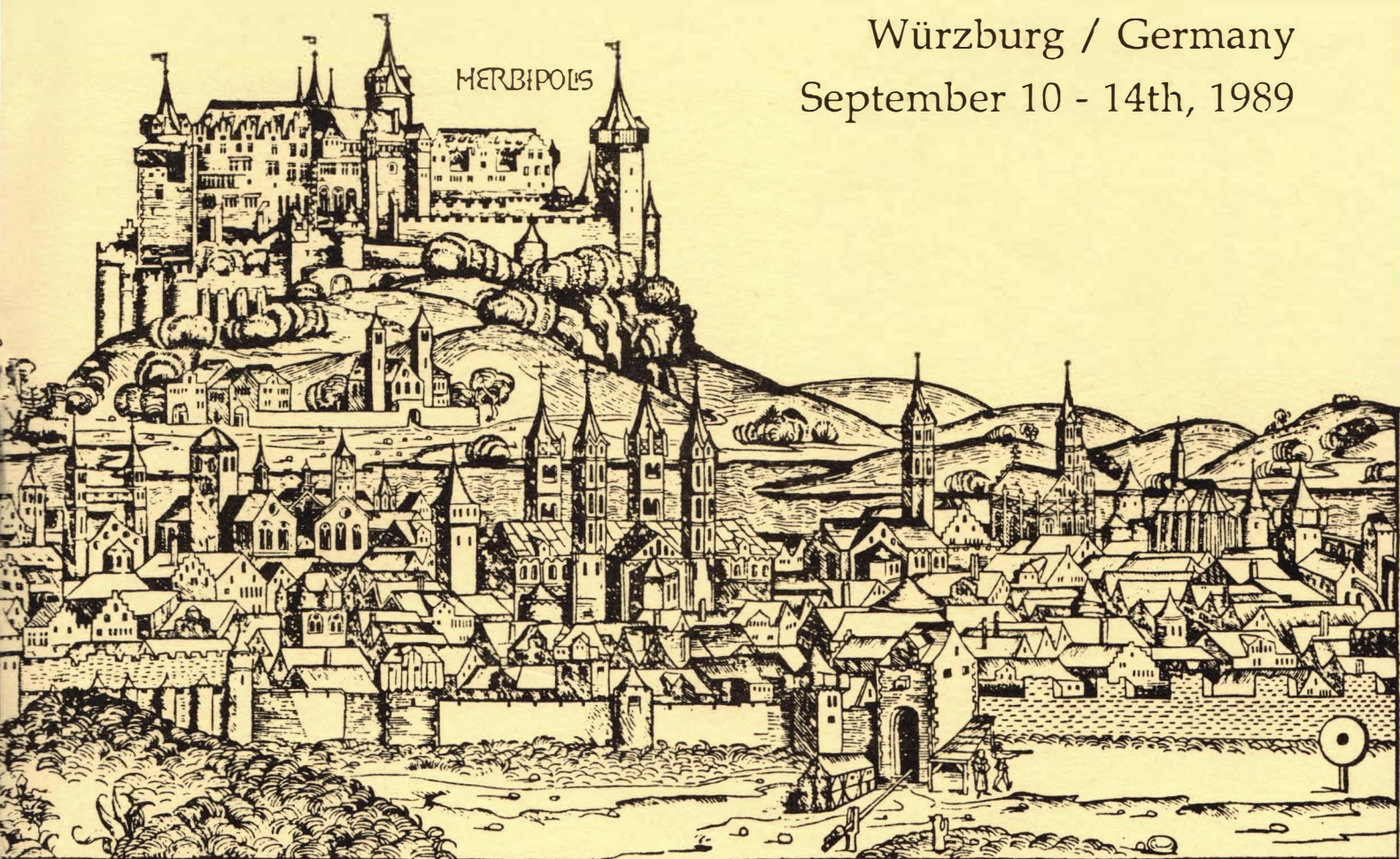


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Abstracts

A COMPARATIVE STUDY OF THE ESSENTIAL OILS FROM *MELALEUCA* SPECIES GROWING IN EGYPT

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Steam-distilled essential oils of the leaves of three *Melaleuca* species growing on Egypt, viz, *M. leucadendron*, *M. armillaris* and *M. ericifolia* were studied. The yield and physical constants were determined. The composition of the oils was studied by capillary gas chromatography (RSL-150 = OVI) coupled to mass spectrometry. Individual components of each oil were identified by their mass spectra and Kovats indices which were compared with those of reference compounds. Quantitation of components was based on integration of peak areas. Methyl eugenol is the main component of *M. ericifolia* (97.7%), while being 1.7% in *M. armillaris* and absent in *M. leucadendron*. 1,8-cineole and limonene constitute 59.6% of the oil of *M. leucadendron*; while being 33.7% in *M. armillaris* and only 0.1% in *M. ericifolia*. Terpinene-4-ol amounts to 24.8% in *M. armillaris* and 0.7% in *M. leucadendron*; while being absent in *M. ericifolia*. *M. armillaris* is the richest in hydrocarbons (32.1%) followed by *M. leucadendron* (13.2%) and *M. ericifolia* (0.8%). The same pattern was found also for alcohols being 27.7, 24.4 and 0.5%, respectively. The oils of these plants are economically important, since they are good sources of methyl eugenol (*M. ericifolia*), terpinen-4-ol (*M. armillaris*) and 1,8-cineole (*M. leucadendron* and *M. armillaris*). The composition of essential oils from these *Melaleuca* species can be of chemotaxonomic significance.

CHIRAL INTERMEDIATES BY MICROBIAL EPOXIDATIONS

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Chiral epoxides are useful intermediates for a multitude of products. With the exception of allyl alcohols - which can be oxidized by means of the Sharpless procedure - direct epoxidations lead to products with low optical purities. For this reason extensive work was done on microbial epoxidations. Epoxy-n-alkanes were produced from the corresponding alkene by microorganisms growing on hydrocarbons whereas the terminal double bond of acyclic terpenoids can be oxidized by some glucose using strains in high optical yields. While most fungi possess hydrolases which open the epoxides to diols we found some microorganisms which let the epoxide unchanged. Alcohols like linalool or nerolidol react intramolecularly with the oxiran moiety to tetrahydrofurans and tetrahydropyrans. The absolute configuration of the epoxides is usually S but depends like their optical purities, which is usually 95% e.e. or better, on the substrate and the strain used.

BREEDING STRATEGIES IN SCENTED GERANIUM

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Scented geranium (*Pelargonium graveolens* L. Herriot) a perennial vegetatively propagated crop is the source of geranium oil highly prized in perfumery industry. Wilt is a major problem limiting its successful cultivation. A breeding project is underway at this institute to develop wilt resistant and superior oil yielding strains. Germplasm evaluation revealed significant herbage yield differences among the natural variants. PG-7 and Algerian were most promising; the latter recording consistently higher oil yield per plant. Though PG-20 was next to Algerian in oil yield, it was deficient in geraniol and had low l-citronellol. Reunion strain, whose oil is most preferred recorded low oil yield. Algerian which showed field tolerance to wilt was susceptible to leaf spot diseases.

Variation induced by tissue culture techniques has been suggested for widening the narrow genetic base. Alternatively we could induce variability in two of the natural variants by an in vivo adventitious bud technique, and obtain four distinct phenotypes. Significant herbage yield differences were observed among the variants and also as compared with the parents. Two Reunion variants had higher oil content while another variant with low oil content recorded higher proportions of l-citronellol and geraniol than that of the parent.

A Reunion variant, pubescent, exhibited gigas traits, formed fully fertile flowers and set seeds. The Algerian variant showing ploidy characters bore stray partially fertile flowers with the number of fertile anthers ranging from 2-4. Among the pubescent plants three distinct types were seen:

- i) Plants bearing flowers with only fertile anthers
- ii) Plants with flowers both fertile and sterile anthers in varying numbers
- iii) Plants with stray fertile flowers

Protandry was observed in fully fertile flowers.

Using Reunion pubescent pollen seed set has been obtained on Algerian variant. Crosses have also been made between, fertile, partially fertile and sterile types. There was no seed set in crosses where the flowers bearing sterile anthers were used as female.

COMMON RELATIONS ON DEVELOPMENT OF ESSENTIAL OIL PLANT PRODUCTION IN BULGARIA

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The cultivation of essential oil-bearing plants has a long tradition in Bulgaria. The present development of the production is realized on the basis of old experience, intensification and the use of achievements of the advance in science and technique.

The basic relations of the essential oil plant production in Bulgaria during the last ten years are considered in the paper. There are shown also the factors increasing the effectiveness of the production of essential raw materials.

ESSENTIAL OIL RESEARCH AND ESSENTIAL OIL SYMPOSIA 1969-1989

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A review is given on some of the problems encountered in the essential oil research 1969-1989, which partly also were discussed on the international symposia in the same period.

The organization of international symposia on essential oil research was discussed in Leiden 1969. The purpose of such symposia should be to create an international forum for discussions of analytical and other problems, exchange of experimental data, mass spectra, authentic samples of pure compounds and essential oils, a.s.o. Actual problems which were discussed in the beginning, were among others length and dimensions of packed GC columns, material of such columns (glass/metal), deactivation of solid support, percentage coating with stationary phase, standardization of columns, use of Kovats' indices, solid sampling, prefractionation of essential oil components prior to GC and prevention of artifact formation. After the introduction of glass and metal capillary columns the coating of such columns, especially with polar stationary phases, was discussed. The introduction of fused silica columns, GC/MS and NMR-techniques as well as head space GC opened quite new ways and created new possibilities in the essential oil research, which also was extended to comprise studies on the biosynthesis of essential oil constituents in intact plants and cell cultures, as well as studies on glycosidic bound essential oil constituents in living plants and the antimicrobial activities of essential oil constituents.

Today - as during 1969-1989 - a better standardization of analytical methods used in the essential oil research should be a great advantage - especially in the rather complex field of sesquiterpene research.

ANALYSIS OF THE ESSENTIAL OIL OF *CRITHMUM MARITIMUM* L.

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Crithmum maritimum L. (Umbelliferae) grows wild along the portuguese coast. Stems and leaves were collected at Costa da Caparica (Setubal) and the essential oil investigated.

The material was heated at 90°C, 0.04 mm Hg, and the volatile cold trapped with liquid nitrogen. The oil was recovered with n-pentane and analysed by FID/HRGC fitted with a single vaporizing injector port into which two separated columns of different polarities were installed (DB-1 and Cp Wax-52) and by GC/MS using a Cp SIL-5 column. The oil is mainly composed of monoterpenes (84%). Nineteen compounds, from the twenty-seven consisting the total oil, were identified: fifteen monoterpene hydrocarbons (83%) and four oxygenated monoterpenes (1%). γ -Terpinene was found to be the major component (41.2%).

Compound	RRT*	%	Compound	RRT*	%
α -Thujene	0.767	0.4	γ -Terpinene	1.060	41.2
α -Pinene	0.785	1.8	Unidentified	1.069	0.2
Camphene	0.815	t	Terpinolene	1.128	0.1
Sabinene	0.866	27.5	Linalool	1.137	0.1
β -Pinene	0.877	2.4	Unidentified	1.194	t
Myrcene	0.898	1.4	Unidentified	1.123	1.5
Unidentified	0.942	t	Unidentified	1.235	t
Δ^3 -Carene	0.953	t	Terpinen-4-ol	1.322	0.4
α -Terpinene	0.963	0.3	α -Terpineol	1.346	0.4
p-Cymene	0.969	3.6	Unidentified	1.428	0.3
Limonene	0.991	} 0.7	Unidentified	1.441	13.8
β -Phellandrene	0.991	}	Unidentified	1.464	t
cis- β -Ocimene	1.000	3.3	Thymol	1.554	0.1
trans- β -Ocimene	1.027	0.2			

* Relative retention time to cis- β -Ocimene on a GC column DB-1 (60m x 0.25 mm, film thickness 0.25 μ m). Injector and detector temperature 200°C, oven: 45°C - 240°C rise 3°C/min., splitter 1/100.

t trace (<0.1%).

