pinaneone (415) predominantly, whereas the fungus converted (+)-2α-hydroxy-3-pinaneone (414¢) mainly to 2α, 5-dihydroxy-3-pinaneone (415¢), 2α,9-dihydroxy-3-pinaneone (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-pinaneone (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-pinaneone (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-pinaneone (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.
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-diol (419ab) by Dr. Radhika Samarasekera, Industrial Technology Institute, Sri Lanka. (-)-2-Hydroxy-3-pinanone (414b¢) showed the mosquito knock-down activity and the mosquitocidal 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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Knock-Down (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-2,5-Dihydroxy-3-pinanone (415, 2%)</td>
<td>27</td>
<td>20</td>
</tr>
<tr>
<td>(+)-2,5-Dihydroxy-3-pinanone (415', 2%)</td>
<td>NT</td>
<td>7</td>
</tr>
<tr>
<td>(+)-2-Hydroxy-3-pinanone (414, 2%)</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>(+)-2-Hydroxy-3-pinanone (414', 2%)</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>(+)-2-Hydroxy-3-pinanone (414', 1%)</td>
<td>53</td>
<td>7</td>
</tr>
<tr>
<td>(+)-Pinane-2,3,5-triol (419, 2%)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>(+)-Pinane-2,3,5-triol (419', 2%)</td>
<td>13</td>
<td>NT</td>
</tr>
<tr>
<td>(+)-Pinane-2,3-diol (418, 2%)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>(+)-Pinane-2,3-diol (418', 2%)</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

* The results are against Culex quinequefasciatus.

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

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC (mg/mL)</th>
<th>Compounds</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>419</td>
<td>415'</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0.5</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0.5</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>0.25</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>0.5</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>0.5</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>MRSA</td>
<td>0.25</td>
<td>0.125</td>
<td>0.125</td>
</tr>
</tbody>
</table>


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 myrcenic 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 isopinocamphene (413), which is further hydroxylated to give 2-hydroxy-3-pinanol (414). Compound 414 is further metabolized to give pinane-2,3-diol (419), 2,5-dihydroxy- (415), and 2,9-dihydroxy-3-pinanol (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-hydroxyfenchene (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 fenchoolquinone (19) to 2-hydroxyfenchene (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-menthol (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-menthene-7-oic acid (84), 2-oxo-8-p-menthene-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; Dhavlikar 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 myrtenoic 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). Myrtenol (8) is metabolized to 3-hydroxy- (9) and 4-hydroxymyrtenol (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-hydrocarvomethenone (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-hydroxycarvomethenone (45) by Aspergillus niger TBUXN-2, the formation of p-menthane-2,8-diol (50) is very high enantio- and diastereoselective in the reduction of 8-hydroxycarvomethenone (Noma, 2007). 8-Hydroxycarvotanacetone (44) is a common metabolite from sobrerol (43) and carvotanacetone (47). Namely, carvotanacetone (47) is metabolized to carvomethone (48), carvomenthol (49), 8-hydroxycarvomethol (50), 5-hydroxy-carvotanacetone (51), 8-hydroxycarvotanacetone (44), 5-hydroxycarvomethone (52), and 2,3-lactone (56) (Gibbon and Pirt, 1971; Gibbon et al., 1972, Noma et al., 1974a; 1985c; 1988b). Carvomethone (48) is metabolized to 45, 8-hydroxycarvomethol (50), 1-hydroxycarvomethenone (53), and p-menthane-1,2-diol (54) (Noma et al., 1985b, 1988b). Compound 52 is metabolized to 6-hydroxymethol (139), which is the common metabolite of menthol (137) (see Figure 14.201). Carvomethol (49) is metabolized to 8-hydroxycarvomethenol (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), carveol (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-oxic 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; Savithry et al., 1997; Van der Werf and de Bont, 1998a, 1998b; Noma et al., 1982, 1992d). Dihydrocarvone (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), carveol (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). Bottropiscatel (92) and 5-hydroxyxcarvone (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, carveol (93) is reduced to carveol (81) (Noma, 1980). Secondly, it is epoxidized to carveol-8,9-epoxide (96), which is further metabolized to dihydroxycarvone-8,9-epoxide (97), dihydroxycarvone-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-methene-1,2-diol (71) via 1-hydroxydihydrocarvone (72), 10-hydroxydihydrocarvone (106), and dihydrocarvone (102), which is metabolized to 10-hydroxydihydrocarveol (107), p-methane-2,8-diol (50), dihydrocarveol-8,9-epoxide (100), p-methane-2,8,9-triol (104), and dihydrobottropsicatal (105) (Noma et al., 1985a, 1985b). In the biotransformation of (+)-carvone by plant pathogenic fungi, Aspergillus niger Tiegh, isodihydrocarveol (101) was metabolized to 4-hydroxysisodihydrocarvone (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-methen-7-ic acid (83). Perillic acid (82) is metabolized degradatively to 84–89 as shown in Figure 14.200 (Swamy et al., 1965; Dhavalikar and Bhattacharya, 1966; Dhavalikar et al., 1966; Ballal et al., 1967; Shukla et al., 1968; Shukula and Bhattacharya, 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-hydroxypiperitone (118). Pulegone (119) is metabolized to 112, 8-hydroxymenthene (121), and 8,9-dehydromenthene (120).

