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Essential Oil Research and Essential Oil Symposia 1969-1989

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The time is ripe, perhaps, to review the development which has taken place since the Essential Oil Symposium started in 1969—and try to see a little ahead. After all, the future will be built on the present, and the present has emerged from the past. Winston Churchill expressed it as follows: "The longer you can look back, the further you can see ahead." This holds true also for us.

In the beginning

On September 30, 1969, four enthusiastic pharmacognosists, F. W. Hefendehl from the University of Freiburg, FRG; K.-H. Kubeczka from the Technical University of Karlsruhe, FRG; J. Karlsen and A. Baerheim Svendsen, both from the State University of Leiden, The Netherlands, came together in Leiden and agreed to organize informal annual meetings to discuss common, mostly practical analytical problems encountered in essential oil research.

Rather than create a new organization or society, the four scientists found it convenient to arrange

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Table I. Essential Oil Symposia 1969-1989

Year	Location
1969	Leiden, NL
1971	Freiburg FRG
1972	Helsinki SF
1973	Freiburg FRG
1974	Freiburg FRG
1975	Leiden NL
1976	Würzburg FRG
1977	Freiburg FRG
1978	Münster FRG
1979	Würzburg FRG
1980	Groningen NL
1981	Marburg FRG
1982	Würzburg FRG
1983	Freising-Weihenstephan FRG
1984	Leiden NL
1985	Holzminden FRG
1986	Bad Bevensen FRG
1987	Leiden NL
1988	Dübendorf CH
1989	Würzburg FRG

meetings in connection with the congresses of the Gesellschaft für Arzneipflanzenforschnung. During the first meeting in Leiden it was decided that only scientists who were personally experimentally involved in research should be invited to participate in the meetings, since only they could contribute substantially to the discussions.

To begin with, only university people were invited. However, as time went on, scientists working in research laboratories of the essential oil industry became interested in participating in meetings. The contributions by such scientists to the development of essential oil research had for several years been substantial.

When compared with many university laboratories, the industrial research laboratories were usually much better equipped—especially some years ago—and the scientists there had gained a lot of experience from which university people could benefit.

The objectives of the two groups of participants differed to some extent. For the industry people, the objectives were primarily to develop better and more exact analytical methods for quality control of essential oils, analysis of essential oils in general and structure elucidation of new interesting constituents. Much fundamental work was therefore performed.

For the university people, the application of adequate isolation and analytical methods in order to solve problems in the biology of essential oil-bearing plants was perhaps the main objective—at least for many pharmacognosists. They studied variation of amount of essential oil components dependent on internal and external factors, detection and characterization of chemotypes of essential oil bearing plants, biosynthesis of essential oil constituents—to mention only a few areas.

In Table I you will see where and when the symposia from 1969 to 1989 have been held. The place chosen for the symposia was always determined by the place where the congress of the "Gesellschaft für Arzneipflanzenforschung" was held, to minimize extra travel time and expense. So, for instance, when this congress was held in Mannheim, FRG, in 1971, the symposium was held in Freiburg, FRG. In 1972 the congress was held in Helsinki, and so was the symposium also—to give two examples.

The symposia usually lasted only one or two days. However, lately there has been a tendency to extend them to three or four days as the meetings have grown. Many participants—especially younger assistants and graduate students—who in several countries get little or no money for attending congresses or symposia in other countries—have not been happy with this tendency. They can not afford to participate, especially not when participation in a

Table II. Topics discussed during the essential oil meeting in Freiburg 1971 as proposed by F. W. Hefendehl and S. Juvonen.

Apparatus and Methods

1. Advantages and disadvantages of various commercial gas chromatographs
2. Coupling of GC/IR, GC/MS with capillary columns
3. Radio gas chromatography
4. Digital integrators
5. Splitting systems for Golay columns
6. Capillary columns
7. Solid support for packed gas chromatographic columns
8. Isomerization caused by some stationary phases
9. Isomerization during the analysis; influence of temperature
10. Retention times of compounds on various polar and nonpolar stationary phases. Agreement on standard columns.
    Exchange of retention values. Kovats Index for identification purposes
11. IR, UV, MS—Exchange of spectra, tables, list of data
12. Exchange of reference substances
13. Reaction gas chromatography
14. Preparative gas chromatography. FID or Catharometer?
15. Efficiency in trapping substances in preparative gas chromatography
16. Prefractionation of essential oils on silica gel columns
17. Separation of groups of compounds occurring in essential oils
18. Isolation of essential oils. Distillation. Extraction.