Granted by I.N.I.C. (Centro de Engenharia Biológica).

INVESTIGATION OF NEPALESE ESSENTIAL OILS I. THE OIL OF *CINNAMOMUM CIECIDODAPHNE* (SUGANDHA KOKILA)

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The oil of *Cinnamomum ciecidodaphne* (Sugandha kokila) which is commercially available from HPPCL (Kathmandu, Nepal) was investigated. The oil which is obtained by steamdistillation of the dried berries has a heavy, but pleasant balsamic, camphoraceous odor with some fruity and minty tones. Its composition was studied by means of GC on two columns and GC-MS after fractionation. Main components were 1,8-cineol (14%) and methyl cinnamate (14.5%). Other important components were 2-nonanone (2%), camphor (1.5%), α -copaene (5%), linalool (3%), 2-undecanone (4%), β -caryophyllene (8%), terpinene-4-ol (4%), α -terpineol (7%), methyl eugenol (1%) and the free acids n-capric acid (4%) and lauric acid (3%). It is entirely different from other *Cinnamomum* oils. Its combination of several known fragrance substances from different chemical groups gives this new oil a characteristic smell which can be of value for the fragrance industry.

SOME APPLICATIONS OF MULTIDIMENSIONAL GAS CHROMATOGRAPHY TO ESSENTIAL OIL ANALYSIS

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The use of multidimensional gas chromatography (MDGC) for the analysis of complex mixtures is gaining in importance.

This communication will illustrate through some examples the possibilities of MDGC in the analysis of essential oils. All the applications described were carried out with the MUSIC system, i.e. a multidimensional chromatography system based on the principle of flow switching proposed about twenty years ago by Deans, and provided with a cold trap between trap column and analytical column.

The examples reported will concern the following topics:

- resolution of overlapping groups of peaks in complex gas chromatographic patterns on a different stationary phase, focused in particular on the separation of components of aromatic importance.
- possibilities of enrichment of minor components: under suitable conditions, the MUSIC cold-trap can store a trapped compound (or a fraction) for a long time. During this time, the same fraction can be heart-cut from several chromatographic runs on the precolumn and stored together in order to accumulate trace compounds; afterwards the accumulated fraction can be injected in the analytical column.

THE CHEMICAL COMPOSITION OF SPANISH MYRTLE OIL

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The chemical composition was studied of the essential oils obtained by hydrodistillation from Spanish cultivated and sylvestre plants from *Myrtus communis* L.

Over 100 components were detected in the oils, from which about 50 could be identified and quantified.

There existed significant differences in the concentrations of the main components of the two oils, e.g. limonene 4.4 and 7.8%, 1,8-cineole 16.0 and 30.0%, linalool 8.5 and 0.7%, α -terpineol 2.1 and 4.1%, linalyl acetate 2.6 and 0.4%, myrtenyl acetate 31.4 and 35.5%, α -humulene 1.1 and 0.05% and eugenyl derivatives 2.4 and 1.4%, in oils from cultivated and sylvestre plant, respectively. Suggestions for the chemical identities of some new constituents will be given.

The chemical composition of two myrtle oils from other origin was also investigated. According to literature and our investigations the chemical composition of Spanish myrtle oils is different from oils from other origin.

POSSIBILITIES AND LIMITS OF STRUCTURE ELUCIDATION BY MODERN NMR-TECHNICS

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Structure elucidation by means of NMR-spectroscopy nowadays is an extremely powerful method. However, in several cases really all technics have to be used to establish the structure and the stereochemistry of compounds with many chiral centers. This will be shown for a few typical examples.

TWO CHEMOTYPES OF *CHAEROPHYLLUM HIRSUTUM* L. AND NEW CONSTITUENTS OF THE RESPECTIVE ESSENTIAL OILS

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Chaerophyllum hirsutum L. is a member of the Apiaceae family occurring in mountaineous regions in Middle-, South- and East-Europe. Preliminary investigations have shown, that the essential oils of different plant parts of *Chaerophyllum hirsutum* consist almost completely of terpenoids with ubiquitous monoterpenes dominating (1). In addition, the above ground parts contain a number of oxygenated sesquiterpenes, among them ketones, alcohols and lactones, partly in appreciable amounts.

A geographical wide ranging study of *Chaerophyllum hirsutum* L. indicated the existence of two different types of fruit oils in respect to their qualitative pattern (2). A thorough analysis revealed the occurrence and absence, respectively of three characteristic constituents as the main feature for distinguishing these two oils.

Isolation of these components by combined liquid and gas chromatography and subsequent detailed structure elucidation by means of mass-, IR- and above all ¹H- and ¹³C-NMR-spectroscopy proved, that these three substances possess an trans- α -bergamotene skeleton with different functional groups in the side chain. The structures of the constituents will be discussed on the basis of their spectral data. A literature search revealed, that these compounds are described for the first time as natural products.

1) Kubeczka, K.-H., Bohn, I., Schultze, W. and Formacek, V.: J.Ess.Oil, Res. (in press)

2) Wörtz, A.: Doctoral dissertation, Würzburg 1987.

BIOSYNTHESIS OF 1-ALKENES IN THE PLANT AND INSECT KINGDOM

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Terminally unsaturated hydrocarbons in the range of C₉-C₂₁ are important alarmones, pheromones, "wetting agents" of defensive secretions from insects, as well as constituent of flower fragrances or plant root oils. Irrespective of their structural diversity (homoterpenes, algal pheromones, porphyrins or fatty acid derived hydrocarbons) their biosynthesis is framed by common features:

- * The precursors always contain oxygen (e.g. alcohols or acids).
- * Enantiospecific attack onto a hydrogen β to the C-O moiety.
- * Fragmentation of the carbon backbone.

In the case of fatty acid derived long chain 1-alkenes the stereochemistry of their biosynthesis is completely unraveled by now using enantiospecifically deuterated 12-phenyldodecanoic acids (1) and germinating safflower (2) (*Carthamus tinctorius*) or insects (3) (*Tribolium confusum*) as a model systems. This biotransformation of acids to olefines is characterized by:

- * Enantiospecific removal of a 3-*pro*(S) hydrogen.
- * Fragmentation via an antiperiplanar transition state geometry into the 1-alkene and CO₂.
- * identical site specificity of enzymes in plants and insects.

The known stereochemical course of vinyl group formation can be further used to monitor the stereochemical course of the enoyl reductase from the fatty acid synthase in plants and insects (3).

1) Görgen, G. and Boland, W.: (1989) *Helv. Chim. Acta*, in press.

2) Görgen, G. and Boland, W.: (1989) *Eur. J. Biochem.*, in press.

3) Görgen, G., Fröhl, C. and Boland, W.: in preparation.

STUDY ON THE COMPOSITION OF SOME DUTCH TANSY OILS BY MEANS OF GC-MS AND GC-FTIR

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Samples of Dutch Tansy (*Tanacetum vulgare* L.), found on different places, could be differentiated in several chemotypes.

By means of GC-MS an artemisia ketone, a chrysanthenol / chrysanthenyl acetate and a β -thujone chemotype could be distinguished.

Furthermore a number of mixed chemotypes was found (1,2).

In several cases identical mass spectra were obtained from compounds with different retention (Kovats) indices on CP-sil 5 (Chrompack), e.g. artemisia ketone and artemisia ketone isomer (RI 1043 and 1169 respectively).

To compare the essential oils of the chemotypes mentioned above, some of these Tansy-samples were analyzed by GC-FTIR as well.

- 1) Hendrics, H., van der Elst, D.J.D., van Putten, F.M.S. and Bos, R.: The essential oil of Dutch Tansy (*Tanacetum vulgare* L.) 19th International Symposium on Essential Oils and other natural substrates, 1988, Greifensee, Switzerland.
- 2) Hendriks, H., van der Elst, D.J.D., van Putten, F.M.S. and Bos, R.: The essential oil of Dutch Tansy (*Tanacetum vulgare* L.). Journal of Essential Oil Research (submitted for publication).

GLANDULAR STUDY IN RELATION TO ESSENTIAL OIL YIELD, EUGENOL CONTENT AND LIGNIN SYNTHESIS OF CLOCIMUM (*OCIMUM GRATISSIMUM*)

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'Clocimum' (clove-scented *Ocimum*), an evolved polycross hybrid strain of *Ocimum gratissimum*, which stand genetically upgraded as 'Clocimum-3c' is rich in Eugenol, forming a dependable alternate source of clove oil of commerce. To optimise the yield attributes and to establish a co-relationship with the trichomal development in the leaves of selected genotypes, a progressive yield study was undertaken at different stages of plant growth during first and subsequent harvesting seasons of the planting year. Based on laminar development, the study revealed the qualitative and quantitative development of the essential oil and eugenol contents in the leaves being directly proportional to the trichomal count per unit area subject to seasonal variations and the year's peak level was observed in the second harvesting period i.e. September month. The graded variation in the yield attributes of the genotypes on co-relation with lignin synthesis in leaves and stem bore direct relationship, which is inversely proportional and corroborates the experimental evidences of Siegel (1955), Higuchi (1957) and Stafford (1960) based on phenylpropanoid amino acids pathway.

ON THE ESSENTIAL OIL OF *LIPPIA JAVANICA* (SPRENG.)

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Lippia javanica Spreng. (= *Lippia asperifolia* Rich.) belonging to the Verbenaceae plant family is a perennial shrub, growing abundant on rocky soils in dry woodlands of South and South-West Africa. The leaves are used in African folk medicine as a remedy against diseases like malaria, indigestion, cold and blackwater fever. Former investigations on the chemical constituents of the leaves showed the presence of amino acids, triterpenes, fatty acids, waxes, and n-alkanes. The essential leaf oil mainly consisted in p-cymene, β -caryophyllene, and linalool (1-4).

Concerning the chemical constituents of the fragrant flowers only the main constituents of the essential oil of flowering tops have been published in the literature: ipsdienone (myrcenone), cis- and trans-tagetenone (cis- and trans-ocimenone) (5). Because of the interesting olfactory properties and a lack of detailed information on the qualitative and quantitative composition, we reinvestigated the steam distillates of the flowering plants by capillary GC- and GC-MS. In addition, several constituents were isolated by the combination of distillation and preparative chromatographic techniques; their structures were further confirmed by IR- and NMR-spectroscopy. Besides myrcenone as the main constituent, 82 compounds were detected, totally representing more than 91% of the oil. Together with myrcenone further oxygenated monoterpenoids as well as mono- and sesquiterpene hydrocarbons, sesquiterpene alcohols, phenols, and aliphatic acids could be identified.

