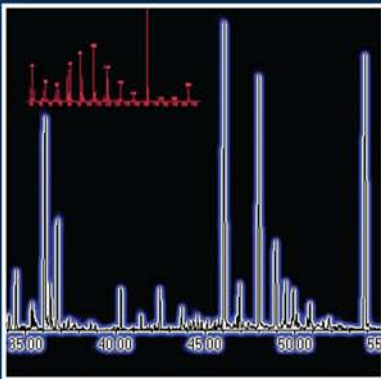


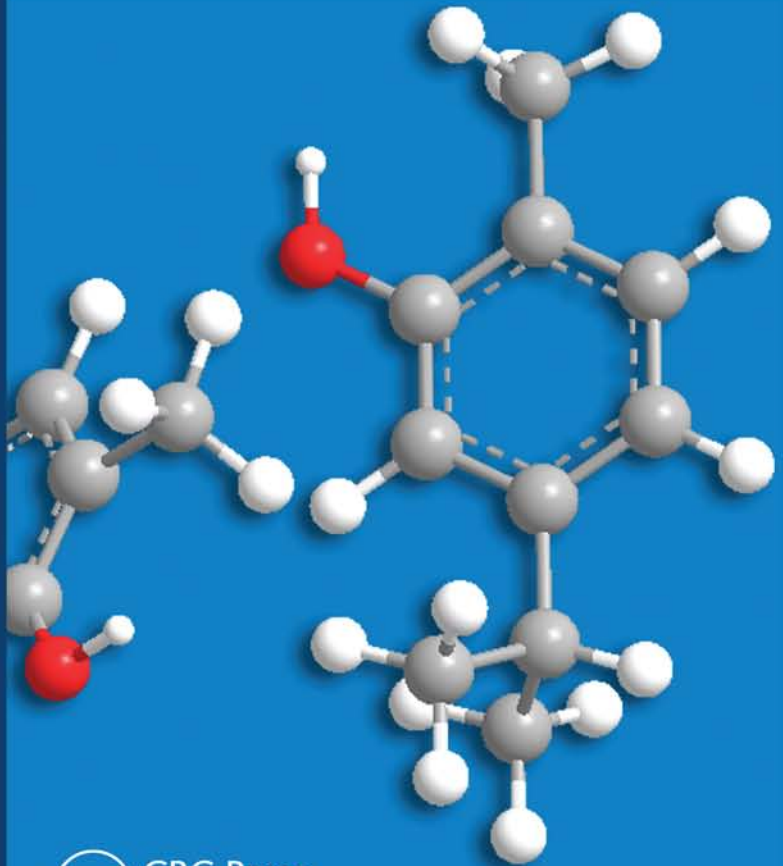
Handbook of

ESSENTIAL OILS

Science, Technology,
and Applications



Edited by
K. Hüsnü Can Başer
Gerhard Buchbauer



 CRC Press
Taylor & Francis Group

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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.

He served as dean of the faculty of pharmacy at Anadolu University (1993–2001), vice-dean of the faculty of pharmacy (1982–1993), head of the department of professional pharmaceutical sciences (1982–1993), head of the pharmacognosy section (1982–present), member of the

University Board and Senate (1982–2001; 2007), and director of the Medicinal and Aromatic Plant and Drug Research Centre (TBAM) (1980–2002) in Anadolu University.

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.

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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.

Among others, he is still a member of the permanent scientific committee of ISEO, a member of the scientific committee of Forum Cosmeticum (1990, 1996, 2002, and 2008), a member of editorial boards (e.g., *Journal of Essential Oil Research*, *The International Journal of Essential Oil Therapeutics*, *Scientia Pharmaceutica*, etc.), assistant editor of *Flavour and Fragrance Journal*, regional editor of *Eurocosmetics*, a member of many scientific societies, for example, *Society of Austrian Chemists*, head of its working group “Food Chemistry, Cosmetics, and Tensides” (2000–2004), *Austrian Pharmaceutical Society*, *Austrian Phytochemical Society*, vicehead of *Austrian Society of Scientific Aromatherapy*, and so on, technical advisor of IFEAT (1992–2008), and organizer of the 27th ISEO (September 2006, in Vienna) together with Professor Dr. Ch. Franz.