Metabolic pathways of menthol (137), menthone (149), thymol (179), and carvacrol 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-hydroxymethyl- (144) and 1,8-dihydroxy- (146) and 7,8-dihydroxymethyl (148) (Asakawa et al., 1991; Takahashi et al., 1994; Van der Werf et al., 1997). Racemic menthol 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-oxidohexenoic acid (154), 1-hydroxymenthene (150), piperitone (156), 7-hydroxymenthene (151), menthone-7-al (163), menthone-7-ic acid (164), and 7-carboxymenthol (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-hydroxyperitone (161), and piperitone-7-ic acid (162) (Lassak et al., 1973). Compound 149 is also formed from menthone (148) by hydrogenation (Mukherjee et al., 1973), which is metabolized to 6-hydroxymenthene (181), 6-hydroxy-4-p-methen-3-one. 6-hydroxymenthene (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-hydroxymenthene (150) and 4-hydroxypiperitone (167) (Lassak et al., 1973; Miyazawa et al., 1991e). Piperitenone oxide (168) is metabolized to 1-hydroxymenthene (150), 1-hydroxyisopulegone (169), and 2,3-seco-p-menthacacetone-3-en-l-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-methane (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-hydroxyepi-camphor (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 CO₂ 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-methane-1,9-diol (254) and p-methane-1,7-diol (255) (Tukamoto et al., 1974, 1975; Noma et al., 1990). Compound 255 is degradatively metabolized via 256–248 to CO₂ 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-Hydroxy-linalool (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), linalool (68), 3,7-dimethyloctane-1,8-diol (266) via 3,7-dimethyl-6-octene-1,8-diol (265), 267, citronellal (261), dihydrocitronellal (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, citronellal acid (262), nerol-6,7-epoxide (273), and nerol (275). Compound 272 is metabolized to neric acid (277). Compounds 275 and 276 are isomerized to each other. Compound 276 is completely decomposed to CO₂ 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-oxocenoic acid (283), 6-methyl-5-heptenoic acid (284), 4-methyl-3-heptenoic acid (284), 4-methyl-3-pentenoic acid (285), and 3-methyl-2-butenolic 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-oxocenoic 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 microorganisms 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 biodegraded 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 esterified 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 degraded 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 *Diploida 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-oxonop-2′,4′-dihydrobenzylether (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. myrtenol; 9. 3-hydroxymyrtenol; 10. 4-hydroxymyrtenol; 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

<table>
<thead>
<tr>
<th>Microbiological Oxidation</th>
<th>Oxidation of alcohols</th>
<th>Oxidation of primary alcohols to aldehydes and acids</th>
<th>Oxidation of secondary alcohols to ketones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-)-trans- Carveol (81a’), (++)-trans-carveol (81a)</td>
<td>(-)-cis-carveol (81b’), (+)-cis-carveol (81b), 2α-hydroxy-1,8-cineole (125b), 3β-hydroxy-1,8-cineole (123b), 3β-hydroxy-1,8-cineole (123a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydroxylation of nonallylic carbon</td>
<td>(-)-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)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydroxylation of allylic carbon</td>
<td>(-)-Isodihydrocarvone (101b), (+)-neodihydrocarvone (102a’), (-)-dihydrocarveol (102b’), (+)-dihydrocarveol (102b), (+)-limonene (68), (-)-limonene (68’)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oxidation of olefins</td>
<td>(+)-Neodihydrocarvone (102a’), (+)-dihydrocarveol (102b), (-)-dihydrocarveol (102b’), (+)-limonene (68), (-)-limonene (68’)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formation of epoxides and oxides</td>
<td>(+)-Neodihydrocarvone (102a’), (+)-dihydrocarveol (102b), (-)-dihydrocarveol (102b’), (+)-limonene (68), (-)-limonene (68’)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formation of diols</td>
<td>(+)-Neodihydrocarvone (102a’), (+)-dihydrocarveol (102b), (-)-dihydrocarveol (102b’), (+)-limonene (68), (-)-limonene (68’)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formation of triols</td>
<td>(+)-Neodihydrocarvone (102a’)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microbiological Reduction</th>
<th>Reduction of aldehydes to alcohols</th>
<th>Reduction of ketones to alcohols</th>
<th>Reduction of saturated ketones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reduction of olefins</td>
<td>Reduction of α,β-unsaturated ketones</td>
<td>(+)-Carvone (93’), (+)-carvone (93), (-)-carvotanacetone (47’), (+)-carvotanacetone (47)</td>
</tr>
<tr>
<td></td>
<td>Hydrogenation of olein conjugated with carbonyl group</td>
<td>Hydrogenation of olein not conjugated with a carbonyl group</td>
<td>(+)-Dihydrocarvone (101a’), (-)-isodihydrocarvone (101b), (+)-carvomenthone (48a’), (-)-isocarvomenthone (48b)</td>
</tr>
</tbody>
</table>
### TABLE 14.19
Microbiological Oxidation, Reduction, and Another Reaction Patterns of Monoterpenoids by *Euglena gracilis* Z