Biogenesis

1. Ontogenetic variability. Comparison of absolute and relative amounts
2. Incorporation of labeled compounds
3. Enzymes and enzyme blockers
4. Tissue cultures

symposium also implies long travels. These aspects should be considered when planning symposia in the years to come. After all, it is the younger generation that carries the future of the essential oil research in its hands.

The very first meeting in Leiden was attended by four persons, and in the beginning the group of participants remained fairly small, generally ten to 15 persons, mainly from universities in Germany, The Netherlands and Finland. But little by little the number increased, especially when industry people started to attend the symposia. So, in 1976 the number of participants had increased to about 40, in 1979 to 70, in 1985 to over 100 and this year to about 160.

These numbers are mentioned to emphasize that organizing such symposia, with participants from countries all over the world, is quite a venture. There is no organization or society which is financially responsible for the symposia. This responsibility rests on the organizing committee alone.

We should all be grateful to those who have been willing to organize these symposia year after year. Nobody knows what it means to organize an international symposium for over 100 participants from countries all over the world, before he has done it himself.

The development of essential oil research

To give an impression of the character of the first meetings, Table II lists the topics on the agenda for the meeting in Freiburg 1971. The topics were proposed by F. W. Hefendehl (Freiburg) and S. Juvonen (Helsinki).

Usually one of the participants gave a short introduction and outlined the problems to be discussed. This was done to stimulate the following discussion in which everybody was supposed to participate. The discussions of the topics—whether analytical or biological—were the main thing at the meeting.

Pharmacognostical research is characterized by its biological problems, whereby accurate and reliable chemical and physical analytical methods often are decisive for the results. So also in essential oil research. Therefore, a topic such as 'incorporation of labeled compounds' was discussed together with 'radio gas chromatography', to give an example.

The character of the meetings changed, however, after some years. Instead of discussing quite informally a number of topics proposed by the participants, the participants were invited to present their research results in short lectures or posters. Thus, gradually the meetings were changed into regular symposia or congresses. This development was regretted by some who participated in the first meetings. However, the increasing number of participants made this development necessary.

To illustrate what was presented in later symposia, selected titles of papers from the period 1976-1985 are listed in Table III.

Table III. Titles of some papers presented during the symposia 1976 to 1985

Analysis

1. Progress in isolation techniques for essential oil constituents
2. Preparation of essential oils for capillary gas chromatography
3. Determination of the enantiomeric composition of natural flavouring agents by ^{1}H-NMR-spectroscopy
4. Isolation and gas chromatographic separation of menthol and menthone enantiomers from natural peppermint oil
5. HPLC-separation of essential oils with chemically bonded stationary phases
6. Negative ion chemical ionization in the essential oil analysis
7. Mass fragmentography in the essential oil analysis
8. Quantitative analysis of essential oils by ^{13}C-NMR-spectroscopy
9. High resolution gas chromatography/Fourier transform infrared spectroscopy investigations on volatile terpenes
10. Ion-trap detector: the technique and its application
11. Application of ^{13}C-NMR spectroscopy in the essential oil analysis
12. Systematic identification and structure elucidation of sesquiterpenes
13. The use of conventional EDP programs for the storage and retrieval of reference mass spectra
14. Distribution of essential oils in the vegetable kingdom
15. Volatile metabolites from microorganisms

Biology

1. Monoterpene glycosides, a discussion about their biological role and experiments in synthesizing these compounds
2. Biotransformation of terpenoids
3. Production and accumulation of essential oils in the whole plant and in tissue cultures
4. Cytology and physiology of gland cells in essential oil bearing plants
5. Genetic, ontogenetic and environmental variability of the constituents of chamomile oil
6. Antimicrobial activities of essential oils

Table III shows that all aspects of essential oil research have been dealt with—and it is evident that a pharmacognostical approach to the problems has been predominant. This is understandable since the meetings were started by pharmacognosists and the organizers of the meetings have been—with a couple of exceptions—pharmacognosists, and so also have most of the participants.

From the tables given, comprising the topics dealt with during the symposia, it is evident that the main interest of the participants was the analysis, improvement and application of analytical methods as well as exclusion of artifact formation. The last mentioned aspect of natural products analysis has often been neglected, but when working with essential oil constituents, many of which are extremely unstable, a closer attention began to be given to artifact formation and its exclusion.