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.

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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.

2 History and Sources of Essential Oil Research

Karl-Heinz Kubeczka

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2.1 FIRST SYSTEMATIC INVESTIGATIONS

The first systematic investigations of constituents from essential oils may be attributed to the French chemist M. J. Dumas (1800–1884) who analyzed some hydrocarbons and oxygen as well as sulfur- and nitrogen-containing constituents. He published his results in 1833. The French researcher

M. Berthelot (1859) characterized several natural substances and their rearrangement products by optical rotation. However, the most important investigations have been performed by O. Wallach, an assistant of Kekule. He realized that several terpenes described under different names according to their botanical sources were often, in fact, chemically identical. He, therefore, tried to isolate the individual oil constituents and to study their basic properties. He employed together with his highly qualified coworkers Hesse, Gildemeister, Betram, Walbaum, Wienhaus, and others fractional distillation to separate essential oils and performed reactions with inorganic reagents to characterize the obtained individual fractions. The reagents he used were hydrochloric acid, oxides of nitrogen, bromine, and nitrosyl chloride—which was used for the first time by W. A. Tilden (1875)—by which frequently crystalline products have been obtained.

At that time, hydrocarbons occurring in essential oils with the molecular formula $C_{10}H_{16}$ were known, which had been named by Kekule *terpenes* because of their occurrence in turpentine oil. Constituents with the molecular formulas $C_{10}H_{16}O$ and $C_{10}H_{18}O$ were also known at that time under the generic name camphor and were obviously related to terpenes. The prototype of this group was camphor itself, which was known since antiquity. In 1891, Wallach characterized the terpenes pinene, camphene, limonene, dipentene, phellandrene, terpinolene, fenchene, and sylvestrene, which has later been recognized to be an artifact.

During 1884–1914, Wallach wrote about 180 articles that are summarized in his book *Terpene und Campher* (Wallach, 1914) compiling all the knowledge on terpenes at that time and already in 1887 he suggested that the terpenes must be constructed from isoprene units. In 1910, he was honored with the Nobel Prize for Chemistry “in recognition of his outstanding research in organic chemistry and especially in the field of alicyclic compounds.”

In addition to Wallach, the German chemist A. von Baeyer, who also had been trained in Kekule's laboratory, was one of the first chemists to become convinced of the achievements of structural chemistry and who developed and applied it to all of his work covering a broad scope of organic chemistry. Since 1893, he devoted considerable work to the structures and properties of cyclic terpenes (von Bayer, 1901). Besides his contributions to several dyes, the investigations of polyacetylenes, and so on, his contributions to theoretical chemistry including the strain theory of triple bonds and small carbon cycles have to be mentioned. In 1905, he was awarded the Nobel Prize for Chemistry “in recognition of his contributions to the development of Organic Chemistry and Industrial Chemistry, by his work on organic dyes and hydroaromatic compounds.” The frequently occurring acyclic monoterpenes geraniol, linalool, citral, and so on have been investigated by F. W. Semmler and the Russian chemist G. Wagner (1899), who recognized the importance of rearrangements for the elucidation of chemical constitution, especially the carbon-to-carbon migration of alkyl, aryl, or hydride ions, a type of reaction that was later generalized by H. Meerwein (1914) as Wagner–Meerwein rearrangement.

More recent investigations of J. Read, W. Hüchel, H. Schmidt, W. Treibs, and V. Prelog were mainly devoted to disentangle the stereochemical structures of menthols, carvomenthols, borneols, fenchols, and pinocampeols, as well as the related ketones (*cf.* Gildemeister and Hoffmann, 1956).