<table>
<thead>
<tr>
<th>Microbiological Oxidation</th>
<th>Microbiological Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidation of alcohols</strong></td>
<td><strong>Reduction of aldehydes</strong></td>
</tr>
<tr>
<td>Oxidation of primary alcohols to aldehydes and acids</td>
<td>Reduction of terpene aldehydes to terpene alcohols</td>
</tr>
<tr>
<td>Oxidation of secondary alcohols to ketones</td>
<td>Reduction of aromatic and related aldehydes to alcohols</td>
</tr>
<tr>
<td><em>Diastereo- and enantioselective dehydrogenation is observed in carveol, borneol, and isoborneol</em></td>
<td>Reduction of aliphatic aldehydes to alcohols</td>
</tr>
<tr>
<td><strong>Hydroxylation</strong></td>
<td><strong>Hydroxylation</strong></td>
</tr>
<tr>
<td>Hydroxylation of nonallylic carbon</td>
<td>Hydroxylation of aliphatic carbon</td>
</tr>
<tr>
<td>Hydroxylation of allylic carbon</td>
<td></td>
</tr>
<tr>
<td><strong>Oxidation of olefins</strong></td>
<td><strong>Reduction of ketones</strong></td>
</tr>
<tr>
<td>Formation of epoxides and oxides</td>
<td>Reduction of saturated ketones</td>
</tr>
<tr>
<td>Formation of diols</td>
<td>Reduction of α,β-unsaturated ketones</td>
</tr>
<tr>
<td>Formation of triols</td>
<td></td>
</tr>
<tr>
<td><strong>Lactonization</strong></td>
<td><strong>Hydrogenation of olefins</strong></td>
</tr>
<tr>
<td><strong>Reduction of aldehydes to acids</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td>Myrtenal (6), myrtanal, (−)-perillaldehyde (78), <em>trans-</em> and *cis-*1,2-dihydroperillaldehydes (261a and 261b), (−)-phellandral (64), <em>trans-</em> and *cis-*tetrahydroperillaldehydes, cuminaldehyde (193), (±)- and (−)-citronellal (261 and 261′)</td>
<td>(−)-Carvone (93′), (±)-carvone (93), (−)-carvotanacetone (47′), (±)-carvotanacetone (47), (−)-carvone-8,9-epoxides (96′), (±)-carvone-8,9-epoxides (96)</td>
</tr>
<tr>
<td><em>Acids were obtained as minor products</em></td>
<td></td>
</tr>
<tr>
<td><strong>Microbiological Reduction</strong></td>
<td><strong>Hydrogenation of olefin not conjugated with a carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Reduction of ketones to alcohols</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Hydrogenation of olefins</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Reduction of aldehydes to acids</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Oxidation of alcohols</strong></td>
<td></td>
</tr>
<tr>
<td>Oxidation of primary alcohols to aldehydes and acids</td>
<td>Oxidation of secondary alcohols to ketones</td>
</tr>
<tr>
<td>(−)-<em>trans</em>-Carveol (81a′), (±)-<em>cis</em>-carveol (81b), (±)-isoborneol (36b)</td>
<td></td>
</tr>
<tr>
<td><strong>Microbiological Oxidation</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Reduction of ketones to alcohols</strong></td>
<td><strong>Hydrogenation of olefin not conjugated with a carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Hyperhydration of olefins</strong></td>
<td><strong>Hydrogenation of olefin not conjugated with a carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Reduction of aldehydes to acids</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Oxidation of alcohols</strong></td>
<td><strong>Hydrogenation of olefin not conjugated with a carbonyl group</strong></td>
</tr>
<tr>
<td>Oxidation of primary alcohols to aldehydes and acids</td>
<td>Oxidation of secondary alcohols to ketones</td>
</tr>
<tr>
<td>(−)-<em>trans</em>-Carveol (81a′), (±)-<em>cis</em>-carveol (81b), (±)-isoborneol (36b)</td>
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</tr>
<tr>
<td><strong>Microbiological Oxidation</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Reduction of ketones to alcohols</strong></td>
<td><strong>Hydrogenation of olefin not conjugated with a carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Hyperhydration of olefins</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Reduction of aldehydes to acids</strong></td>
<td><strong>Hydrogenation of olefin not conjugated with a carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Oxidation of alcohols</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td>Oxidation of primary alcohols to aldehydes and acids</td>
<td>Oxidation of secondary alcohols to ketones</td>
</tr>
<tr>
<td>(−)-<em>trans</em>-Carveol (81a′), (±)-<em>cis</em>-carveol (81b), (±)-isoborneol (36b)</td>
<td></td>
</tr>
<tr>
<td><strong>Microbiological Oxidation</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Reduction of ketones to alcohols</strong></td>
<td><strong>Hydrogenation of olefin not conjugated with a carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Hyperhydration of olefins</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Reduction of aldehydes to acids</strong></td>
<td><strong>Hydrogenation of olefin not conjugated with a carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Oxidation of alcohols</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td>Oxidation of primary alcohols to aldehydes and acids</td>
<td>Oxidation of secondary alcohols to ketones</td>
</tr>
<tr>
<td>(−)-<em>trans</em>-Carveol (81a′), (±)-<em>cis</em>-carveol (81b), (±)-isoborneol (36b)</td>
<td></td>
</tr>
<tr>
<td><strong>Microbiological Oxidation</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Reduction of ketones to alcohols</strong></td>
<td><strong>Hydrogenation of olefin not conjugated with a carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Hyperhydration of olefins</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Reduction of aldehydes to acids</strong></td>
<td><strong>Hydrogenation of olefin not conjugated with a carbonyl group</strong></td>
</tr>
<tr>
<td><strong>Oxidation of alcohols</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td>Oxidation of primary alcohols to aldehydes and acids</td>
<td>Oxidation of secondary alcohols to ketones</td>
</tr>
<tr>
<td>(−)-<em>trans</em>-Carveol (81a′), (±)-<em>cis</em>-carveol (81b), (±)-isoborneol (36b)</td>
<td></td>
</tr>
<tr>
<td><strong>Microbiological Oxidation</strong></td>