Developments in chromatography

The analytical method of choice for essential oils and their constituents was primarily gas liquid chromatography, although other chromatographic techniques, such as thin layer chromatography and various types of column chromatography had been applied with some success. However, because of the large number of possible components as well as their volatility, gas chromatography became the method of choice. Its separation capability exceeded all other separation methods 20 years ago, when packed columns were used, and it still does when fused silica capillary columns are used.

Although capillary columns had been introduced in essential oil research already before 1960,[1] it was many years before this analytical technique became common in university laboratories. There, packed 2 to 4 m long columns with an inner diameter of 2 to 4 mm were mostly used. Often, in order to obtain the best possible separation of the components present in an oil, a long series of stationary phases of various polarities were used.

The percentage of the stationary phases varies strongly—from 10 to 25 percent, and even more. Therefore, relatively high column temperatures often had to be used in order to obtain reasonably short retention times.

Since the gas chromatographic conditions applied in different laboratories varied so much, it was difficult to compare results obtained in various laboratories. Therefore, it was decided at one of our early meetings to limit the number of stationary phases to two or three and to standardize the gas chromatographic conditions as much as possible.

Some of the participants had experience with low loaded packed columns; that means columns with a coating of the solid support with 1.5 to 2 percent of stationary phase. By means of such columns rather low column temperatures could be used, when compared with columns with 20 to 25 percent of stationary phase.[2] High column temperatures were regarded as a source of artifact formation.

However, the low loaded columns could also create artifacts, not because of the temperature used, but because of uncoated active points on the solid support. Several deactivation treatments of the solid support—among others silylation—were therefore used prior to the coating with the stationary phase. When using a percentage of stationary phase of five to ten, such a deactivation seemed not to be necessary, since the solid support was regarded sufficiently covered by the stationary phase.

The length of the packed column was often discussed. Although the length of the column plays a minor role according to the theory, since the number of theoretical plates does not increase with the length of the column, but with the square root of the length, practical experience showed that an increase of the length to 8 m often had a quite favourable influence on the resolution.

However, the packing of 8 m long, coiled metal columns with an inner diameter of 2 mm could cause difficulties. But with some experience, 8 m columns with a fairly good resolution, sufficient for

several studies on essential oils, could be prepared. Such columns were used for many years in university laboratories with good results, especially when applied in combination with a solid-liquid column chromatographic prefractionation of the oil on deactivated silica gel columns.

To facilitate the gas chromatographic identification of the constituents of an essential oil, a separation of hydrocarbons and oxygen-containing compounds by the method of Kirchner and Miller[3] was often carried out. Several modifications of the method were developed. However, many of them had some disadvantages, particularly because they consisted of many steps, each of which might generate artifacts, as repeatedly described in the literature.

Because of the large variations in the relative amounts of the many different constituents in an essential oil, problems often arose in the gas chromatographic separation and identification of minor components when packed columns were used, even after prefractionation according to the principle of Kirchner and Miller. In order to detect minor or trace components it was necessary to inject relatively large amounts ob the oil for a gas chromatographic analysis. This resulted often in overlapping of the peaks of the main components with those of minor ones.

Several column chromatographic prefractionation methods were developed in order to solve this problem so, Kubeczka in 1973 introduced such a separation using a dry silica gel column and successive elution with solvents of increasing polarity, first pentane and then diethyl ether.[4] Scheffer, et al. in 1976-77[5-7] applied liquid-solid chromatography on a silica gel column containing five percent of water, and was able to separate mixtures of monoterpene hydrocarbons and of oxygen-containing monoterpenes by elution with pentane and a gradient of pentane and diethyl ether, respectively. A number of small fractions were collected and subsequently gas chromatographed.

This column chromatographic separation led to an enrichment of several minor compounds in the fractions collected so the subsequent gas chromatographic identification was facilitated. For oxygen-containing monoterpenes a significant tendency in the elution sequence was observed: first, esters were eluted, then aldehydes and ketones, and finally alcohols. Thus, some information of the functional group of a compound could be obtained in addition to an enrichment of the various constituents within one group.

It was found important to treat the silica gel with hydrochloric acid to remove traces of metals, which caused a drastic isomerization of some compounds. The hydrochloric acid must be removed quantitatively for the same reason. Since it had been observed that dry silica gel could cause isomerization, five percent of water had to be added prior to use.