- 1) Neidlein, R., Staehle, R.: Dtsch.Apoth.Ztg., **113** (26), 993 (1973)
- 2) Neidlein, R., Staehle, R.: Dtsch.Apoth.Ztg., **113** (32), 1219 (1973)
- 3) Neidlein, R., Staehle, R.: Dtsch.Apoth.Ztg., **114** (40), 1588 (1974)
- 4) Neidlein, R., Staehle, R.: Dtsch.Apoth.Ztg., **114** (49), 1941 (1974)
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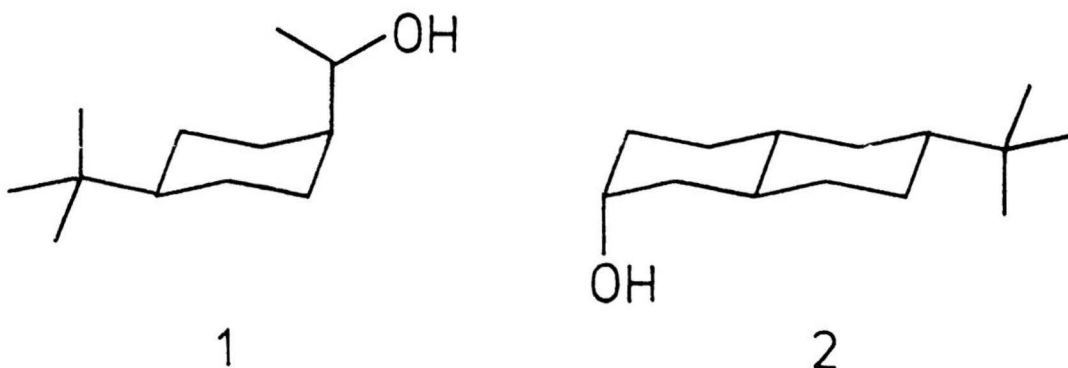
CONFORMATIONAL CALCULATIONS ON SANDALWOOD ODOUR MOLECULES

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Among fragrance compounds with sandalwood odour, **1** belongs to the very interesting group of cyclohexanol derivatives. In this case only secondary alcohols possess this precious fragrance and only the *cis*-isomer, as shown in the formula, is able to evoke this odour sensation. In order to gain more insight into structure-odour-relationships of these compounds at first we analysed their conformations by force field calculations to establish the accurate geometries of the molecules. In addition we calculated by means of the semiempirical method AM 1 their electron densities. Then two by two we compared the best conformations of these cyclohexanols with 8-*t*-butyl-bicyclo(4.4.0)decan-3-ol **2**, thus looking for the best congruence especially in the region of the functional group. **2** is known to exhibit the typical sandalwood odour and we use it because of its rigidity as a standard. Because the surface area of an odour molecule seems to be most important to create the odour sensation at the receptor site, we also compared the van der Waals surfaces of these cyclohexanols by statistical methods.



ANTIMICROBIAL ACTIVITY AND CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *THYMBRA SPICATA* CULTIVATED IN TURKEY

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Thymbra spicata (Lamiaceae) is used as a spice and in folk medicine in some eastern mediterranean countries. In order to find out about its antibiotic activity, the essential oil was tested against 4 microorganisms (*Escherichia coli*, *Klebsiella pneumoniae*, β -Haemolytic streptococcus, *Staphylococcus aureus*) which had been isolated from patients. Essential oil emulsions were prepared in 1/5 ratio using Tween 80 (5%) on filter paper discs of 6 mm of diameter. Antibiotic discs of amoxycillin, ceftizoxime and tobramycin (Oxoid, England) were used to compare inhibition zones. Bauer-Kirby technique (1) was applied for this purpose. As a result, the essential oil of *Thymbra spicata* had antibacterial activity to *Staphylococcus aureus* and was stronger compared to amoxycillin.

The essential oil had been obtained by hydrodistillation and analysed using capillary gas chromatography. There were thirtyfour components from which twenty-one could be identified by coinjection with authentic samples and by comparison of their MS-data (2). Carvacrol and γ -terpinene appeared in considerable amounts: 67 and 14%. Thymol acetate, carvacrol acetate and trans-sabinene hydrate were identified in traces.

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- 2) Burkhardt-Sischka, S.: Einfluß ökologischer Faktoren auf Qualität und Quantität des ätherischen Öles bei einigen Lamiaceen. Diss. Univ., Hohenheim 1989

RESULTS OF THE FIRST CULTURES OF THE *OCIMUM BASILICUM* L. IN PORTUGAL

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The *Ocimum basilicum* L. is an annual plant from the family of lamiaceae with green stems and leaves and a pleasant smell.

It is used in food industry and perfumery and it is very common in Portugal to offer this plant in the St.Johns festivity, in June.

We show results of experiences of the last three years cultivation of *Ocimum basilicum* L. in our country. The techniques permit harvesting three or even four times if the plants were transplanted in May.

The oil is obtained by steam distillation and the best harvesting time regarding the oil content is investigated.

The samples of the first cultures indicate linalool as the main component, significant amounts of 1,8-cineol, terpinen-1-ol-4 and caryophyllene, methyl chavicol and methyl cinnamate, and a weak quantity of eugenol. The *Ocimum basilicum* L. seems to be a linalool type, but it is not according to the similar oils from other geographic origines.

ANTIMICROBIAL ACTIVITY OF PLANT VOLATILE OILS AND THEIR CONSTITUENTS

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(abstract not available)

ANNUAL VARIATION OF MONOTERPENE CONCENTRATIONS IN NEEDLES AND BUDS OF *PICEA ABIES* (L.) KARST.

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The annual variation of monoterpene concentrations was investigated with needles and buds of four- to five-year-old clonal Norway spruce trees in 1987 and 1988. The main compounds in current-year and last-year needles are camphene, bornyl acetate, α -pinene, 1,8-cineole and limonene. Further are present tricyclene, sabinene, β -pinene, myrcene, Δ -3-carene, β -phellandrene, terpinolene, camphen hydrate, camphor, borneol, α -terpineol, piperiton and terpinyl acetat. The total amounts of needle monoterpenes range from 2 to 4 mg/g fresh mass.

The terpene concentrations of both clones take the same alteration pattern over the year with the exeption of a generally higher level of terpene concentrations in needles of the year 1988. The results can be summarized in the following way: One-year-old needles maintain an approximately constant terpene-level with a slight depression during winter-time. In the buds only a small amount of terpenes is deposited, limonene being the main compound with up to 30% of the total terpene content, while oxygenated monoterpenes are scarcely present. A strong increase of the terpene concentrations in new needles ends in July; during the remaining months no great changes in regard of terpene pattern and concentration occur. According to these results the period from August to October seems to be the best sampling time for new and one-year-old needles of Norway spruce.

DISTRIBUTION AND COMPOSITION OF ESSENTIAL OIL
CONSTITUENTS AS CHEMOTAXONOMIC MARKERS IN THREE
MENTHA L. SPECIES IN YUGOSLAVIA

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Taxonomically, the genus *Mentha* L. is very complex due to different sex forms and ploidy levels, long history of clonal cultivation, naturalisation of cultivated forms followed by hybridisation with native species and morphological plasticity. *Mentha arvensis* L., *M. longifolia* (L.) Hudson and *M. spicata* L. in particular have in the past posed difficult problems in classification due to either lack of reliable morphological characters or large variation in leaf and inflorescence morphology. The taxa under study are distributed in the temperate regions of the old world and like many Mediterranean labiates are rich in essential oils.

The analysis of the essential oil components: -piperitone, linalool, menthol, geraniol, limonene, carvone, pulegone, menthone, p-cymene, caryophyllene and γ -terpene, by GLC of three wild species of genus *Mentha* collected from the River Danube ravine north east of Yugoslavia has been carried out. High levels of variation between species were observed among some of essential oil components. This variation may be a useful additional character in delimiting these species is discussed; in relation to the use of these oils as chemical markers.

APPLICATION OF GC/UV-COUPLING TO THE ANALYSIS OF ESSENTIAL OILS

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The hyphenated techniques GC-MS and more recently GC-FTIR have become powerful tools in the analysis of essential oils and are nowadays well established techniques. In contrast, the application of a GC-UV coupling is relatively seldom. In spite of the higher sensitivity of UV-spectroscopy as compared to IR-spectroscopy, this may be attributed to the relatively low information which can be deduced from a UV- spectrum.

By means of examples from the field of essential oils analysis the applicability of a GC/UV-spectrometer coupling will be demonstrated.

In this study a commercially available UV-VIS diode-array spectrometer (MCS 220, C.Zeiss, Oberkochen/FRG) was used, which can provide continuous monitoring in the range from 200 - 600 nm in intervals of approx. 1 nm. By interfacing the spectrometer via fibre optics to a thermostated flow cell which was connected to the gc-column effluent, a complete spectrum could be monitored within 70 ms using a personal computer (HP Vectra) for data storage and handling.

THE INVESTIGATION OF THE STEREOCHEMISTRY OF FLAVOUR COMPOUNDS USING CHIRAL CAPILLARY GAS CHROMATOGRAPHY WITH CYCLODEXTRIN STATIONARY PHASES

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The importance of stereochemistry with regard to flavour and fragrance is well-known from many examples. The determination of the configuration of a constituent of complex mixtures is particularly difficult and can be achieved by chiral capillary gas chromatography. We have recently developed methods to prepare highly enantioselective lipophilic derivatives of cyclodextrins (1). They are thermally stable up to 400°C and can be used as chiral stationary phases to separate the enantiomers of many compounds classes. Since enantioselective interaction of a chiral substrate and a cyclodextrin phase is based mainly on inclusion into the macrocyclic cavity and not on hydrogen bonding and other polar effects the spectrum of enantiomers susceptible to chiral recognition and resolution could be greatly enhanced. Regioselectively alkylated and acylated β - and γ -cyclodextrins proved to be highly suitable to separate the enantiomers of compounds related to flavour and fragrance like olefins (α -pinene, camphene, limonene), alcohols (borneol, isoborneol, β -citronellol, myrtenol, trans-pinocarveol, 1-octene-3-ol, grandisol), carbonyl compounds (menthone, isomenthone, fenchone, carvone, camphor, α -ionone, α -damascone, myrtenal), γ - and δ -lactones. The absolute configuration of compounds present as trace constituents in complex mixtures like α -ionone and α -damascone in black tea extracts could be determined. It could be shown that many chiral constituents of essential oils are present as mixtures and not as pure enantiomers. It can be anticipated that many other chiral flavour constituents will be separated as soon as more racemic reference compounds will be available.

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CROSSING RESULTS OF MARJORAM (*MAJORANA HORTENSIS*
MOENCH.) WITH DIFFERENT ODOUR AND FLAVOUR PROFILE

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(abstract not available)

BIOSYNTHESIS OF HOMOTERPENES IN HIGHER PLANTS: STEREOCHEMICAL IMPLICATIONS

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The two homoterpenes 4,8-dimethylnona-1,3,7-triene and 4,8,12-trimethyltrideca-1,3,7,11-tetraene are widespread flower fragrances (1); they also act as mediators in insect/plant interactions (2). The present work provides evidence, that these compounds are biosynthesized from the two regular terpene alcohols nerolidol and geranyllinalool by oxidative fragmentation into the homoterpene and a still unknown C₄ fragment (3). During this reaction a single hydrogen from C-5 of nerolidol is removed and the segment (C-1)-(C-3) is lost (but-3-en-2-one?). Further work with enantiospecifically deuterated (5*R*)- and (5*S*)-[4,4',5-²H₃]nerolidol reveals the hydrogen abstraction as an exclusive *Si*-attack (4). Artificial [²H]-nerolidol analogues with ethyl- or phenyl substituents instead of the vinyl group are also converted to 4,8-dimethyl-[²H]nona-1,3,7-triene. However, the expected deuterated ketone fragments could not be isolated as yet.

The same stereochemical events were observed with specimens of *Magnolia liliiflora nigra*, *Philadelphus coronarius* and *Robinia pseudoacacia*.

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AGROBOTANICAL STUDY ON DIFFERENT BASIL VARIETIES GROWN INDOORS IN FINLAND

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Twenty different basil varieties of Canadian, Hungarian and Greek origin were cultivated in a greenhouse in Puumala, Finland during 1987-1989. The plants were sown in 3-5 liter pots and the growing period was April-September. The agrobotanical features, such as growth habits, plant height and weight, number of branches, colour and the variability of the leaves were studied. Important factors for the production of herbs; fresh and dry yields, leaf/stem ratio and the volatile oil content and composition were also of interest.

The basil varieties studied can be divided into four chemotypes on the basis of gas chromatographic analysis of the oils. The chemotypes found in this study were: linalol-, methylchavicol/linalool-, 1,8-cineole/linalool- and methyl-cinnamate-type.

There is no clear connection between the chemotype and the agrobotanical features. The variation of the morphological features was great. By dividing the material into groups on the basis of for example the leaf size the variation of the other morphological features is smaller within the group than between the groups.