A significant improvement in structure elucidation was the application of dehydrogenation of sesqui- and diterpenes with sulfur and later with selenium to give aromatic compounds as a major method, and the application of the isoprene rule to terpene chemistry, which have been very efficiently used by L. Ruzicka (1953) in Zurich, Switzerland. In 1939, he was honored in recognition of his outstanding investigations with the Nobel Prize in chemistry for his work on “polymethylenes and higher terpenes.”

The structure of the frequently occurring bicyclic sesquiterpene β -caryophyllene was for many years a matter of doubt. After numerous investigations W. Treibs (1952) has been able to isolate the crystalline caryophyllene epoxide from the autoxidation products of clove oil and F. Šorm et al. (1950) suggested caryophyllene to have a 4- and 9-membered ring on bases of infrared (IR) investigations. This suggestion was later confirmed by the English chemist D. H. R. Barton (Barton and Lindsay, 1951), who was awarded the Nobel Prize in Chemistry in 1969.

The application of ultraviolet (UV) spectroscopy in the elucidation of the structure of terpenes and other natural products was extensively used by R. B. Woodward in the early forties of the last century. On the basis of his large collection of empirical data, he developed a series of rules (later called the Woodward rules), which could be applied to finding out the structures of new natural substances by correlations between the position of UV maximum absorption and the substitution pattern of a diene or an α,β -unsaturated ketone (Woodward, 1941). He was awarded the Nobel Prize in Chemistry in 1965. However, it was not until the introduction of chromatographic separation methods and nuclear magnetic resonance (NMR) spectroscopy into organic chemistry, that a lot of further structures of terpenes were elucidated. The almost exponential growth in our knowledge in that field and other essential oil constituents is essentially due to the considerable advances in analytical methods in the course of the last half century.

2.2 RESEARCH DURING THE LAST HALF CENTURY

2.2.1 ESSENTIAL OIL PREPARATION TECHNIQUES

2.2.1.1 Industrial Processes

The vast majority of essential oils are produced from plant material in which they occur by different kinds of distillation or by cold pressing in the case of the peel oils from citrus fruits.

In water- or hydrodistillation, the chopped plant material is submerged and in direct contact with boiling water. In steam distillation, the steam is produced in a boiler separate of the still and blown through a pipe into the bottom of the still, where the plant material rests on a perforated tray or in a basket for quick removal after exhaustive extraction. In addition to the aforementioned distillation at atmospheric pressure, high-pressure steam distillation is most often applied in European and American field stills and the applied increased temperature significantly reduces the time of distillation. The high-pressure steam-type distillation is often applied for peppermint, spearmint, lavandin, and the like. The condensed distillate, consisting of a mixture of water and oil, is usually separated in a so-called Florentine flask, a glass jar, or more recently in a receptacle made of stainless steel with one outlet near the base and another near the top. There the distillate separates into two layers from which the oil and the water can be separately withdrawn. Generally, the process of steam distillation is the most widely accepted method for the production of essential oils on a large scale.

Expression or cold pressing is a process in which the oil glands within the peels of citrus fruits are mechanically crushed to release their content. There are several different processes used for the isolation of citrus oils; however, there are four major currently used processes. Those are Pellatrice and Sfumatrice—most often used in Italy—and the Brown Peel Shaver as well as the FMC extractor, which are used predominantly in North and South America. For more details see for example Lawrence 1995. All these processes lead to products that are not entirely volatile because they may contain coumarins, plant pigments, and so on; however, they are nevertheless acknowledged as essential oils by the International Organization for Standardization (ISO), the different pharmacopoeias, and so on.

In contrast, extracts obtained by solvent extraction with different organic solvents, with liquid carbon dioxide or by supercritical fluid extraction (SFE) may not be considered as true essential oils; however, they possess most often aroma profiles that are almost identical to the raw material from which they have been extracted. They are therefore often used in the flavor and fragrance industry and in addition in food industry, if the chosen solvents are acceptable for food and do not leave any harmful residue in food products.