When these precautions were taken, no detectable isomerization took place. On dry silica columns, however, drastic isomerization of some monoterpene hydrocarbons was found. Sabinene was completely isomerized to γ-terpinene (53%), α-terpinene (29%), terpinolene (9%), α-phellandrene (3%), β-phellandrene (3%), p-cymene (2%) and limonene (1%) for example.

Because in many cases the amounts of an essential oil available for analysis are small, the prefractionation can be carried out using so-called mini-extraction columns of silica gel, of a volume of 1 ml or 3 ml, respectively. Amounts of 10 μl and 30μl respectively of an oil can be analyzed without traces of isomerization.[8]

Although 8 m long packed gas chromatographic columns gave rather satisfactory results for many purposes, especially in combination with a column chromatographic prefractionation, quite another resolution was achieved with capillary columns. The coating of metal and glass capillary columns, especially with polar stationary phases, was one of the problems often discussed at our meetings. Since it was easier to achieve a total coating with a non-polar phase, such phases were often preferred..

To begin with, mostly metal capillaries were used, but they often caused catalytic isomerization of some of the compounds to be analyzed. Deactivated glass capillaries were more inert and offered a better separation power.[9] But the real breakthrough of capillary gas chromatography came in 1982 with the introduction of fused silica capillary columns. Such columns are also ideally suited for coupling with a mass spectrometer.

Compared to packed columns, the capillary columns offered a much higher efficiency in a shorter analysis time. Also, rather low column temperatures usually could be used. The precision of retention times increased sharply, a very important property in connection with identification. A disadvantage is the low sample capacity of capillary columns, which made it necessary to use sensitive detectors. However, with the development of thick film columns, larger samples can be injected.

A valuable development during the last years has been the so-called 'two dimensional gas chromatography', whereby the sample to be analyzed is split in the injector, and one half is brought into a polar capillary column, the other half in a slightly polar capillary column. The same temperature programming and the same gas flow are used. Each flame ionization detector is coupled to a recorder and an integrator, allowing a simultaneous quantification of all peaks on both chromatograms.

Because the retention times of many components differ on two columns of different polarities, quite different elution sequences are obtained. Combined with the quantitative data obtained by both integrators, a rather complete picture of the oil is obtained, facilitating the identification of the many constituents.

Isolation Procedures

Most investigations on essential oils have been performed on such oils isolated by steam or hydrodistillation of the plant material in which they occur. However, isomerization, hydrolysis, polymerization and other reactions may take place during the isolation procedure. Therefore, results of the following analysis, no matter how good, may not give a correct picture of the composition of the essential oil present in the living plant, but of the isolated oil. This is in many cases the only goal of the investigation. However, when the goal is to study the composition of the oil as it occurs in the living plant, then the problems are more complicated.

Therefore, extensive investigations have been carried out on the isolation procedures, such as hydrodistillation, using a Clevenger type apparatus,[10] as well as a Likens-Nickerson type apparatus for combined hydrodistillation-solvent extraction.[11] Several factors that could cause isomerization and other artifact reactions may be brought under control as a result of the investigations.

The Likens-Nickerson type apparatus is often used, since the apparatus, according to the literature, permits a many thousand-fold concentration of volatile compounds from an aqueous medium in one step. However, this seems to hold true only for rather non-polar volatile compounds. An investigation on the recovery of some aliphatic and aromatic alcohols, monoterpene alcohols and phenols revealed remarkable differences.

The recoveries of slightly polar compounds, such as borneol, linalool, menthol and carvacrol were about 80 percent after two hours of distillation, whereas for rather polar compounds, such as benzyl alcohol and β-phenylethanol, they were only 6 percent and 13 percent respectively. However, an extension of the distillation time to 4 or 8 hours in order to increase the recoveries of the more polar compounds decreased the recoveries of some of the other compounds, possibly because of polymerization or other reactions taking place during the long distillation time.[12] The Likens-Nickerson apparatus should, therefore, be applied with some care.

To avoid artifact formation during the isolation procedures involving a lengthy hydrodistillation, devices were developed allowing the introduction of small amounts of living plant material (10 to 20 mg) into the injection port of the gas chromatograph. At a temperature of 200°C, the volatile compounds distill off immediately and are swept into the gas chromatographic column by the inert carrier gas, mostly nitrogen.

Several types of such devices for solid injection of fresh plant material were developed[13,14] and interesting results obtained, qualitative as well as quantitative. A draw back of the technique is, however, that only small amounts of plant material can be analyzed, and therefore, no complete picture of the composition of the essential oil of a whole plant can be obtained, only of the small pieces of it, which are brought into the injection port for analysis.