The fresh yield varied from 1.79 to 6.49 kg/m². The yield is determined by the proportion of stems, i.e. the more stems, the bigger yield.

Due to the different shape and colour (light green, deep green, purple) and also chemical composition basil finds use as a fresh and dry condiment as well as an ornamental plant.

YIELD AND VOLATILE OIL OF FOUR PERENNIAL HERBS GROWN IN FINLAND (*HYSSOPUS OFFICINALIS*, *ORIGANUM VULGARE*, *SALVIA OFFICINALIS*, *THYMUS VULGARIS*)

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Hyssop, wild marjoram, sage and thyme were cultivated on small and semilarge experimental plots (size 2-20 or 100-1000 m²) during 1984-1988 in Puumala, South Finland (61°40'N, 28°15'E). Of these plants wild marjoram occurs naturally in Finland. The wintering properties, yields and volatile oil were studied.

Sage and thyme were totally destroyed by the frost every winter, so they could be cultivated only as annuals. Hyssop and marjoram wintered very well.

The dry crop of sage varied from 0.17 to 0.38 kg/m² during the experimental years, with an average of 0.23 kg/m². The corresponding crop of thyme varied from 0.07 to 0.32 kg/m², being on average 0.17 kg/m². The crop of both species was quite small and varied depending on the temperature of the growing season. The crops of hyssop and wild marjoram were much higher, 0.73 and 0.54 kg/m² in the second year, respectively. Hyssop and wild marjoram seemed not to be as sensitive to the temperature as sage and thyme.

The volatile oil composition of sage resembles that of Dalmatian sage, i.e. the main components were α - and β -thujone, camphor and borneol. The oil content varied between 0.8-2.0% (at least 1.5%, DAB 8). Thyme contained 1.2-2.4% volatile oil, which main component was thymol (DAB 8; at least 1.2%, with 40-50% thymol). Hyssop contained 0.8-1.2% volatile oil, with β -pinene, pino- and isopinocampheol and pinocarvone. This is in accordance with the data given by Leung (1). Wild marjoram had an oil content of 0.4-0.6%, with thymol and carvacrol 0.4-22% (Leung; 0.1-1.0% oil, thymol and carvacrol 0-90%).

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HEAVY METAL CONTENT OF HERBS GROWN IN FINLAND AND CENTRAL EUROPE

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Lead and cadmium contents of 14 herb species grown in three experimental farms in southern, central and northern Finland were compared with those collected from commercial sources in Hungary and Czechoslovakia. Samples from experimental Czech farms were analysed as well. Samples were determined for lead and cadmium by electrothermal atomic absorption spectrometry using Zeeman background correction, well established methods (1) and certified plant reference materials to ensure analytical quality control.

The results obtained showed that the average lead and cadmium contents of Central European samples were approximately three times higher compared to Finnish samples (Table 1).

Table 1. Average lead and cadmium contents ($\mu\text{g/kg}$ dry weight) of 14 herb species grown in Finland and Central Europe.

Sampling area	Lead content			Cadmium content		
	Mean	-/+SD	N	Mean	+/-SD	N
Finland	463	232	50	64	61	46
Central Europe	1437	740	50	132	110	46
t-test, paired samples:	p<0.0003			p<0.001		

There appeared to be differences in lead and cadmium contents among herb species as well, although this could not be confirmed statistically because of the limited number of samples available.

Due to the generally low average level of consumption of herbs their contribution to total heavy metal intake is limited, however. The present results support the earlier data showing that Finnish foods are low in heavy metals (2).

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GROWTH AND DEVELOPMENTAL PHYSIOLOGY IN RELATION TO
ESSENTIAL OIL SYNTHESIS OF *MENTHA PIPERITA* L. UNDER
VARYING FERTILIZER, PHOTOPERIOD AND GIBBERELIC ACID
TREATMENTS IN THE GANGETIC PLAINS OF WEST BENGAL, INDIA

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N:P:K combination with 200:100:50 kg/ha, Nitrogen-225 kg/h, Long-day-20 photoinductive cycles and GA₃-10 µg/ml treatments had been found most effective for maximum augmentation of growth, metabolism and essential oil formation in *M. piperita* plant under the agroclimatic conditions of gangetic plains of West Bengal, India.

Studies clearly revealed that though the vegetative phase exhibited maximum rates of growth and essential oil formation, the yield of oil per unit laminar tissue registered maximum prevalence during reproductive phase. Analysis of growth and essential oil formation had clearly shown that the rates of leaf formation and dry matter accumulation were accompanied by the rate of the formation of essential oil in the leaves.

The correlation between an extended duration of reproductive phase and shortened duration of senile phase with higher essential oil content during the entire life history of the plant had also been well established. The quanta of shortening and lengthening of the developmental phases had a direct bearing on the effectiveness of a particular treatment. Besides, some sort of morphological indexing on the basis of blooming condition vis-a-vis essential oil formation had been also worked out. Biochemical indexing like increase in soluble nitrogen and total carbohydrate content accompanied by increase of essential oil formation was established.

Analysis of menthol content during different developmental phases had clearly shown that the constituent increased up to reproductive phase and declined thereafter. Effective treatments of fertilizer and photoperiod could induce the plant to produce more menthol content. It is interesting to note that GA₃ could not augment the synthesis of menthol in *M. piperita* oil.

A CHEMICAL STUDY ON VARIOUS BASIL CHEMOTYPES CULTIVATED IN FINLAND

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Basil (*Ocimum basilicum* L., Lamiaceae) is a plant with many subspecies, varieties and chemotypes. The occurrence of the following five chemotypes has been reported: 1. European or Mediterranean type with linalool and methylchavicol as the main components, 2. Reunion type with methylchavicol, 3. Bulgarian or methyl cinnamate type, 4. Eugenol type and 5. Linalool + eugenol type (1). In this study 13 basil cultivars of different origin were analysed by headspace gas chromatography (HSGC) and by gas chromatography-mass spectrometry (GC-MS).

From the oils of these basil cultivars 49 compounds could be identified. Of these 14 were monoterpene hydrocarbons, 11 sesquiterpene hydrocarbons, 15 oxygenated monoterpenes, 4 oxygenated sesquiterpenes, 5 phenylpropanes and 3 oxygenated aliphatic compounds. Determined by the main component five of the studied oils belonged to the European type, three to the Bulgarian type and one to the linalool-eugenol type. Three oils had linalool as a sole main component and one oil contained huge amounts of 1,8-cineole. The last mentioned type with 1,8-cineole as the main component is not reported earlier in the literature. The plant-to-plant variation among the same cultivar was, however, great, which can be due to the fact that basil plants are easily cross pollinated if given the opportunity. Seeds of the same origin are also reported to produce varying oil compositions if cultivated under different conditions (2).

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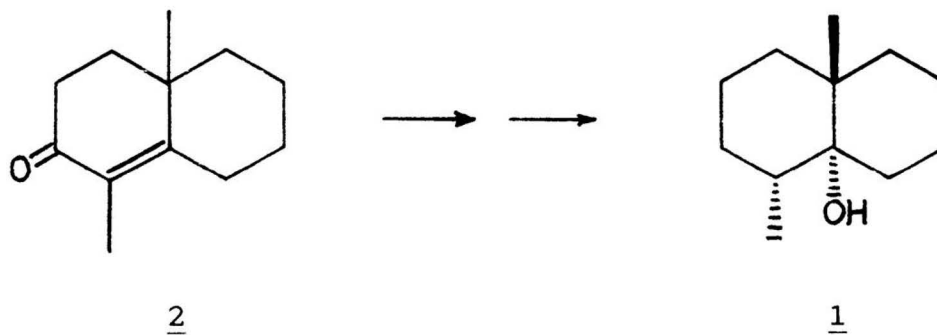
A STEREOSELECTIVE SYNTHESIS OF (±)-GEOSMIN

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The strongly earthy-smelling compound geosmin **1** is obtained stereospecifically in four steps and 42% overall yield from 1,4a β-dimethyl-4,4a,5,6,7,8-hexahydronaphtalen-2(3H)-one **2**.



The key step involves a new one-pot double reduction of an epoxytosylate.

This successful synthetic methodology has now opened the way to enantiomerically pure (-)- (natural) or (+)-geosmin starting from (+)- or (-)-octalone **2** respectively.

Revial, G.: Tetrahedron Letters (1989)

COMPOSITION OF THE LEAF ESSENTIAL OILS OF VARIOUS HOP CULTIVARS

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Various cultivars of hop, *Humulus lupulus* L., are of great interest for the breweries, among others because of the essential oil from the hop cones. In a previous study, we analysed the volatile compounds isolated from cones of ten hop cultivars (1). Since the oils of the various cultivars represented different genotypes, it was concluded that the plant material should be suitable for our investigations on the possible formation of hop flavour components by means of plant cell cultures.

We were also interested to know whether volatile compounds are present in other parts of hop plants, however, a search of the literature did not yield any publication on the occurrence of volatiles in hop leaves. Therefore, we subjected leaves of ten hop cultivars to hydrodistillation. Subsequently the oils obtained were analysed by GC - using a dual channel gas chromatograph equipped with capillary columns of different polarities - and their composition compared.

Although the oil amounts were small, we were able to detect many components, about 25 of which - found in almost all samples - were identified. Like in the oils from the hop cones, sesquiterpene hydrocarbons clearly predominated. α -Humulene (3-23%) and β -caryophyllene (5-29%) were main components present in all samples; the former component predominated in four of the oil samples, the latter in two of them. In contrast to the oils from the cones, β -myrcene was hardly present or in small amounts (<1%) only.

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ANALYSIS OF THE VOLATILE COMPOUNDS FROM CONES OF TEN *HUMULUS LUPULUS* L. CULTIVARS

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Hop, *Humulus lupulus* L., belongs to the order Urticales and, to the family Moraceae or Cannabaceae (1). Various cultivars of hop are of great interest for the breweries. The lupulin glands of the hop cones contain a resinous fraction and an essential oil which are responsible for the bitter taste of beer and contribute to the flavour of it, respectively (2,3).

Our aim was to analyse and compare the essential oils from cones of ten hop cultivars: 'Brewers Gold', 'Eroica', 'Hallertau', 'Hersbrücker', 'Hugo de Groot', 'Olympic', 'Wye Challenger', 'Wye Northdown', 'Wye Target' and 'Yeoman'.

The dried hops of each cultivar were submitted to hydrodistillation. Since 'Hallertau' is known as an aromatic cultivar and its oil consisted of more components than the other oils, this oil was chosen for a complete analysis by LSC, GLC and GC-MS. GC analyses were carried out on a dual channel gas chromatograph equipped with capillary columns. GC-MS data were obtained on a gas chromatograph interfaced with a Finnigan MAT 700 ion trap detector.

The yields of the essential oils isolated by hydrodistillation from cones of the various hop cultivars studied, varied from <0.1% to 2.0% (vol/dry wt). Of the many components detected in the oil of the cultivar 'Hallertau', 62 were identified which represented more than 95% of the oil. Subsequently, the oils isolated from the other cultivars which were grown under the same conditions, were compared with the 'Hallertau' oil using the two GC columns of different polarities. In all oil samples, which showed to be rather complex, mono- and sesquiterpene hydrocarbons clearly predominated. β -Myrcene, α -humulene and β -caryophyllene were characteristic main components present in all samples, although variations in their relative amounts did exist.

The oils isolated from the various hop cultivars represented different genotypes: in two oils α -humulene was found as main component (ca. 40%), in seven oils β -myrcene (42-58%) clearly predominated, whereas in one oil almost equal amounts of β -myrcene (25%) and α -humulene (27%) were found.

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STUDY ON THE EFFECT OF IRRIGATION OF MINT (*MENTHA PIPERITA*) VARIETY "SOFIA" BY MEANS OF VARIOUS IRRIGATION TECHNIQUES

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During the period 1985-1988 in the institute for scientific and research work of roses essential-oils and medicinal plants in the town of Kazanlik was made on experiment over the leached-maroon soils with *Mentha piperita* variety "Sofia". With the aim of establishing the irrigation techniques effect on the mint yield and the most economical use of water resources.