<td><strong>Hydrogenation of olefin conjugated with carbonyl group</strong></td>
</tr>
<tr>
<td>TABLE 14.19 (continued)</td>
<td>Microbiological Oxidation, Reduction, and Another Reaction Patterns of Monoterpenoids by <em>Euglena gracilis</em> Z</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| **Hydrolysis**           | Hydrolysis of ester  
(+)-trans- and cis- Carveyl acetates (108a and b),  
(−)-cis-carveyl acetate (108b'), (−)-cis-carveyl propionate, geranyl acetate (270) |
| **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**        | Geraniol (271), nerol (272)  
9-hydroxyfenchone; 17, fenchone-9-al; 18, fenchone-9-oic acid; 19, fenchquinone; 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, thujol alcohol; 30, 1-hydroxythujone; 31, 1,3-dihydroxythujone; 32, pinyln cation; 33, 1-p-methene-8-cation; 34, α-terpineol; 35, bornyl cation; 36, bornel; 37, camphor; 38, α-pinene epoxide; 39, isonovalal; 40, novalal; 41, 2-hydroxypinyl cation; 42, 6-hydroxy-1-p-methene-8-cation; 43, *trans*-soberol; 44, 8-hydroxycurvotanacetone; (carvonehydrate); 45, 8-hydroxacarvomenthone; 46, 1-p-methen-2-ol; 47, carvotanacetone; 48, carvomenthone; 49, carvomenthol; 50, 8-hydroxycarvomenthol; 51, 5-hydroxycurvotanacetone; 52, 5-hydroxycarvomenthone; 53, 1-hydroxycurvomenthone; 54, p-methane-1,2-diol; 55, p-methane-2,9-diol; 56, 2,3-lactone; 57, diepoxide; 58, 8,9-epoxy-1-p-menthol; 59, 1-p-methene-4-cation; 60, terpine hydrate; 61, oleuropic acid (8-hydroxyperilic acid); 62, 1-p-methene; 63, phellandrol; 64, phellandral; 65, phellandric acid; 66, 7-hydroxy-p-methane; 67, 2-(4-methyl-3-cyclohexenylidene)-propionic acid; 68, limonene; 69, limonene-1,2-epoxide; 70, 1-p-methene-9-oic acid; 71, limonene-1,2-diol; 72, 1-hydroxy-8-p-methene-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-methene-8,9-diol; 80, 4-hydroxy-p-menth-1,8-diene (4-terpineol); 81, carveol; 82, perillic acid; 83, 4,9-dihydroxy-1-p-methene-7-oic acid; 84, 2-hydroxy-8-p-methen-7-oic acid; 85, 2-oxo-8-p-methen-7-oic acid; 86, β-isopropyl pimelic acid; 87, isopropenylglutaric acid; 88, isobutenoic acid; 89, isobutyric acid; 90, 1-p-methene-8,9-diol; 91, carveol-8,9-epoxide; 92, bottropsicatol; 93, carvone; 94, 5-hydroxycurveol; 95, 1-p-methene-6,9-diol; 96, carvone-8,9-epoxide; 97, dihydrocarvone-8,9-epoxide; 98, 5-hydroxycurveol; 99, 5-hydroxydihydrocurveol; 100, 5-hydroxydihydrocarveol; 101, dihydrocarveol; 102, dihydrocarveol; 103, dihydrocarveol-8,9-epoxide; 104, p-methene-2,8,9-triol; 105, dihydrobottropsicatol; 106, 10-hydroxydihydrocurveol; 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-hydroxyisopiperitenone; 118, 7-hydroxyisopiperitenone; 119, pulegone; 120, 8,9-dehydromethenone; 121, 8-hydroxymethenone; 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, menthone; 149, menthone; 150, 1-hydroxymenthone; 151, 7-hydroxymenthone; 152, 3,7-dimethyl-6-hydroxyoctanoic acid; 153, 3,7-dimethyl-6-octanoic acid; 154, 2-methyl-2,5-dioxoheptanoic 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-oic acid; 165, 7-carboxymenthol; 166, piperitone oxide; 167, 4-hydroxypiperitone; 168, piperitone oxide; 169, 1-hydroxypulegone; 170, 2,3-seco-p-methylacetone-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, 6-hydroxymenthylbenzenone, 6-hydroxy-4-p-methen-3-one; 182, 3-hydroxythymol, 4-quinol; 183, 2-hydroxythymoquinone; 184, 2,4-dimethyl-6-oxy-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, cuminic acid; 195, cis-2,3-dihydroxy-2,3-dihydro-p-cumaric acid; 196, 3-hydroxycumaric acid; 197, 2,3-dihydroxy-p-cumaric acid; 198, 2-hydroxy-6-oxy-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-hydroxyperilyl 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-octene-6-oic acid; 213, phellandric acid-8-cation; 214, 2,3-epoxylinalool; 215, 5-menthy-5-vinyltetrahydro-2-furanol; 216, 5-menthyl-5-vinyltetrahydro-2-furanone; 217, 2,2,6-trimethyl-3-hydroxy-6-vinyltetrahydropyran; 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, isobutyratic 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-pyrene; 232, isohydroxycamphor acid; 233, isoketocamphor 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-pyrene; 242, 5-hydroxy-3,4,4-trimethyl-2-heptene-1,7-dioic acid; 243, 3-hydroxycamphor; 244, camphorquinone; 245, 2-hydroxyecipcamphor; 246, 2-hydroxy-p-menthane-7-oic acid; 247, 2-oxo-p-menthane-7-oic acid; 248, 3-isopropylheptane-1,7-dioic acid; 249, 3-isopropylpentane-1,5-dioic acid; 250, 4-methyl-3-oxopentanolic acid; 251, methylisopropyl 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-methene-7-al; 257, 1-hydroxy-p-menthene-7-oic acid; 258, citronellol; 259, dihydrocitronellol; 260, 2,8-dihydroxy-2,6-dimethyl octane; 261, citronellall; 262, citronelic acid; 263, pulegol; 264, 7-hydroxymethyl-6-octene-3-ol; 265, 3,7-dimethyl-6-octene-1,8-diol; 266, 3,7-dimethyloctane-1,8-diol; 267, isopulegol; 268, 6,7-epoxyctronellol; 269, citronellyl acetate; 270, geranyl acetate; 271, geraniol; 272, nerol 273, nerol-6,7-epoxide; 274, 6,7-epoxygeraniol; 275, neral; 276, geraniol; 277, neric acid; 278, geranic acid; 279, 2,6-dimethyl-8-hydroxy-7-oxy-2-octene; 280, 6-methyl-5-heptenoic acid; 281, 7-methyl-3-carboxymethyl-2,6-octadiene-1-oic acid; 282, 7-methyl-3-hydroxycarboxymethyl-6-octen-1-oic acid; 283, 7-methyl-3-oxy-6-octanoic 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-
m-cymene-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-caranne; 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-methen-8-ol; 454, 3-oxonapol; 455, nopol benzyl ether; 456, 4-oxonopl-2',4'-dihydroxybenzyl ether; 457, 7-hydroxymethyl-1-p-methen-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-oi 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-oi acid; 473, 8,9-dehydrocarvacrol; 474, 8-hydroxycarvacrol; 475, 7-hydroxycarvacrol; 476, carvacrol-7-oi acid; 477, 8,9-dihydroxyarvacrol; 478, 7,9-dihydroxyarvacrol methyl ether; 479, 7-hydroxythymol; 480, 9-hydroxythymol; 481, thymol-7-oi acid; 482, 7,9-dihydroxythymol; 483, thymol-9-oi acid; 484, (1R,2R,3S,4S,5R)-3,4-pinanediol.