An isolation technique for the gas chromatographic analysis of very small amounts of essential oil was developed by Kubeczka,[15] whereby a mini-extraction kiselguhr column is used. A homogenate of 2 g fresh plant material in 20 ml water was brought on the mini-column of porous kiselguhr, a so-called Extrelut-Fertigsäule. Subsequently the essential oil was eluted by means of pentane or diethyl ether, and after concentration of the sample to 0.5 ml, small amounts were injected for a capillary gas chromatographic analysis.

The development of head space gas chromatography opened new ways for the analysis of volatile compounds occurring in fresh plant material. Chialva, et al.[16] compared the composition of the volatiles of aromatic plants with that of the essential oil. They observed that the volatile compounds detected in the head space often were absent in the essential oil, although they contributed strongly to the plant fragrance. Dynamic head space capillary gas chromatography was applied.

This technique must, however, be applied with utmost care, since contamination by the laboratory atmosphere may occur. In the case of a strawberry flavour, among 38 components identified in the head space, 17 may have come from the laboratory atmosphere.

Equilibrium head space gas chromatography was extensively dealt with during the 15th essential oil symposium in 1984. I will not go into details here, but only emphasize that in equilibrium head space gas chromatography several parameters such as the temperature and the equilibrium period, have to be carefully and precisely controlled to achieve reproducible results.

Hiltunen and coworkers[17] carried out comparative studies by equilibrium head space gas chromatography and conventional hydrodistillation gas chromatography and found for plant material such as flowers and leaves (chamomile, peppermint, rosemary) similar patterns of the essential oil constituents for both methods. But for hard plant material, such as some fruits (anise, fennel, juniper), the results obtained with head space gas chromatography were about 50 percent lower than for conventional hydrodistillation gas chromatography. A temperature of 120°C and an equilibrium period of 30 min were applied by the investigation.

Chialva and Gabri,[18] on the other hand, applied an equilibrium temperature of 60°C and an equilibrium period of 1 hour, and observed that gas chromatograms of the essential oil isolated by hydrodistillation contained a greater number of compounds than the head space. So, whereas the head space of peppermint contained about 24% of menthone and 15% of menthol, the corresponding values for the isolated oil was 20% and 31% respectively.

In conclusion, Head space gas chromatography is a technique which has opened quite new possibilities in the essential oil research, but the technique should be applied with care.

Identifying essential oil constituents

The identification of essential oil constituents in gas chromatography was often quite a problem before mass spectrometers coupled to gas chromatographs became commonly available. Identification was usually achieved via retention times obtained on columns of different polarities, using pure reference substances for co-chromatography. In cases of doubt about the identity—and such doubt often existed—the compound in question had to be isolated, often via preparative gas chromatography, and subsequently its mass spectrum could be recorded.

Exchange of reference substances and their mass spectra became an important task for the participants in the first meetings. Although so-called pure reference substances were available from some essential oil firms, the instability of some of them could play tricks on us. For instance, a sample of pure sabinene contained only traces of sabinene when we received it. Most of the sabinene had been isomerized to other compounds, and it took us some time until we became aware of it. Therefore, we often preferred to exchange essential oil samples containing the compound in question as a main constituent, since many sensitive compounds are quite stable in the essential oil in which they occur. The sample was always accompanied by a gas chromatogram.

When GC/MS suited also for capillary gas chromatography became more common in university laboratories, it meant a great step forward. The in-

troduction of the ion trap detector (ITD) as a relatively inexpensive mass spectrometer was important for many institutes.

More sophisticated detection equipment, such as the Fournier transform infrared detector, a non-destructive detector, allows on-line coupling with other detectors, and gives additional valuable information.

The application of ^{13}C-NMR-spectroscopy, as demonstrated by Formáček and Kubeczka[19] needs only to be mentioned here. The method is extremely well suited to essential oil research, and it should be recommended for use in sesquiterpene research. Too often papers occur in which the identification of sesquiterpenes has been based solely on their mass spectra. This is not at all sufficient.

A well-planned cooperation between some laboratories might be beneficial for many working in this field. Laboratories equipped for two dimensional capillary gas chromatography/mass spectroscopy should analyse a series of selected essential oils rich in sesquiterpenes on two selected capillary columns of different polarities, one polar and one slightly polar. Retention times on both columns as well as the mass spectra should be carefully recorded.