Two irrigation techniques were studied - sprinkling and dripping with the dripping having been done in two ways surface and subsurface in maintaining an optimum pre-irrigation soil humidity 85% in the experimental plot. The highest yield was achieved through the dripping irrigation technique, combined with the most economical use of water resources. The values of evapotranspiration have been established with the three irrigation techniques along with the biophysical coefficients - R according to Alpatev, Z to Delibaltov, Christov, Tzonev and K to Blany and Criddle. The data that were obtained will be used in the regional distribution of the irrigation technique and in designing the new irrigation systems.

PHYSIOLOGY AND BIOCHEMISTRY OF THE ALLIINASE SYSTEM FROM GARLIC (*ALLIUM SATIVUM* L.)

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The central alliin lyase protein system (alliinase) converts the amino acid S-allyl-L-cysteine sulfoxide (alliin) into allyl thiosulfinate (allicin). A maximal specific activity in the range of 220 (25°C) and 490 (37°C) μ moles per min and mg enzyme protein can be achieved. Besides alliin, the alliinase accepts S-ethyl- and, likewise, S-propyl-L-cysteine sulfoxide as substrates with an activity of about 16.5% and 9.8%, respectively, if compared to alliin. Km-values for the different substrates are in the mM range. The pH optimum for the enzymatic cleavage of alliin was determined to be at pH 6.5. The temperature optimum for the alliin lyase is focussed at 33°C. The determination of activation energies of alliinase-catalyzed alliin cleavage resulted in 14.7 kJ/mol, and the corresponding value for S-ethyl-L-cystein sulfoxide as substrate was 17.8 kJ/mol.

The enzymatically generated allicin is converted into the dithiines, ajoenes and di- or poly-sulfides, respectively, depending on the environmental conditions. A broad variety of sulfides is detected in the gase phase enriched above crushed garlic.

The alliinase-dependent generation of allicin and its decay products can be inhibited by specific components. Hydroxylamine sulfate, with pyridoxal-5'-phosphate as the main target group, decreases the production of allicin by 90% at a 50 μ M concentration. The isoflavonoid rotenone reveals the same inhibition rate even at a concentration of 10 nM. It can be demonstrated that rotenone attacks a flavin part of the alliinase enzyme protein. - Whereas pyridoxal-5'-phosphate has been known to be part of the alliin lyase system, the existence of a further coenzyme, a flavin, of the yellow appearing alliinase protein has not been reported yet.

Purification of the yellow alliinase protein, involving liquid chromatography on hydroxylapatite, on an anion exchanger and on a chromatofocussing medium, finally resulted in SDS gelelectrophoretic homogeneity. The enzyme was characterized by a relative molecular mass of 108.000, and was found to consist of two equal subunits. A simulated gastricintestinal passage by a modified "half change-test" revealed a high acid lability of the purified alliinase protein.

COMPOSITION AND ACCUMULATION SITES OF THE ESSENTIAL OIL FROM DIFFERENT PARTS OF *PONCIRUS TRIFOLIATA* RAF.

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Poncirus trifoliata is an arborescent Rutaceae, growing wild in Northern and Central China, but is also cultivated in North America and mediterranean countries.

While the essential oil of the leaves and fruits has been subject of several investigations (1-3) there exists no report on the composition of the volatiles of the twigs and the underground parts of *P. trifoliata*.

The essential oil of all parts of *P. trifoliata* obtained by hydrodistillation was analyzed by us by means of capillary GC-MS. As a result we found different oil patterns which can be correlated to some extent to different glandular structures: plant parts with glandular complexes such as petals, sepals, leaves, twigs and the exocarp of the fruit contain an oil which consists almost exclusively of monoterpene hydrocarbons (up to 90%). The essential oil of glandular cells, which can be found in the pulp, comprises mainly sesquiterpene hydrocarbons and oxygenated compounds.

The essential oil of the roots which is accumulated in individual oil cells has a quite different make up. It is characterized by trinor-sesquiterpenoids, mainly by pregeijeren which has previously been found in the underground parts of several other plants belonging to the Rutaceae family.

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THE STRUCTURE OF CYPROTENE, A NOVEL C₁₄ HYDROCARBON RELATED TO SESQUITERPENOIDS

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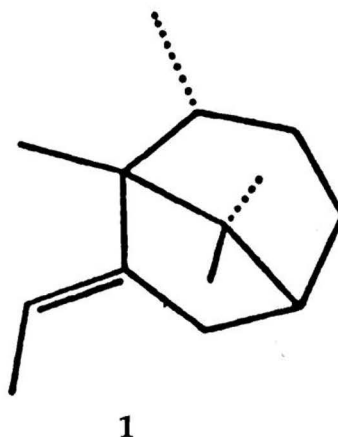
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During the study of the essential oil from *Cyperus rotundus*, which is produced in China by industrial scale, a hydrocarbon with the unusual formula C₁₄H₂₄ was observed many years ago (1).

In this study we report on the isolation and structure elucidation of this constituent **1**, which we propose to name "cyprotene".

Isolation was achieved by fractional distillation and subsequent liquid column chromatography, using silica gel and n-pentane as a stationary and a mobile phase, respectively. The structure of the white crystalline compound (m.p. 22-23°C), which seems to be related to sesquiterpenoids, was established by means of IR-, ¹H- and ¹³C-NMR spectroscopy.



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BERGAMOT OIL AND MANDARIN OIL – TWO RAW MATERIALS OF SPECIAL INTEREST TO THE FRAGRANCE AND FLAVOR INDUSTRY

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In comparison to other citrus oils, the production capacity for bergamot and mandarin oil seems to be limited. Availability and price fluctuation are therefore two problems which cannot be either neglected nor accepted without any reaction by the fragrance and flavor industry.

As a matter of fact, especially bergamot oil has been used since a long time as a very important ingredient in perfume compositions of the EdC-type. But the cold-pressed oil is known to contain phototoxic substances which have to be eliminated prior to the incorporation into a perfume and its topic application to the skin. This may create another problem aggravating the supply situation and the cost figures of this oil.

The intimate knowledge of the chemical composition of both the mentioned oils rendered it possible to make olfactorily nice substitutes on the basis of synthetically available chemicals like linalool and its derivatives or methyl N-methyl anthranilate, respectively. These substitutes will no longer show such dramatic price increases as observed for the naturals and give us the opportunity to strictly keep the latter materials for flavor purposes for which naturalness is required.

We will discuss some of the trace components in more detail and compare our own results with data available from literature.

CHEMICAL IONIZATION MASS SPECTROMETRY OF SESQUITERPENOIDS

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CI (chemical ionization) mass spectrometry is widely used as a complementary additional technique to EI (electron impact) mass spectrometry for analyzing complex mixtures.

The reaction behaviour of a number of sesquiterpene hydrocarbons (mwt 204) and sesquiterpene alcohols (mwt 222) under the conditions of CI is studied, using i-butane and ammonia, respectively, as the reagent gases.

With i-butane the hydrocarbons produce intense $(M+1)^+$ ($= m/z$ 205) ions while many of the alcohols form the species $(M+1)^+$ ($= m/z$ 223) with only very low intensity. Both the compound groups display very different spectral patterns. Classification of the compound classes is not absolutely unambiguous.

With ammonia as the reagent gas the molecular mass can be determined unequivocally for the sesquiterpene hydrocarbons and the alcohols as well by means of the cluster ions $(M+18)^+$ and $(M+35)^+$, respectively. Compounds of both types can be localized in balm oil, (from *Melissa officinalis*) by applying mass chromatography in CI mode. This example also demonstrates, that CI can be utilized successfully to detect unresolved GC peaks.

SOME CONSTITUENTS FROM PHILIPPINE CULTIVARS OF *ALLIUM CEPA* L. AND THEIR ORAL HYPOGLYCEMIC EFFECT IN RABBITS

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Gas chromatography of the volatile oil from the ethereal extract of the skin-free bulbs of four cultivars (red, yellow, purple, and white) of onion yielded several peaks of which only propyl disulfide was identified. The headspace gas of the four cultivars showed the presence of methyl sulfide and propyl disulfide and other unidentified compounds. In addition, methyl disulfide was identified in the headspace gas of the red and yellow cultivars.

The petroleum ether-insoluble fraction of the volatile oil from the red and yellow cultivars produced a significant hypoglycemic effect when administered orally to normal rabbits, the effect being 71.8% and 41.5%, respectively of that of tolbutamide, a known oral hypoglycemic agent.

SFE/SFC FOR THE ANALYSIS OF AROMATIC PLANTS

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SFE is a selective sampling method, which in certain cases has advantages over conventional selective sampling methods, such as liquid extraction, static or dynamic headspace extraction and thermal desorption. The combined SFE/SFC is comparable to static headspace gas chromatography (HSGC) and was used for the analysis of dragonhead (*Dracocephalum moldavica* L.) herb samples.

The plant material used was dried and coarsely ground. Before the analysis it was finely ground and 10 mg or 50 mg weighed into the extraction vessel, which had a volume of 3 ml. The extraction system consisted of two 10-port valves (Valco) and the extraction vessel. It was on-line coupled to a Lee Scientific series 600 supercritical fluid chromatograph equipped with FID (320°C). The column used was SB-octyl-50 (Lee Scientific, 10 m x 50 µm i.d.). CO₂ was used as the supercritical fluid.

In order to find the best analytical conditions different extraction temperatures (60, 80, 90 and 100°C) and different densities of CO₂ were tested. The maximum total peak area was obtained at 80°C and at this temperature the terpene composition is comparable to that obtained from the distilled oil by GC. At 90 and 100°C the proportion of the main compound, geranyl acetate, is significantly smaller than at 80°C. The resolution for the main compounds in SFE/SFC is satisfactory, but the resolution for minor and trace components is not as good as in capillary GC. The main advantage of SFE/SFC over static HSGC seems to be the use of a lower extraction temperature, since supercritical fluids have high solute diffusivities and excellent solvating power. However, SFE/SFC includes numerous variables which still have to be optimized before routine use in volatile oil analysis.

MICROPROPAGATION OF LAVENDER

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In recent years the interest in Lavender Oil used in pharmaceutical preparations, perfumery and cosmetic increases. A new cultivar *Lavandula vera* D.C. with high average biological essential oil yield 15,7 kg/dka was obtained at the Institute.

The purpose of this work was to obtain plants, produced in vitro for rapid building up mother stock of the new cultivar. In this report a method for rapid propagation of *Lavandula vera* D.C. cultivar "Iubileina" will be presented. The method involve tip-culture, shoot elongation, multiplication and root initiation by small cuttings and transfer of plants to soil. The in vitro produced lavender plants by this method were planted in the field in May 1988/89.

CHIRALITY EVALUATION OF ESSENTIAL OIL COMPOUNDS USING MULTIDIMENSIONAL GAS CHROMATOGRAPHY (MDGC)

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Chiral discrimination is well appreciated as an important principle in odour perception (1-5). Consequently, the evaluation of enantiomeric compositions and absolute configurations of chiral compounds from essential oils and from other complex flavour matrices is highly warranted.

In our studies on structure-function relationships of stereoisomeric flavour compounds the chirospecific analysis has been carried out for a number of chiral molecules (6-9).

Ni(II)-bis[3-heptafluorobutanoyl-(1R)-camphorate] and Heptakis(3-O-acetyl-2,6-di-O-pentyl)- β -cyclodextrin (Lipodex D) respectively are proved to be powerful tools to differentiate chiral 1-alken-3-yl esters. From commercially available lavender oils chiral 1-alken-3-yl esters are directly stereoanalyzed by multidimensional gas chromatography (MDGC), employing heart-cutting techniques from OV-1701 as the pre column onto the chiral main column.