2.2.1.2 Laboratory-Scale Techniques

The following techniques are used mainly for trapping small amounts of volatiles from aromatic plants in research laboratories and partly for determination of the essential oil content in plant material. The most often used device is the circulatory distillation apparatus, basing on the

publication of Clevenger in 1928 and which has later found various modifications. One of those modified apparatus described by Cocking and Middleton (1935) has been introduced in the European Pharmacopoeia and several other pharmacopoeias. This device consists of a heated round-bottom flask into which the chopped plant material and water are placed and which is connected to a vertical condenser and a graduated tube, for the volumetric determination of the oil. At the bottom of the tube a three-way valve permits to direct the water back to the flask, since it is a continuous closed-circuit distillation device, and at the end of the distillation process to separate the essential oil from the water phase for further investigations. The length of distillation depends on the plant material to be investigated; however, it is usually fixed to 3–4 h. For the volumetric determination of the essential oil content in plants according to most of the pharmacopoeias, a certain amount of xylene—usually 0.5 mL—has to be placed over the water before running distillation to separate even small droplets of essential oil during distillation from the water. The volume of essential oil can be determined in the graduated tube after subtracting the volume of the applied xylene.

Improved constructions with regard to the cooling system of the above-mentioned distillation apparatus have been published by Stahl (1953) and Sprecher (1963), and in publications of Kaiser et al. (1951) and Mechler et al. (1977), various apparatus used for the determination of essential oils in plant material are discussed and depicted.

A further improvement was the development of a simultaneous distillation–solvent extraction device by Likens and Nickerson in 1964 (*cf.* Nickerson and Likens, 1966). The device permits continuous concentration of volatiles during hydrodistillation in one step using a closed-circuit distillation system. The water distillate is continuously extracted with a small amount of an organic and water-immiscible solvent. Although there are two versions described, one for high-density and one for low-density solvents, the high-density solvent version using dichloromethane is mostly applied in essential oil research. It has found numerous applications and several modified versions including different microdistillation devices have been described (e.g., Bicchi, 1987; Chaintreau, 2001).

A sample preparation technique basing on Soxhlet extraction in a pressurized container using liquid carbon dioxide as extractant has been published by Jennings (1979). This device produces solvent-free extracts especially suitable for high-resolution gas chromatography (HRGC). As a less time-consuming alternative, the application of microwave-assisted extraction has been proposed by several researchers, for example by Craveiro et al. (1989), using a round-bottom flask containing the fresh plant material. This flask was placed into a microwave oven and passed by a flow of air. The oven was heated for 5 min and the obtained mixture of water and oil collected in a small and cooled flask. After extraction with dichloromethane the solution was submitted to gas chromatography–mass spectrometry (GC-MS) analysis. The obtained analytical results have been compared with the results obtained by conventional distillation and exhibited no qualitative differences; however, the percentages of the individual components varied significantly. A different approach yielding solvent-free extracts from aromatic herbs by means of microwave heating has been presented by Lucchesi et al. (2004). The potential of the applied technique has been compared with conventional hydrodistillation showing substantially higher amounts of oxygenated compounds at the expense of monoterpene hydrocarbons.

2.2.1.3 Microsampling Techniques

2.2.1.3.1 Microdistillation

Preparation of very small amounts of essential oils may be necessary if only very small amounts of plant material are available, and can be fundamental in chemotaxonomic investigations and control analysis but also for medicinal and spice plant breeding. In the past, numerous attempts have been made to minimize conventional distillation devices. As an example, the modified Marcusson device may be quoted (Bicchi et al., 1983) by which 0.2–3 g plant material suspended in 50 mL water can be distilled and collected in 100 μ L analytical grade pentane or hexane. The analytical results proved to be identical with those obtained by conventional distillation.