Laboratories equipped also with a ^{13}C NMR-spectrometer should in addition provide the identification of the sesquiterpenes in the selected oils by ^{13}C NMR-spectroscopy. The data obtained from all participating laboratories should make it possible by means of two dimensional capillary gas chromatography, retention times and mass spectra, to perform an identification of those sesquiterpenes for which the data have been recorded in other essential oils. For new sesquiterpenes detected in an oil, their isolation and identification by means of ^{13}C NMR-spectroscopy is necessary.

Because many sesquiterpenes are relatively stable in the essential oils in which they occur, but often not in pure form, a series of sesquiterpene containing oils should be recommended as standard oils for identification purposes.

At present, the data recorded by means of the identification methods mentioned are often so many that it is difficult to use them properly. Different data systems are used in different laboratories for the storage of analytical data. If also in this area some kind of standardization of the data systems could be agreed upon, it would certainly facilitate the exchange of data—to the benefit of everyone working with essential oils.

More standardization of experimental conditions was one of the main objectives of our meetings in the begin. Now, more standardization of the recorded data might be an objective for the participants of the current symposia.

Not only have investigations on the chemistry of the essential oils been discussed during our meetings, but also several biological aspects of such oils, among others the antimicrobial activities of essential oils and their main components. . . . Because the antimicrobial test methods which had been used previously differed rather much, the data given in the literature about the antimicrobial activities of essential oils were not at all comparable. Therefore, quite extensive investigations were carried out on these test methods[20] and the mechanism of action.[21]

Screening of a relatively large number of essential oils and their main constituents for a possible antimicrobial activity showed such an activity for several oils. Most interesting was perhaps the effect against some dermatophytes.[22]

It should be emphasized that, sometimes, traces of impurities occurring in so-called pure monoterpene hydrocarbons were responsible for the antimicrobial activity observed. Therefore, careful gas chromatographic control of the compounds is a must.

The future of essential oil research

Which areas of research will be most important in the years to come, and what should the aims of our symposia be?

The screening of the vegetable kingdom for essential-oil-bearing plant species should be continued in order to find oils containing new interesting compounds of value for the perfumery industry as well as for pharmacy, to find new oils with a high content of already known, valuable compounds, and to apply the results obtained as to the chemical composition of essential oils for chemosystematic purposes. Investigations to develop better analytical methods should be continued.

Many pharmacognosists will certainly lay special emphasis on the study of sesquiterpenes occurring in essential oils. In their work they should consult the excellent dissertation of Schmaus.[22]

Studies on the biosynthesis of essential oil components will be continued, and perhaps the results obtained for glycosidically bound monoterpenes and other volatile compounds occurring in living plants will be of importance here.[23,24]

The aims of the symposia should be as before: namely, to bring together scientists who are interested in all aspects of essential oil research, for discussions of common problems and presentation of their results, to the benefit of essential oil research in general.

The 20th international symposium on essential oils, with about 160 participants from 24 countries all over the world, clearly demonstrates the great interest for essential oil research nowadays. Let us hope that it will continue so in the years to come.