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BIOSYNTHESIS OF ALGAL PHEROMONES: STEREOCHEMICAL COURSE OF FATTY ACID CYCLIZATION

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Unsaturated C₁₁ and C₈ hydrocarbons are secreted as pheromones by female gametes of many marine brown algae (1). Without exception, these compounds are metabolites of multiply unsaturated C₁₂ or C₉ fatty acids. Their production from these precursors is framed by the following observations (2):

1. Specific embedding of the fatty acid into the active center of the various enzymes determines absolute configuration of the products.
2. Hydrogen abstraction from C(5), C(8) or C(11) determines the ring size of the products.
3. Ring closure occurs between the activated methylene group at C(8) or C(11) and the $\Delta^{3,4}$ double bond with concomitant decarboxylation.
4. Linear hydrocarbons result from attack onto C(5) and subsequent decarboxylation.

Further studies with the composite *Senecio isitadeus* and enantiospecifically labelled (8*R*)- and (8*S*)-trideca-3,6,9-[8-²H]trienoic acids indicates *Re*-specificity of the plants enzyme(s). This result is in agreement with the absolute configurations of the same compounds from marine brown algae.

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THE COMPOSITIONS OF *ANGELICA* ROOT OILS OBTAINED BY SUPERCRITICAL CO₂ EXTRACTION AND STEAM DISTILLATION

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Supercritical CO₂ extraction was used for isolating the flavour compounds from angelica root. The extractions were conducted at 40°C, with pressures ranging from 80 to 400 bar. Depending upon the extraction conditions, the appearance of the product varied from a pale yellow mobile oil to a dark yellow viscous liquid. The extracts were analyzed by capillary gas chromatography and mass spectrometry. For comparisons the flavour compounds were also isolated from angelica root by steam distillation.

A total of 101 components were determined quantitatively. α -Pinene, sabinene, myrcene, β -phellandrene, 3-carene, p-cymene, β -phellandrene, chrysanthenyl acetate, α -copaene, pentadecanolide, 7-methoxy-8-(3-methyl-2-butenyl)-2H-1-benzopyran-2-one, and a tentatively identified furanocoumarin with a molecular weight of 286 were among the major volatile components in the supercritical extracts. For monoterpenoids, e.g., α -pinene, β -phellandrene, 3-carene, and chrysanthenyl acetate, the maximum yield was obtained already at 100 bar.

The total amount of volatiles obtained by supercritical CO₂ extraction at various pressures varied from 0.13% to 0.55%. The highest yield was obtained at 200 bar, particularly because of the high amount of 7-methoxy-8-(3-methyl-2-butenyl)-2H-1-benzopyran-2-one. The amount of total volatiles obtained by steam distillation was 0.10%. The extracts obtained at pressures of 100 to 200 bar were sensorially acceptable.

A noticeable difference between the supercritical extracts and the oil isolated by steam distillation was found in the content of chrysanthenyl acetate. In the supercritical extracts it was present in amounts of 74 to 94 mg/kg of angelica root, whereas the oil isolated by steam distillation contained only 1 mg/kg. In contrast, p-menth-2-en-1-ol, limonene-1,2-oxide, 4(10)-thujen-3-ol, Z-piperitol, carvone, and piperitone were absent from the supercritical extracts but found in the oil obtained by steam distillation.

COMPOSITION OF THE HERB ESSENTIAL OIL OF *HYPTIS SPICIGERA*

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Aerial parts of *Hyptis spicigera* Lam. are used in soaps, lotions and perfumes by the people of northern Nigeria, and they are included in baths or decoctions for the treatment of various skin diseases. Some Senegalese tribes rub the plant on corpses, for embalmment. The plant is used in the treatment of headaches and migraine, as well as in the control of bruchid beetles. The seeds are made into a pulp and used in stews and gravies in Sudan and in central Africa.

The seed is reported to yield a yellow, edible, drying fixed oil which is composed mainly of linolenic acid. The quality of the fatty oil has been found to be comparable with those of linseed and conophor oils.

Aerial parts of *H. spicigera* growing wild in Ile-Ife (Nigeria), were collected in August 1988. Two frozen samples of the plant material were subjected to hydrodistillation and to distillation-extraction respectively. The essential oils were analysed by GLC using two capillary columns of different polarities and by GC-MS. LSC prefractionation over silica gel using disposable solid phase extraction columns was carried out to facilitate the GC analysis.

The essential oil samples obtained by the two different isolation procedures were qualitatively similar; they were characterized by a high content of sesquiterpene hydrocarbons (74%), the major one being β -caryophyllene (68%). Besides these, only α -pinene (5%) occurred in substantial amounts. The occurrence of these two components was in agreement with reports on other *Hyptis* species. Long chain fatty alcohols and fatty acid methyl esters were also detected in the essential oil, high amounts of related fatty acids have been found in the fixed oil. Trace amounts of precocene I, which was until recently only found in the family Asteraceae, were also detected. We found large amounts of this compound in *Platostoma africanum* P. Beauv., family Lamiaceae, and a related chromene was recently detected in *Orthosiphon stamineus* Benth., again family Lamiaceae. Three oxygen-containing sesquiterpenes were detected but could not be identified to date. The analysis and chemical composition of the oils will be discussed.

COMPOSITION OF THE ESSENTIAL OIL OF *HOSLUNDIA OPPOSITA* COLLECTED IN NIGERIA AND RWANDA

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Hoslundia opposita Vahl. is found widespread throughout tropical Africa; it is mentioned to be one of the Lamiaceous plants rich in essential oil, and to have an odour resembling that of vanillin. In Nigeria and Rwanda as well as in some other parts of Africa, the plant is used in traditional remedies. However, phytochemical studies on *H. opposita* have not been reported. Because of the various uses of the plant, we were interested in analysing its essential oil.

Twigs of *H. opposita* growing wild in Rwanda were collected in June 1984 and twigs from plants growing wild in Nigeria in August 1987 and in August 1988. The samples were subjected to hydrodistillation and/or to distillation-extraction. The essential oils were analysed by GLC and by GC-MS. Prefractionation over silica gel using disposable solid phase extraction columns was carried out to facilitate the GC analysis.

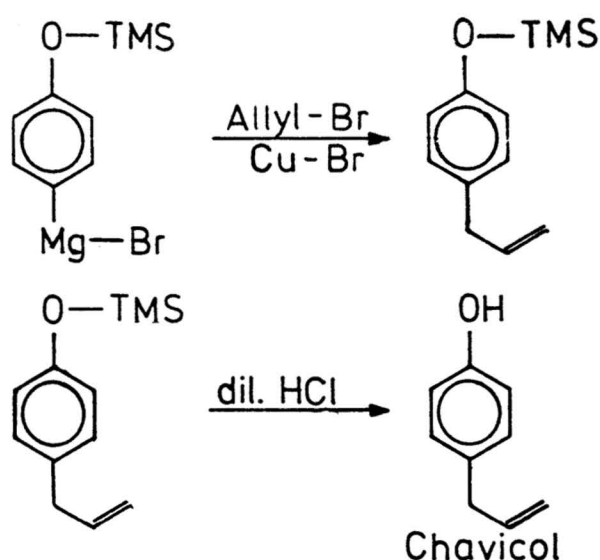
The essential oils of both geographical origins were found to consist mainly of sesquiterpenes hydrocarbons (45-68%), the major components being germacrene-D (ca. 20%) and β -caryophyllene (11-22%). α -Humulene (14%) and δ -cadinene (8%) occurred in substantial quantities only in the Rwandan oil. Oct-1-en-3-ol (9.5%), cis-hex-3-en-1-ol (8%) and a compound identified as α -humulene oxide (5%) occurred in substantial quantities in the distilled-extracted Nigerian 1987 oil sample. Some alkylbenzenes (1.9%) were also detected in this sample, but smaller amounts or traces were found in the hydrodistilled 1987 sample as well as in the distilled-extracted 1988 sample. These alkylbenzenes and some long GC retention time components which were found to be diterpenes (10-20% of Nigerian oils; percentages given above are excluding diterpenes), the major of which was tentatively identified as phytol, were not detected in the Rwandan oil. In conclusion, it can be stated that *H. opposita* normally produces sesquiterpene hydrocarbons as its main volatile constituents. The geographical origin of the plants or the occurrence of chemical varieties may explain the differences in the oil composition.

A SIMPLE AND EFFECTIVE SYNTHESIS OF PHENYLPROPANE DERIVATIVES OCCURRING IN ESSENTIAL OILS

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By treatment of a mixture of 4-phenoxytrimethylsilylmagnesium bromide and copper(I) bromide as a catalyst (1) with allyl bromide a silylated phenylpropane derivative was synthesized. After refluxing and standing cool overnight the formed trimethylsilylether was hydrolyzed under mild conditions yielding the phenolic phenylpropane.



This reaction proved to be suitable for the formation of several phenolic phenylpropanoids occurring in essential oils such as chavicol, chavibetol etc.

Isomerization of the obtained phenolics with potassium tert-butoxide in dimethyl sulfoxide (2) at 4°C led to the respective 1-propenyl derivatives in very good yields.

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ESSENTIAL OIL PLANT PREPARATIONS AGAINST RHEUMATISM (DEVELOPMENT AND STANDARDIZATION)

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(abstract not available)

HRGC OF ESSENTIAL OIL FROM A *Satureja montana* L. ECOTYPE GROWN IN NORTHERN ITALY

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Winter Savory (*Satureja montana* L.) is a perennial semishrub which grows wildly in rocky, sunny and calcareous soils of Middle and Southern Europe, of Caucasus and of boreal Africa.

It is commonly used as a spicy herb in flavoring condiments, sausages and meats. It also possesses pharmacological properties and is employed in folk medicine as astringent, digestive, expectorant, laxative, sedative and tonic.

It is important to analyse *S. montana* essential oil in order to choose its best utilisation (pharmacology, food).

Aim of this work was to determine the composition of the essential oil of an ecotype of *Satureja montana* wildly grown in Northern Italy, while establishing what chemical race this species belong to. Essential oil, obtained by steam distillation, was analysed by high resolution gaschromatography (HRGC) and the identification of the compounds was obtained by comparison with gaschromatographic retention times of standards and by mass spectrometric data.

We will report chromatographic profiles and quali-quantitative data.

A HEADSPACE GAS CHROMATOGRAPHIC SCREENING METHOD FOR THE VOLATILES IN SCOTS PINE (*PINUS SYLVESTRIS* L.) NEEDLES

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An application of the static headspace capillary gas chromatographic (HSGC) analysis method was developed to determine the volatile constituent levels in solid, fresh pine needle matrix.

The aim of the work was to determine whether HSGC techniques can be used to elucidate needle terpene contents directly in Scots pine (*Pinus sylvestris* L.) trees and seedlings instead of first isolating the volatile oil from the plant material by hydrodistillation followed by conventional GC analysis.

The effects of sample size, HS sampler bath temperature and sample equilibration time on HSGC analyses were investigated.

The HSGC chromatogram profiles for the needle volatiles and the GC profiles for the hydrodistilled needle volatile oil clearly differed, and were characteristic when examined as a whole. Constituents with high volatility in the initial zone of the HSGC chromatogram were small, missing or obscured by the solvent in the GC chromatogram. Furthermore, the last eluting constituents with low volatility in GC were small or missing in the HSGC.

When 60 mg of fresh, finely sliced pieces of pine needles were equilibrated in a 20 ml headspace vial for 30 minutes at 120°C in the HS sampler, the terpene constituents were qualitatively and quantitatively almost identical in both the HSGC and GC chromatograms.

The mean relative standard deviations (c.v.%) for the percentage concentrations were 1.0 for the main needle monoterpene and 6.2 for the main needle sesquiterpene constituents in the GC, and 7.5 and 8.4 in the HSGC respectively. The HSGC values include the biological between sample variation.