Microversions of the distillation–extraction apparatus, described by Likens and Nickerson, have also been developed as well for high-density solvents (Godefroot et al., 1981), as for low-density solvents (Godefroot et al., 1982). The main advantage of these techniques is that no further enrichment by evaporation is required for subsequent gas chromatographic investigation.

A different approach has been presented by Gießelmann et al. (1993) and Kubeczka et al. (1995). By means of a new developed microhydrodistillation device the volatile constituents of very small amounts of plant material have been separated. The microscale hydrodistillation of the sample is performed using a 20 mL crimp-cap glass vial with a Teflon®-lined rubber septum containing 10 mL water and 200–250 mg of the material to be investigated. This vial, which is placed in a heating block, is connected with a cooled receiver vial by a 0.32 mm I.D. fused silica capillary. By temperature-programmed heating of the sample vial, the water and the volatile constituents are vaporized and passed through the capillary into the cooled receiver vial. There, the volatiles as well as water are condensed and the essential oil collected in pentane for further analysis. The received analytical results have been compared to results from identical samples obtained by conventional hydrodistillation showing a good correlation of the qualitative and quantitative composition. Further applications with the commercially available Eppendorf MicroDistiller® have been published in several papers, for example, by Briechle et al. (1997) and Baser et al. (2001).

A simple device for rapid extraction of volatiles from natural plant drugs and the direct transfer of these substances to the starting point of a thin-layer chromatographic plate has been described by Stahl (1969a) and in his subsequent publications. A small amount of the sample (ca. 100 mg) is introduced into a glass cartridge with a conical tip together with 100 mg silica gel, containing 20% of water, and heated rapidly in a heating block for a short time at a preset temperature. The tip of the glass tube projects ca. 1 mm from the furnace and points to the starting point of the thin-layer plate, which is positioned 1 mm in front of the tip. Before introducing the glass tube it is sealed with a silicone rubber membrane. This simple technique has proven useful for many years in numerous investigations, especially in quality control, identification of plant drugs, and rapid screening of chemical races. In addition to the aforementioned microhydrodistillation with the so-called TAS procedure (T = thermomicro and transfer; A = application; S = substance), several further applications, for example, in structure elucidation of isolated natural compounds such as zinc dust distillation, sulfur and selenium dehydrogenation, and catalytic dehydrogenation with palladium have been described in the microgram range (Stahl, 1976).

2.2.1.3.2 Direct Sampling from Secretory Structures

The investigation of the essential oils by direct sampling from secretory glands is of fundamental importance in studying the true essential oil composition of aromatic plants, since the usual applied techniques such as hydrodistillation and extraction are known to produce in some cases several artifacts. Therefore only direct sampling from secretory cavities and glandular trichomes and properly performed successive analysis may furnish reliable results. One of the first investigations with a kind of direct sampling has been performed by Hefendehl (1966), who isolated the glandular hairs from the surfaces of *Mentha piperita* and *Mentha aquatica* leaves by means of a thin film of polyvinyl alcohol, which was removed after drying and extracted with diethyl ether. The composition of this product was in good agreement with the essential oils obtained by hydrodistillation. In contrast to these results, Malingré et al. (1969) observed some qualitative differences in course of their study on *Mentha aquatica* leaves after isolation of the essential oil from individual glandular hairs by means of a micromanipulator and a stereomicroscope. In the same year Amelunxen et al. (1969) published results on *Mentha piperita*, who separately isolated glandular hairs and glandular trichomes with glass capillaries. They found identical qualitative composition of the oil in both types of hairs, but differing concentrations of the individual components. Further studies have been performed by Henderson et al. (1970) on *Pogostemon cablin* leaves and by Fischer et al. (1987) on *Majorana hortensis* leaves. In the latter study, significant differences regarding the oil composition of the hydrodistilled oil and the oil extracted by means of glass capillaries from the trichomes