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16 Industrial Uses of Essential Oils

W. S. Brud

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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 archaeological 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).
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

### TABLE 16.1

<table>
<thead>
<tr>
<th>Company Name</th>
<th>Country</th>
<th>Established</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antoine Chiris</td>
<td>France (Grasse)</td>
<td>1768</td>
</tr>
<tr>
<td>Cavallier Freres</td>
<td>France (Grasse)</td>
<td>1784</td>
</tr>
<tr>
<td>Dodge &amp; Olcott Inc.</td>
<td>USA (New York)</td>
<td>1798</td>
</tr>
<tr>
<td>Roure Bertrand Fils and Justin Dupont</td>
<td>France (Grasse)</td>
<td>1820</td>
</tr>
<tr>
<td>Schimmel &amp; Co.</td>
<td>Germany (Leipzig)</td>
<td>1829</td>
</tr>
<tr>
<td>J. Mero-Boyveau</td>
<td>France (Grasse)</td>
<td>1832</td>
</tr>
<tr>
<td>Stafford Allen and Sons</td>
<td>United Kingdom (London)</td>
<td>1833</td>
</tr>
<tr>
<td>Robertet et Cie</td>
<td>France (Grasse)</td>
<td>1850</td>
</tr>
<tr>
<td>W.J. Bush</td>
<td>United Kingdom (London)</td>
<td>1851</td>
</tr>
<tr>
<td>Payan-Bertrand et Cie</td>
<td>France (Grasse)</td>
<td>1854</td>
</tr>
<tr>
<td>A. Boake Roberts</td>
<td>United Kingdom (London)</td>
<td>1865</td>
</tr>
<tr>
<td>Fritsche-Schimmel Co</td>
<td>USA (New York)</td>
<td>1871</td>
</tr>
<tr>
<td>V. Mane et Fils</td>
<td>France (Grasse)</td>
<td>1871</td>
</tr>
<tr>
<td>Haarman&amp;Reimer</td>
<td>Germany (Holzminden)</td>
<td>1874</td>
</tr>
<tr>
<td>R.C. Treatt Co.</td>
<td>United Kingdom (Bury)</td>
<td>1886</td>
</tr>
<tr>
<td>N.V. Polak und Schwartz</td>
<td>Holland (Zaandam)</td>
<td>1889</td>
</tr>
<tr>
<td>Ogawa and Co.</td>
<td>Japan (Osaka)</td>
<td>1893</td>
</tr>
<tr>
<td>Firmenich and Cie</td>
<td>Switzerland (Geneve)</td>
<td>1895</td>
</tr>
<tr>
<td>Givaudan S.A.</td>
<td>Switzerland (Geneve)</td>
<td>1895</td>
</tr>
<tr>
<td>Maschmeijer Aromatics</td>
<td>Holland (Amsterdam)</td>
<td>1900</td>
</tr>
</tbody>
</table>

*Note:* Companies continuing to operate under their original name are printed in bold.
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. 0.3 l (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**

<table>
<thead>
<tr>
<th>Oil Name</th>
<th>Consumption (tons)</th>
<th>Approximate Value (€ million)</th>
<th>Major Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>50,000</td>
<td>275</td>
<td>Soft drinks, sweets, fragrances</td>
</tr>
<tr>
<td>Cornmint (Mentha arvensis)</td>
<td>25,000</td>
<td>265</td>
<td>Oral care, chewing gum, confectionery, fragrances, menthol crystals</td>
</tr>
<tr>
<td>Peppermint</td>
<td>4500</td>
<td>120</td>
<td>Oral care, chewing gum, confectionery, liquors, tobacco, fragrances</td>
</tr>
<tr>
<td>Eucalyptus (Eucalyptus globulus)</td>
<td>4000</td>
<td>22</td>
<td>Oral care, chewing gum, confectionery, pharmaceuticals, fragrances</td>
</tr>
<tr>
<td>Lemon</td>
<td>3500</td>
<td>21</td>
<td>Soft drinks, sweets, diary, household chemicals</td>
</tr>
<tr>
<td>Citronella</td>
<td>3000</td>
<td>33</td>
<td>Perfumery, toiletries, household chemicals</td>
</tr>
<tr>
<td>Eucalyptus (E. citriodora)</td>
<td>2100</td>
<td>10</td>
<td>Confectionery, oral care, chewing gum, pharmaceuticals, fragrances</td>
</tr>
<tr>
<td>Clove leaf</td>
<td>2000</td>
<td>24</td>
<td>Condiments, sweets, pharmaceuticals, household chemicals</td>
</tr>
<tr>
<td>Spearmint (Mentha spicata)</td>
<td>2000</td>
<td>46</td>
<td>Oral care, chewing gum, confectionery</td>
</tr>
<tr>
<td>Cedarwood (Virginia)</td>
<td>1500</td>
<td>22</td>
<td>Perfumery, toiletries, household chemicals</td>
</tr>
<tr>
<td>Lime</td>
<td>1500</td>
<td>66</td>
<td>Soft drinks, sweets, diary, fragrances</td>
</tr>
<tr>
<td>Lavandin</td>
<td>1000</td>
<td>15</td>
<td>Perfumery, cosmetics, toiletries</td>
</tr>
<tr>
<td>Litsea cubeba</td>
<td>1000</td>
<td>20</td>
<td>Citral for soft drinks, fragrances</td>
</tr>
<tr>
<td>Cedarwood (China)</td>
<td>800</td>
<td>11</td>
<td>Perfumery, toiletries, household chemicals</td>
</tr>
<tr>
<td>Camphor</td>
<td>700</td>
<td>3</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>Coriander</td>
<td>700</td>
<td>40</td>
<td>Condiments, pickles, processed food, fragrances</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>700</td>
<td>9</td>
<td>Soft drinks, fragrances</td>
</tr>
<tr>
<td>Star anise</td>
<td>700</td>
<td>7</td>
<td>Liquors, sweets, bakery, household chemicals</td>
</tr>
<tr>
<td>Patchouli</td>
<td>600</td>
<td>69</td>
<td>Perfumery, cosmetics, toiletries</td>
</tr>
<tr>
<td>Basil</td>
<td>500</td>
<td>12</td>
<td>Condiments, processed food, perfumery, toiletries</td>
</tr>
<tr>
<td>Mandarine</td>
<td>500</td>
<td>30</td>
<td>Soft drinks, sweets, liquors, perfumery, toiletries</td>
</tr>
</tbody>
</table>

---

*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
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.
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

---

**Rose Blanche**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rose oil</td>
<td>10 g</td>
</tr>
<tr>
<td>Rose absolute</td>
<td>20 g</td>
</tr>
<tr>
<td>Patchouli oil</td>
<td>25 g</td>
</tr>
<tr>
<td>Bergamot oil</td>
<td>50 g</td>
</tr>
<tr>
<td>Linalool</td>
<td>60 g</td>
</tr>
<tr>
<td>Benzyl acetate</td>
<td>7 g</td>
</tr>
<tr>
<td>Phenylethyl acetate</td>
<td>75 g</td>
</tr>
<tr>
<td>Citronellol</td>
<td>185 g</td>
</tr>
<tr>
<td>Geraniol</td>
<td>200 g</td>
</tr>
<tr>
<td>Phenylethyl alcohol</td>
<td>300 g</td>
</tr>
</tbody>
</table>
### TABLE 16.4
Prices of Selected Standard and “Organic” Essential Oils