Considerable variation was found in the terpene constituent levels when needles of pine trees and seedlings of different genetic background were analysed by HSGC techniques.

With carefully selected analytical conditions the developed HSGC application method is sensitive and precise enough for the direct screening of volatile constituent levels in solid, fresh pine tree or seedling matrices. Moreover, the ease of sample preparation and small sample size needed for the analysis makes the HSGC techniques far more applicable than the usual hydrodisillation-GC-techniques.

ESSENTIAL OIL FROM *SALVIA POTENTILLIFOLIA* BOISS. ET HELDR.

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The genus *Salvia* (Labiatae) grows in the Mediterranean area, and some species are endemic in Anatolia (1). *Salvia potentillifolia* Boiss. et Heldr. is an endemic species of C. and S.W. Anatolia. The plant used in folk medicine. The composition of the essential oil of *S. potentillifolia* was hitherto unknown.

The flowering plants were collected in Elmalı (Antalya, Turkey) in June 1985. The plant material was subjected to hydrodistillation for 2.5 h using a Clevenger apparatus and it yielded a yellowish essential oil (0.80%).

The oil was fractionated by LSC on a silicagel column (2). The fractions were analyzed by GLC.

Thirty constituents of the essential oil were identified. The essential oil contained a higher proportion of oxygenated compounds (81.9%) than hydrocarbonated ones (18.1%). The main constituents of the oil analyzed were α -terpineol (10.2%), terpinen-4-ol (8.8%), camphor (8.3%), borneol (6.1%), 1,8-cineole (5.9%) and an unknown compound (5.8%).

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PARTITION COEFFICIENTS OF PLANT CUTICLES FOR D-LIMONENE

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The sorption of monoterpenes in plant cuticles is an interesting aspect especially from the point of view of pathogen repelling by terpenes. Moreover, the reactive terpenes may interact with gaseous pollutants even within the cuticle itself.

Partition experiments were carried out with ^{14}C d-limonene, using astomatous cuticles of *Citrus*, *Ficus* and *Abies* and stomatous cuticles of *Picea*. Due to the high volatility of limonene and its very effective sorption in lipophilic materials, the quantification of the terpenes in the gas phase is difficult. For this reason partition coefficients were determined for the system isolated cuticle/water, using meltable glass vials. As the low water solubility of limonene (0.9×10^{-4} mol/l) made direct partition experiments impossible, an extrapolation and a desorption method were applied. The partition coefficients were calculated from the ratio of the limonene concentration in both phases, which was measured by liquid scintillation counting.

The coefficients for all types of cuticles tested are in the same order of magnitude, about 10^5 , which indicates a high accumulation of terpenes in the cuticle. While the values for *Picea* and *Abies* cuticles of one-month-old needles are similar to those of the angiosperm cuticles, the partition coefficients for cuticles of elder needles (seven months) are lower - a fact, that is attributed to problems during cuticle isolation due to the higher lignin content of elder needles. The increase of the coefficients after removal of polar and phenolic components by acid hydrolysis approve this interpretation.

INVESTIGATIONS ON THE UNDERGROUND PARTS OF BALM (*MELISSA OFFICINALIS* L.): COMPOSITION OF THE ESSENTIAL OIL AND MICROSCOPICAL INVESTIGATIONS

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Balm (*Melissa officinalis* L., Lamiaceae) is an old, well known medicinal plant, the main active principles of which belong to the essential oil and the tannins (literature is reviewed in (1) and (2)).

While several investigations have been done on the essential oil of the herb and leaves of this plant, only very few information exists concerning the volatiles of the underground parts (3).

Therefore, we want to report on the essential oil of the roots and rhizomes of balm and compare its composition with that of the essential leaf oil. We found that the underground parts contain small amounts (about 0.002%) of a very complex essential oil, of which more than 100 compounds could be identified.

Because of the small quantity of essential oil gained, identification of the components could nearly exclusively be performed by so-called "hyphenated techniques" above all by GC-FTIR-MS.

Particularly, the on-line coupling with a FTIR spectrometer proved to be a powerful analytical tool, e.g. for distinguishing several stereo isomers and to determine the position of the double bond of 4,5-dimethyl-2-cyclohexen-1-one.

The oil of the underground parts contains about 37% monoterpenoids (e.g. α -pinene, 1,8-cineole, citronellal, camphor, linalool, isobornyl acetate, neral, α -terpineol, geranial, citronellol, geraniol), 33% sesquiterpenoids (e.g. β -caryophyllene, E- β -farnesene, α -humulene, δ -cadinene, germacrene B, caryophyllene epoxide) and 30% components belonging to different compound classes (e.g. 2-pentylfuran, trimethyloxazole, *cis*- and *trans*-2,3-dimethylcyclohexanone, trimethylthiazole, nonanal, 1-octen-3-ol, benzaldehyde).

This oil differs clearly in composition from the essential leaf oil of balm though these two oils have several compounds in common, too. In particular, in the oil of the underground parts we found numerous oxygenated mono- and sesquiterpenoids which could not be detected in the leaf oil.

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NEW DEVELOPMENTS AND APPLICATIONS OF MASS SPECTROMETRY IN ESSENTIAL OIL RESEARCH

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In this lecture we will mainly focus on two subjects:

1. The direct introduction of plant material and essential oils into a mass spectrometer
2. The GC/FTIR/MS on-line coupling

Ad 1:

Some examples will be presented, showing that this method can be a valuable tool, e.g. for detecting adulterations of plant drugs and essential oils, distinguishing chemotypes and for recognizing some toxic plant constituents.

Ad 2:

The results of some studies of essential oils by means of a GC/FTIR/MS system are introduced.

In particular, the following topics will be discussed:

- System configuration
- Display and report of GC/MS and GC/FTIR data
- Comparison of TIC and TRC
- Sensitivity
- Combination of GC/MS and GC/IR library search results
- Advantages and possibilities of the integrated GC/FTIR/MS system in essential oil analysis
- Automation
- Headspace/GC/FTIR/MS

VARIATIONS IN THE ESSENTIAL OIL COMPOSITION OF *MINTHOSTACHYS ANDINA* (BRETT.) EPLING

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The essential oil of *Minthostachys andina* (Brett) Epling, (Labiatae), extracted from the aerial part of wild-growing plants, contains mostly oxygenated compounds (more than 80%), of which (R) - pulegone and menthone are the major ones. When the essential oil of individual plants is examined, a great variation in the essential oil composition can be noticed, (R)-pulegone content varies between 60,88 and 25.44%, while menthone ranges between 26.64 and 62.40%. Other major and minor constituents of this essential oil will also be discussed.

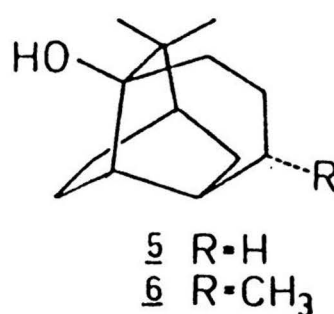
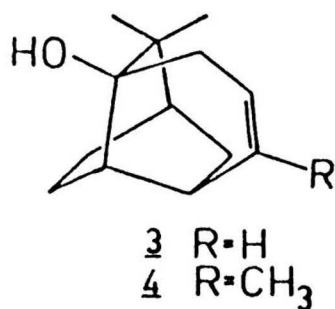
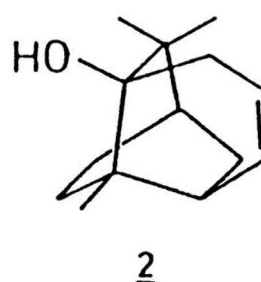
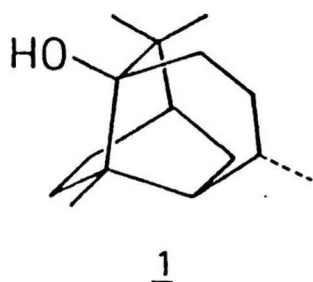
SYNTHESES AND ODOUR OF PATCHOULOL-ANALOGUES

H. Spreitzer and G. Buchbauer

Institute of Pharmaceutical Chemistry, University of Vienna,
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The harmonious playing together of the woody, earthy and camphoraceous notes renders patchouli oil to a favourite and important ingredient in perfume compounding. The two sesquiterpenic alcohols patchoulol **1** and norpatchoulol **2** are the principal cause of patchouli odour.

In continuation of studying structure-activity relationship of odorous compounds we study the influence of the bridge head bonded methyl group of patchoulol **1** and norpatchoulol **2** and the olfactory properties of the corresponding unsaturated resp. saturated derivatives **3-6**.



VARIATIONS IN THE ESSENTIAL OIL COMPOSITION OF *MINTHOSTACHYS ANDINA* (BRETT.) EPLING

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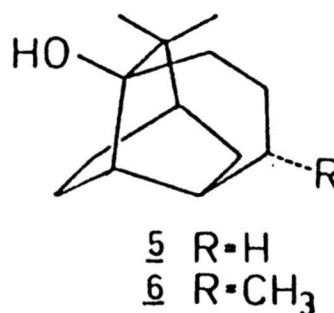
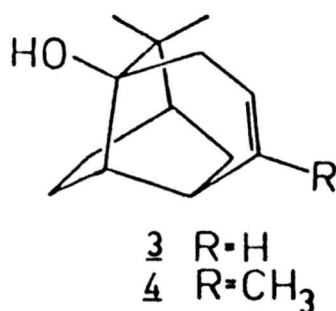
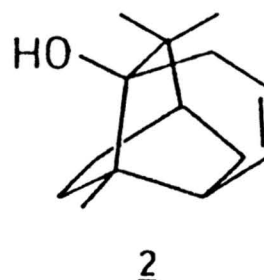
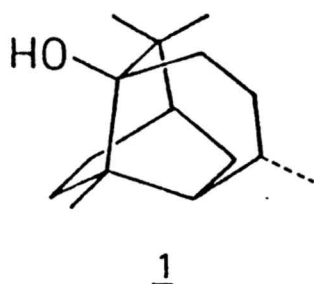
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DETERRENT AND INSECTICIDAL CHROMENES AND SESQUITERPENES FROM THE ASTERACEAE

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Different species from the genera *Ageratum*, *Ageratina* or *Encelia* (all Asteraceae) contain several important natural products, the major being chromene, benzofuran and sesquiterpene derivatives. Their feeding deterreny and insecticidal activity was tested against two phytophagous insects- the Mexican bean beetle, *Epilachna varivestis* (Muls.) and the noctuid tobacco caterpillar, *Spodoptera littoralis* (Boisduval). Insecticidal activity was investigated by topical application of the compounds to dorsum in larval stages of both the insects and ventrum to adults of *E. varivestis* via a microliter syringe or by the contact residue bioassay using glass vials. Encecalin and precocene II (both chromenes) proved to be the most toxic whereas sesquiterpenes and benzofurans largely non-toxic to both insect species in this bioassay. The tolerance of the insects to the topically applied dosage of encecalin and precocene II increased with their increasing age and developmental stages. For examples, the LD₅₀ of encecalin to 48h old grubs of *E. varivestis* was 0.05 μmol (= 12 μg) per insect, 24h after treatment, whereas for 10d old grubs it was just double i.e. 0.1 μmol per insect at the same exposure period. In 24h old adult beetles the LD₅₀ of encecalin still increased up to 1.0 μmol per insect, which may partially be attributed to the relatively tougher chitinous covering in the beetles.

The leaf disc bioassay, where discs of the fresh bean leaves were covered with known amounts of the different substances under test, interestingly enough the most toxic chromenes exhibited the highest feeding deterreny to the adult beetles (ca. 0.5d old) of *E. varivestis*. The ED₅₀ (effective dose at which feeding is reduced to 50% compared to controls) values of encecalin and precocene II in this bioassay were 0.05 and 0.1 μmol per disc, respectively. Encelin, a sesquiterpene lactone exhibited almost equal antifeedant activity against adult beetles of *E. varivestis*.