References

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1. E Guenther, J A Rogers Jr, G. Gilbertson, R T Koenig, Essential Oils and Related Products, *Anal Chem* **37** 46R-66R (1965)
2. A Baerheim Svendsen, J Karlsen, Gaschromatographie von Monoterpenkohlenwasserstoffen aus ätherischen Ölen an gepackten Trennsäulen mit niedrigem Gehalt an flüssiger, stationärer Phase, *Planta Med* **15** 1-5 (1967)
3. J G Kirchner, J M Miller, Preparation of terpeneless essential oils—a chromatographic process, *Ind Eng Chem* **44** 318-21 (1952)
4. K-H Kubeczka, Vortrennung ätherischer Öle und ähnlich komplexer Stoffgemische für die GC-Analyse durch modifizierte Trockensäule-Chromatographie, *Chromatographie* **6** 106-8 (1973).
5. J J C Scheffer, A Baerheim Svendsen, Improved gas chromatographic analysis of naturally occurring monoterpene hydrocarbons following pre-fractionation by liquid-solid chromatography, *J Chromatogr* **115** 607-11 (1976)
6. J J C Scheffer, A Koedam, A Baerheim Svendsen, Occurrence and prevention of isomerization of some monoterpene hydrocarbons from essential oils during liquid-solid chromatography on silica gel, *Chromatographie* **9** 425-32 (1976)
7. J J C Scheffer, A Koedam, M Th I W Schüsler, A Baerheim Svendsen, Improved gas chromatographic analysis of naturally occurring oxygen-containing monoterpenes following prefractionation by liquid-solid chromatography, *Chromatographie* **10** 669-77 (1977)
8. J J C Scheffer, et al., Prefractionation of essential oils using silica gel 'mini extraction columns', to be published
9. H Grob, Polare Impregnierung von Glasskapillären für die Gaschromatographie *Helv Chim Acta* **48** 1362-70 (1965)
10. J F Clevenger, Apparatus for the determination of volatile oils, *J Am Pharm Ass* **17** 345-49 (1928)
11. S T Likens, G W Nickerson, Detection of certain hop oil constituents in brewing-products, *Am Soc Brew Chem Proc* 5-13 (1964)
12. A Baerheim Svendsen, Y M Merkx, Recovery of some polar volatile compounds by combined hydrodistillation-solvent extraction using a Likens-Nickerson apparatus, *Sci Pharm* (Vienna) in press
13. A Baerheim Svendsen, J Karlsen, Direkte Gaschromatographie der Bestandteile von ätherischen Ölen in Pflanzenmaterial, *Planta Med* **14** 367-80 (1966)
14. A Baerheim Svendsen, J Karlsen, New aspects of the gas chromatographic analysis of lower terpenes in plant material, in *Pharmacognosy and Phytochemistry,* Ed H Wagner and L Hörhammer, Springer-Verlag: Berlin-Heidelberg-New York 1971, pp 17-40
15. K-H Kubeczka, Neue Techniken zur Isolierung kleinster ätherischer Ölmengen für gaschromatographishe Analysen, in *Ätherische Öle, Analytik, Physiologie, Zusammensetzung,* Ed K-H Kubeczka, Georg Thieme Verlag: Stuttgart-New York 1982, pp 116-122
16. F Chialva, G Gabri, P A P Liddle, F Ulian, Qualitative evaluation of aromatic herbs by direct headspace GC analysis. Application of the method and comparison with the traditional analysis of essential oils, *J High Resolut Chromatogr Chromatogr Commun.* **5** 182-88 (1982)
17. R Hiltunen, H Vuorela, I Laakso, Quantitative headspace gas chromatography in the analysis of volatile oils and aromatic plants, in *Essential oils and aromatic plants,* Ed A Baerheim Svendsen, J J C Scheffer, Martinus Nijhoff/dr W Junk Publishers: Dordrecht-Boston-Lancaster 1984, pp 3-21
18. F Chiala, G Gabri, Headspace versus classical analysis, in *Capillary gas chromatography in essential oil analysis,* Ed P Sandra, C Bicchi, Dr Alfred Huethig Verlag: Heidelberg-Basel-New York 1987, pp 123-54
19. V Formácvek, K-H Kubeczka, *Essential oils analysis by capillary gas chromatography and carbon-13 NMR-spectroscopy,* John Wiley & Sons: Chichester-New York-Brisbane-Toronto-Singapore 1982
20. A M Janssen, J J C Scheffer, A Baerheim Svendsen, Antimicrobial screening of essential oils—aspects of the agar overlay technique, in *Progress in essential oils research,* Ed E-J Brunke, Walter de Gruyter: Berlin-New York 1986, pp 401-428
21. K Knobloch, H Weigand, N Weis, H-M Schwarm, H Vigenschow, Action of terpenoids on energy metabolism, in *Progress in essential oil research,* Ed E-J Brunke, Walter de Gruyter: Berlin-New York 1986, pp 429-445
22. G Schmaus, Untersuchungen über die Zusammensetzung der ätherischen Wurzelöle verschiedener mittel- und westeuropäsischer Peucedanum-Arten (Apiaceae) unter besonderer Berücksichtigung von Peucedanum palustre (L.) Moench and Peucedanum lancifolium Lange, PhD Thesis, University of Würzburg, 1988
23. J M A van den Dries, A Baerheim Svendsen, A simple method for detection of glycosidic bound monoterpenes and other volatile compounds occurring in fresh plant material, *Flavour Fragr J* **4** 59-61 (1989)
24. A Baerheim Svendsen, Y M Merkx, A simple method for screening of fresh plant material for glycosidic bound volatile compounds, *Planta Med* **55** 38-40 (1989)