<table>
<thead>
<tr>
<th>Oil Name</th>
<th>Standard Quality (€/kg)a</th>
<th>Organic Quality (€/kg)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>5.50</td>
<td>35</td>
</tr>
<tr>
<td>Cornmint (M. arvensis)</td>
<td>10.50</td>
<td>50</td>
</tr>
<tr>
<td>Peppermint</td>
<td>27.00</td>
<td>100</td>
</tr>
<tr>
<td>Eucalyptus (E. globulus)</td>
<td>5.50</td>
<td>26</td>
</tr>
<tr>
<td>Lemon</td>
<td>6.00</td>
<td>30</td>
</tr>
<tr>
<td>Citronella</td>
<td>11.00</td>
<td>23</td>
</tr>
<tr>
<td>Eucalyptus (E. citriodora)</td>
<td>5.00</td>
<td>34</td>
</tr>
<tr>
<td>Clove leaf</td>
<td>12.00</td>
<td>60</td>
</tr>
<tr>
<td>Spearmint (M. spicata)</td>
<td>23.00</td>
<td>40</td>
</tr>
<tr>
<td>Cedarwood (Virginia)</td>
<td>15.00</td>
<td>58</td>
</tr>
<tr>
<td>Lime</td>
<td>44.00</td>
<td>92</td>
</tr>
<tr>
<td>Lavandin</td>
<td>15.00</td>
<td>36</td>
</tr>
<tr>
<td>Litsea cubeba</td>
<td>20.00</td>
<td>44</td>
</tr>
<tr>
<td>Cedarwood (China)</td>
<td>14.00</td>
<td>53</td>
</tr>
<tr>
<td>Camphor</td>
<td>4.50</td>
<td>24</td>
</tr>
<tr>
<td>Coriander</td>
<td>57.00</td>
<td>143</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>13.00</td>
<td>170</td>
</tr>
<tr>
<td>Patchouli</td>
<td>115.00</td>
<td>250</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Position</th>
<th>Product</th>
<th>Average Dosage of Fragrance Compound in Product (%)</th>
<th>Average Content of Essential Oils in Fragrance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Perfumes</td>
<td>10.0–25.0</td>
<td>5–30a</td>
</tr>
<tr>
<td>2</td>
<td>Toilet waters</td>
<td>3.0–8.0</td>
<td>5–50a</td>
</tr>
<tr>
<td>3</td>
<td>Skin care cosmetics</td>
<td>0.1–0.6</td>
<td>0–10</td>
</tr>
<tr>
<td>4</td>
<td>Deodorants (inclusive deoparfum)</td>
<td>0.5–5.0</td>
<td>0–10</td>
</tr>
<tr>
<td>5</td>
<td>Shampoos</td>
<td>0.3–2.0</td>
<td>0–5</td>
</tr>
<tr>
<td>6</td>
<td>Body cleansing products (liquid soaps)</td>
<td>0.5–3.0</td>
<td>0–5</td>
</tr>
<tr>
<td>7</td>
<td>Bath preparations</td>
<td>0.5–6.0</td>
<td>0–10</td>
</tr>
<tr>
<td>8</td>
<td>Soaps</td>
<td>0.5–3.0</td>
<td>0–5</td>
</tr>
<tr>
<td>9</td>
<td>Toothpastes</td>
<td>0.5–2.5</td>
<td>10–50b</td>
</tr>
<tr>
<td>10</td>
<td>Air fresheners</td>
<td>0.5–30.0</td>
<td>0–20</td>
</tr>
<tr>
<td>11</td>
<td>Washing powders and liquids</td>
<td>0.1–0.5</td>
<td>0–5</td>
</tr>
<tr>
<td>12</td>
<td>Fabric softeners</td>
<td>0.1–0.5</td>
<td>0–10</td>
</tr>
<tr>
<td>13</td>
<td>Home care chemicals</td>
<td>0.5–5.0</td>
<td>0–5</td>
</tr>
<tr>
<td>14</td>
<td>Technical products</td>
<td>0.1–0.5</td>
<td>0–5</td>
</tr>
<tr>
<td>15</td>
<td>Aromatherapy and organic products</td>
<td>0.1–0.5</td>
<td>100</td>
</tr>
</tbody>
</table>

a Traditional perfumery products contained more natural oils than modern ones.
b Mainly mint oils.
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

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**TABLE 16.6**

Average Content of Flavors in Food Products and of Essential Oils in Flavors

<table>
<thead>
<tr>
<th>Position</th>
<th>Food Products</th>
<th>Flavor Dosage in Food Product (%)</th>
<th>Essential Oils Content in Flavor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alcoholic beverages</td>
<td>0.05–0.15</td>
<td>3–100</td>
</tr>
<tr>
<td>2</td>
<td>Soft drinks</td>
<td>0.10–0.15</td>
<td>2–5</td>
</tr>
<tr>
<td>3</td>
<td>Sweets (confectionery, chocolate, etc.)</td>
<td>0.15–0.25</td>
<td>1–100</td>
</tr>
<tr>
<td>4</td>
<td>Bakery (cakes, biscuits, etc.)</td>
<td>0.10–0.25</td>
<td>1–50</td>
</tr>
<tr>
<td>5</td>
<td>Ice creams</td>
<td>0.10–0.30</td>
<td>2–100</td>
</tr>
<tr>
<td>6</td>
<td>Diary products, desserts</td>
<td>0.05–0.25</td>
<td>1–50</td>
</tr>
<tr>
<td>7</td>
<td>Meat and fish products (also canned)</td>
<td>0.10–0.25</td>
<td>10–20</td>
</tr>
<tr>
<td>8</td>
<td>Sauces, ketchup, condiments</td>
<td>0.10–0.50</td>
<td>2–10</td>
</tr>
<tr>
<td>9</td>
<td>Food concentrates</td>
<td>0.10–0.50</td>
<td>1–25</td>
</tr>
<tr>
<td>10</td>
<td>Snacks</td>
<td>0.10–0.15</td>
<td>2–20</td>
</tr>
</tbody>
</table>
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.

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Unilever: http://www.unilever.com/aboutus
Aroma-Vital Cuisine
Healthy and Delightful Consumption by the Use of Essential Oils

Maria M. Kettenring and Lara-M. Vucemilovic-Geeganage

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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 (amyloytic 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 savorness 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 disposition 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

<table>
<thead>
<tr>
<th>Basic Essential Oils</th>
<th>Mixtures</th>
<th>Emulsifier Seasonings</th>
<th>Recipes Example</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EURO ASIA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lime (<em>Citrus aurantiifolia</em>)</td>
<td>5 drops</td>
<td>1. Oil 50 mL sesame oil</td>
<td>Asian style</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Dairy prod. 50 mL mayonnaise</td>
<td>Eggs</td>
</tr>
<tr>
<td>Coriander seed (<em>Coriandrum sativum</em>)</td>
<td>1 drop</td>
<td>3. Vinegar 50 mL rice vinegar</td>
<td>Sushi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Sweetener 50 mL agave syrup</td>
<td>Chutney</td>
</tr>
<tr>
<td>Ginger (<em>Zingiber officinalis</em>)</td>
<td>2 drops</td>
<td>5. Salt 50 mg sea-salt</td>
<td>Spice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6. Tofu and co 50 mL soy sauce</td>
<td>Marinated fried tofu</td>
</tr>
<tr>
<td>Lemongras (<em>Cymbopogon citratus</em>)</td>
<td>1 drop</td>
<td>7. Vegetables and fruits 50 mL coconut milk</td>
<td>Rice and curry</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8. Nuts and seeds 50 mg sesame seeds</td>
<td>Spice</td>
</tr>
<tr>
<td>Green pepper (<em>Piper nigrum</em>)</td>
<td>1 drop</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>O SOLE MIO</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyme linalool (<em>Thymus vulgaris</em>)</td>
<td>1 drop</td>
<td>1. 50 mL olive oil</td>
<td>Pasta</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. 50 mL egg yolk</td>
<td>Omelette</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. 50 mL balmy vinegar</td>
<td>Salad</td>
</tr>
<tr>
<td>Rosemary cineole (<em>Rosmarinus officinalis</em>)</td>
<td>1/2 drop</td>
<td>4. 50 mL honey</td>
<td>Cuisine Provencal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5. 50 mg sea-salt</td>
<td>Spice</td>
</tr>
<tr>
<td>Clementine (<em>Citrus deliciosa</em>)</td>
<td>5 drops</td>
<td>6. 50 mg tofu</td>
<td>Grilled tofu</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7. 50 mg avocado</td>
<td>Guacamole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.</td>
<td>Pesto</td>
</tr>
<tr>
<td><strong>CAPRI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange (<em>Citrus sinensis</em>)</td>
<td>5 drops</td>
<td>1. 50 mL hazelnut oil</td>
<td>Desserts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. 50 mL buttermilk</td>
<td>Drink</td>
</tr>
<tr>
<td>Lemon (<em>Citrus limon</em>)</td>
<td>3 drops</td>
<td>3. 50 mL cider vinegar</td>
<td>Salad</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. 100 mL maple syrup</td>
<td>Desserts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5. 50 mg sea-salt</td>
<td>Spice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6. 50 mL apple vinegar</td>
<td>Fruit salad</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7. 50 mg avocado</td>
<td>Sauce</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8. 50 mg walnuts</td>
<td>Cakes</td>
</tr>
<tr>
<td><strong>BERGAMOT-GRAND MANIER</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grapefruit (<em>Citrus paradisi</em>)</td>
<td>5 drops</td>
<td>1. 50 mL walnut oil</td>
<td>Salad</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. 50 mg butter</td>
<td>Cake</td>
</tr>
</tbody>
</table>

continued
### TABLE 18.1 (continued)

Basic Spice Rack of Essential Oils: How to Prepare Essential Oil Mixtures and Essential Oil Seasonings

<table>
<thead>
<tr>
<th>Basic Essential Oils</th>
<th>Mixtures</th>
<th>Emulsifier Seasonings</th>
<th>Recipes Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange (<em>Citrus sinensis</em>)</td>
<td>5 drops</td>
<td>3. 1 L white wine</td>
<td>Beverage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. 50 mg raw sugar</td>
<td>Sweets</td>
</tr>
<tr>
<td>Limon (<em>Citrus limon</em>)</td>
<td>2 drops</td>
<td>5. 50 mg sea-salt</td>
<td>Spice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6. 50 mL tamarind sauce</td>
<td>Thai cuisine</td>
</tr>
<tr>
<td>Bergamot (<em>Citrus bergamia</em>)</td>
<td>2 drops</td>
<td>7. 50 mL lemon juice</td>
<td>Drink</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8. 50 mg pumpkin seeds</td>
<td>Soup</td>
</tr>
</tbody>
</table>

**MAGIC ORANGE**

| Orange (*Citrus sinensis*) | 5 drops | 1. 50 mL almond oil | Sweets |
| Vanilla extract (*Vanilla planifolia*) | 3 drops | 3. 50 mL raspberry vinegar | Fruit salad |
| Kakaao extract (*Theobroma cacao*) | 3 drops | 4. 100 mL honey/treacle | Sweets |
| Rose (*Rosa damascena*) | 1/2 drop | 7. 50 mg bananas | Desserts |

**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 |

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### 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 inter-
     mixture (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.

![image of bergamot and lime](image)

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.

*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.
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).
Handbook of Essential Oils

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 Chèvre 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 apple's 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.
**Aroma-Vital Cuisine**

**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

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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>
<thead>
<tr>
<th>English Name</th>
<th>Latin Name</th>
<th>Plant Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anise oil</td>
<td>Anisi aetheroleum</td>
<td>Pimpinella anisum L. fruits</td>
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<tr>
<td>Bitter-fennel fruit oil</td>
<td>Foeniculi amari fructus aetheroleum</td>
<td>Foeniculum vulgare Miller subsp. vulgare var. vulgare</td>
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<td>Cinnamomum cassia Blume (Cinnamomum aromaticum Nees)</td>
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<td>Cinnamomum zeylanicum Nees</td>
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<td>Cinnamomum verum J.S. Presl.</td>
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<td>Cymbopogon winterianus Jowitt</td>
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<td>Clove oil</td>
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<td>Pinus mugo Turra.</td>
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<td>Citri reticulatae aetheroleum</td>
<td>Citrus reticulata Blanco</td>
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<td>Matricariae aetheroleum</td>
<td>Matricaria recutita L. (Chamomilla recutita (L.) Ranschert)</td>
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<td>Mint oil, partly dementholized</td>
<td>Menthae arvensis aetheroleum</td>
<td>Mentha canadensis L. (Mentha arvensis L. var. glabrata (Benth.) Fern, Mentha arvensis L. var. piperascens Malinv. ex Holmes) Japanese mint</td>
</tr>
<tr>
<td></td>
<td>partim mentholi privum</td>
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</tr>
<tr>
<td>Neroli oil (formerly bitter-orange flower oil)</td>
<td>Neroli aetheroleum (formerly Aurantii amari floris aetheroleum)</td>
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<td>Nutmeg oil</td>
<td>Myristicae fragransis aetheroleum</td>
<td>Mentha × piperita L.</td>
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<tr>
<td>Peppermint oil</td>
<td>Menthae piperitae aetheroleum</td>
<td>Pinus silvestris L.</td>
</tr>
<tr>
<td>Pine silvestris oil</td>
<td>Pini silvestris aetheroleum</td>
<td>Rosmarinus officinalis L.</td>
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<td>Lavandula lanfolia Medik.</td>
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<td>Spicae aetheroleum</td>
<td>Illicium verum Hooker fil.</td>
</tr>
<tr>
<td>Star anise oil</td>
<td>Anisi stellati aetheroleum</td>
<td>Citrus sinensis (L.) Osbeck (Citrus aurantium L. var. dulcis L.) Cheel, Melaleuca linearifolia Smith, Melaleuca dissitiflora F. Mueller and other species</td>
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<td>Sweet orange oil</td>
<td>Aurantii dulcis aetheroleum</td>
<td>Melaleuca alternifolia (Maiden et Betch) Cheel, Melaleuca linearifolia Smith, Melaleuca dissitiflora F. Mueller and other species</td>
</tr>
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<td>Melaleucae aetheroleum</td>
<td>Thymus vulgaris L., T. zygis L.</td>
</tr>
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<td>Thyme oil</td>
<td>Thymi aetheroleum</td>
<td>Pinus pinaster Aiton.</td>
</tr>
<tr>
<td>Turpentine oil, <em>Pinus pinaster</em> type</td>
<td>Terebinthini aetheroleum ab pinum pinastrium</td>
<td>(Maritime pine)</td>
</tr>
</tbody>
</table>
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. Monoterpane-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 a 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 (Moscetti, 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 repellant 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 (Syzygium 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 (Syzygium 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 masticha*, *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 masticha* 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 Herbornix 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 ethereal 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


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.
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.
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.

![Florida grapefruit oil price graph](image)

**FIGURE 20.7** Price graph of grapefruit oil.

![Yearly average price for piperita peppermint oil](image)

**FIGURE 20.8** Price graph of peppermint oil (piperita).
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.

**FIGURE 20.9** Price graph of peppermint oil (arvensis).
21 Storage and Transport of Essential Oils

Klaus-Dieter Protzen

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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):

- 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 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.


Because of their composition essential oils are classified by regulatory authorities in the EU as “Natural” but also as “Chemical Substances” (Dueshop, 2007).


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 Registration, Evaluation, Authorization of Chemicals. 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
Storage and Transport of Essential Oils

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 “unknown or variable composition, complex reaction products, and biological 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.
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.
**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)
(VL123) 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 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/](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:

![Warning labels](image)

The following warning labels cover the majority of risks:

<table>
<thead>
<tr>
<th>Code</th>
<th>Label</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>190</td>
<td>Xn</td>
<td>Harmful—a St. Andrew’s Cross (Xn)</td>
</tr>
<tr>
<td>174</td>
<td>N</td>
<td>Dangerous for the environment</td>
</tr>
<tr>
<td>60</td>
<td>Xi</td>
<td>Irritant—a St. Andrew’s Cross (Xi)</td>
</tr>
<tr>
<td>12</td>
<td>T</td>
<td>Toxic—a skull and cross-bones (T)</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Corrosive—the symbol showing the damaging effect of an acid</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Code</th>
<th>R-Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>205</td>
<td>R-43</td>
<td>May cause sensation by skin contact</td>
</tr>
<tr>
<td>158</td>
<td>R-65</td>
<td>Harmful—may cause lung damage if swallowed</td>
</tr>
<tr>
<td>103</td>
<td>R-51/53</td>
<td>Toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment</td>
</tr>
<tr>
<td>95</td>
<td>R-38</td>
<td>Harmful if swallowed</td>
</tr>
<tr>
<td>88</td>
<td>R-50/53</td>
<td>Very toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment</td>
</tr>
<tr>
<td>80</td>
<td>R-10</td>
<td>Flammable</td>
</tr>
<tr>
<td>40</td>
<td>R-52/53</td>
<td>Harmful to aquatic organisms—may cause long-term adverse effects on the aquatic environment</td>
</tr>
<tr>
<td>38</td>
<td>R-22</td>
<td>Harmful if swallowed.</td>
</tr>
</tbody>
</table>
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

  *OJ L 259, 15.10.1979, p. 10–28 (DA, DE, EN, FR, IT, NL)*

  *OJ L 200, 30.7.1999, p. 1–68 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)*

  *OJ L 184, 15.7.1988, p. 61–66 (ES, DA, DE, EL, EN, FR, IT, NL, PT)*

  *OJ L 43, 14.2.1997, p. 1–6 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)*

  *OJ L 109, 6.5.2000, p. 29–42 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)*

  *OJ L 31, 1.2.2002, p. 1–24 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)*

  *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)*

  *OJ L 104, 8.4.2004, p. 1–35 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)*

  *OJ L 123, 24.4.1998, p. 1–63 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)*


  *(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)

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**Amended by**

- A1: Act of Accession of Greece
- A2: Act of Accession of Spain and Portugal

**Corrected by**

- C1: Corrigendum, OJ L 255, 25.9.1984, p. 28 (84/415/EEC)
- C7: Corrigendum, OJ L 58, 26.2.2004, p. 28 (2003/83/EC)

**REFERENCES**


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 squeezer waiting for fruits.
FIGURE 4.7  FMC extractor.

FIGURE 4.18  Oil and muddy water in the Florentine flask.
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.