In feeding bioassay, where known amounts of the various test compounds were incorporated into an artificial diet and feeding deterreny was tested by allowing the neonate larvae of *S. littoralis* to feed on it, again encecalin and precocene II proved to be the most active amongst chromenes. The LD₅₀ of encecalin and precocene II, 7d after feeding was 2.0 and 1.0 $\mu\text{mol/g}$ diet, respectively. It was observed that almost all the substances used in the present investigation (including those that did not significantly reduce survivalship of the larvae) showed considerable growth-inhibitory effect on the larvae of *S. littoralis*.

Acknowledgements: This study was supported by a grant of the DFG to P.P. Further support by the DAAD (travel grant to R.P.S) is gratefully acknowledged.

ESSENTIAL OIL POLYMORPHISM IN FINNISH *THYMUS* SPECIES

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Chemical polymorphism concerning the essential oils of the genus *Thymus* is a widespread phenomenon, especially in the northern species. The two Finnish species, *T. serpyllum* ssp. *serpyllum* and *T. serpyllum* ssp. *tanaensis*, turned out to form four different chemotypes each, with hedycaryol, germacra-1(10), 5-dien-4-ol, germacra-1(10),4-dien-6-ol, linalool, and linalyl acetate as type characterizing compounds. Otherwise the oils of the two subspecies were similar containing myrcene, trans- β -ocimene, β -caryophyllene, and germa-crene D as main terpene hydrocarbons. 1,8-cineol and camphor represented another great portion in both oils.

If Finland is regarded as an area of *T. serpyllum* (s.l.), total of six types of plants can be defined with regard to the essential oil chemistry only. Including the frequency of these six types at the four areas investigated, a certain gradient from the south to the north can be seen. A most interesting aspect is the fact that the most frequent, linalyl acetate containing chemotype of the northern Lapland has nearly the same oil composition as *T. praecox* ssp. *arcticus* in Island, Norway, and Greenland.

STUDY OF THE ESSENTIAL OILS OF *SATUREJA MONTANA* AND *SATUREJA SUBSPICATA*

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Nine species, five subspecies and seven varieties of the *Satureja* genus belong to the flora of Yugoslavia.

The most abundant species, *S. montana*, represents a valuable aromatic and medicinal plant. We have investigated the variation in the oil content and composition of the specimens collected in five locations. In addition, specimens from two selected locations were analysed during the vegetative period.

It was found that the oil content varied from 0.8% to 1.8%, being highest just prior to flowering. The composition of oils was analysed applying gas-chromatography. Depending upon the location or vegetative stage of the plant, the oils are characterized by considerable differences in the content of individual components, e.g. p-cymene (2.22-8.94%), terpinolene (3.06-16.91%), caryophyllene (2.16-14.37%), bornyl acetate (2.38-5.10%), α -terpineol+borneol (1.48-12.15%), thymol (1.02-61.04%) and carvacrol (4.80-57.94%).

Essential oil of the endemic species *S. subspicata* subsp. *liburnica* has not been investigated until now. The oil (0.1%) was found to contain carvacrol (21.85%) and bornyl acetate (13.57%) as the main components along with α -pinene, camphene, sabinene, α -terpinene, limonene, 1,8-cineole, p-cymene, terpinolene, octanol, nonanol, linalool, linalyl acetate, caryophyllene, α -terpineol+borneol, citronellol, carvone, copaene, ylangene, γ -muurolene, methyl eugenol, thymol and cadinol.

VOLATILE CONSTITUENTS OF BIRD CHERRY FLOWERS (*PADUS AVIUM* MILL.)

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Padus avium Mill. (syn. *Prunus padus* L., Rosaceae, bird cherry) is a small to medium sized tree, often planted for ornament, with white fragrant flowers on drooping and wide spreading racemes about 10 cm long.

The flowers have an interesting clear cherry flower-like odour with distinct aspects of jasmin, orange flower and elder flower; characteristic is a pronounced undertone of anthranilate-like compounds.

Vacuum steam distillation (1) as a very useful method for the investigation of flower volatiles gave an odour concentrate which was analysed by GC-MS. More than 80 components could be identified. Main constituents were benzaldehyde, benzyl alcohol and 2-phenylethyl alcohol. Remarkable was the detection of a number of oxygenated linalool derivatives (e.g. 8-oxo-linalool). In addition, numerous nitrogen containing compounds were identified. Some of them proved to be important for the typical odour (e.g. anthranilic aldehyde, 2-aminoacetophenone, methyl anthranilate, indole, nicotinic aldehyde). Several compounds have not previously been found in nature.

- 1) Joulain, D.: in: Progress in Essential Oil Research, p. 57, W. de Gruyter, Berlin 1986

BIOCHEMICAL AND PHYSIOLOGICAL STUDIES ON *SATUREJA*
HORTENSIS L.GROWN IN SCOTLAND

K.P. Svoboda and S.G. Deans

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(abstract not available)

COMPARATIVE ANALYSIS BY DYNAMIC HEADSPACE AND DIRECT INJECTION OF BLACK TRUFFLE ALCOHOLIC EXTRACT

T. Talou, M. Delmas and A. Gaset

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Black Truffles (*Tuber melanosporum*) are underground mushrooms that grow in symbiosis with certain trees, especially oaks. One finds them in several regions of Europe, particularly in France where they are very appreciated by gourmets for their typical aroma. Indeed, used in the culinary field, they impart to dishes they are added a flavour that was always very sought after. Although the french food industry, especially the cooked meats industry, traditionnally uses Black Truffles to flavor its products, it presently looks for natural and artificial truffle flavourings. After having set up the first nature-identical Black Truffle flavoring, which is a synthetic aroma reconstitution based on a dynamic headspace analysis of fresh truffles, we carried out alcoholic extractions of Black Truffles in order to propose a natural truffle extract. The analysis of the volatile composition of the extracts was carried out using dynamic headspace and direct injection methods. We used a modified gas chromatographic device equipped both with a D.C.I system (Desorption - Concentration - GC Introduction) based on a cryogenic adsorption of the volatiles on a Tenax trap followed by a thermal desorption, and with a split injector. The identification of the major volatile compounds was carried out by combined capillary gas chromatography - mass spectrometry. The qualitative and quantitative comparison of the different chromatographic profiles allowed us to characterize such extracts. A lot of degradation products, due to the solvolysis of the vegetable matrix, could be identified. Moreover, a sensory analysis carried out by a panel of specialists reported a large difference between the alcoholic extract odor and the characteristic Black truffle aroma showing in this sense the difficulty to use such a product as a natural Black Truffle flavoring, even diluted in vegetable oil.

CRITERIA FOR APPLICATION OF COMPUTERISED METHODS TO THE STUDY OF FLAVOURING MIXTURE COMPOSITION

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Some criteria for the employment of computer programmes, including the use of a GC/MS hook-up, are proposed for the study of the composition of complex mixtures of essential oils and natural-identical compounds. In many cases, these criteria make it possible to identify the essential oils present in various formulations. Logic diagrams, which can be computerised, also make it possible to obtain useful information for the selection of essential oils and natural-identical compounds for the formulation of flavourings. This work also provides actual examples of the application of computer programmes.

COMPARATIVE STUDIES OF VARIOUS ARTEMISIA OILS

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The essential oils from *Artemisia laciniata* (two different specimen), *A. moorcroftiana*, and *A. salsoloides* from the Himalaya region of India have been analyzed on a preparative scale to isolate the compounds responsible for the typical odor of these oils. All new constituents have been fully characterized by means of spectroscopic data.

Although there is a common pattern of typical compounds of these four oils (1,8-cineol, artemisia ketone, thujone, camphor, piperitone) the total sum of constituents for each of the oils exhibit great differences.

This behavior is reflected in the complex odor of those *Artemisia* oils which is formed by combination of several constituents.

ESSENTIAL OIL OF *POLYGONUM* SPP.

Karim b Yaacob

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Department of Chemistry, Faculty of Physical & Applied Sciences
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor, Malaysia

The genus *Polygonum* (Polygonaceae) is made up of several species which hitherto has not been examined before for its essential oils. Only two species, *P. minus* and *P. hydropiper* were found to be aromatic and produced essential oils on conventional steam distillation. The chemical constituents of the two essential oils were examined in detail using routine GC-MS and spectroscopic methods. Results obtained from the chemical analysis of the essential oils and also some preliminary plant propagation studies indicated that the two essential oils has tremendously prospects for commercialisation as new products for the flavour and fragrance industries.

GEIGERIA – A SOURCE OF INTERESTING COMPOUNDS

C. Zdero

Institut für Organische Chemie der TU Berlin,
Straße des 17. Juni 135, D-1000 Berlin, FRG

The investigation of seven species of the mainly South African genus *Geigeria* afforded in addition to known compounds 28 new sesquiterpene lactones, including two dimeric ones and a fulvene lactone, as well as six new nerolidol derivatives, a geranyl linalool, a myrcene glucoside and two acids derived from ivaxillaric acid. The structures were elucidated by high field technics.

AROMATIC ACIDS FROM THE LEAVES OF *CITRUS LIMON*

H. Ziegler¹, G. Spiteller²

1 Aromachemie, D-8551 Aufsess, FRG

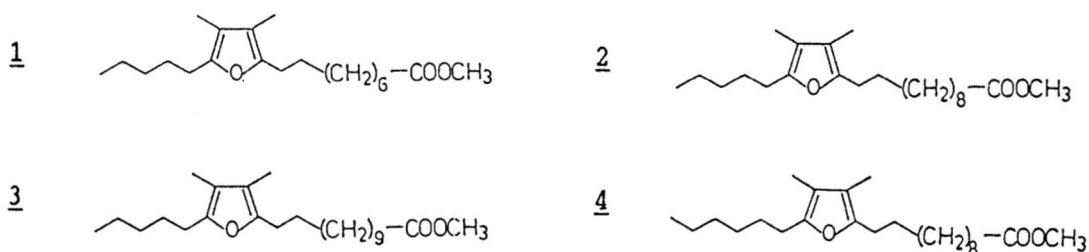
2 Institut für Organische Chemie, Universität Bayreuth,
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The analysis of lipid extracts of lemon leaves led to the detection of two groups of aromatic fatty acids:

1. Furan fatty acids
2. Aromatic monocarboxylic acids

For the first time we wish to report 4 furan fatty acids as minor constituents of lemon leaves. An especially developed work-up procedure permitted their detection within the unpolar fraction.

The compounds were detected via their methyl esters using GC/MS. Compound 3 and 4 have only been reported in 1988 as constituents in the liver of crustacea. Investigations on a variety of plants points to the conclusion that these acids are introduced into the organism via the food chain. Their biological importance is so far unknown.



Within the medium polar fatty acids a number of hydroxylated cinnamic acid and benzoic acid derivatives have been detected after chromatographic separation. Phenolic carboxylic acids are frequently detected constituents in higher plants.

As far as we know no complete review of these derivatives in lemon leaves has been published.

ESSENTIAL OIL OF *POLYGONUM* SPP.

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COMPARATIVE STUDY OF THE ESSENTIAL OILS OBTAINED UNDER DIFFERENT
TREATMENTS FROM JUNIPERUS COMMUNIS BERRIES

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This study reviews the influence of the grinding of the *Juniperus communis* berries, the recovery and the composition of the volatile compounds using the method of headspace technology. Unlike traditional analytical methods, the headspace method can be used to analyze volatile flavour compounds with almost negligible compound alteration or degradation. The method minimizes changes in composition of volatiles and does not introduce solvent interferences. Comparative analysis of gas chromatographic profiles of the berries is more direct approach to obtain the information on their aroma constituents when compared to studying *Juniperus communis* berries essential oils obtained by hydro-distillation. This is only possible because of the sensitivity and reproducibility of this analytical method. The headspace volatile compounds from the *Juniperus* berries were studied by investigating the influence of the parameters of temperature and extracting time ranging from 80 to 140°C and 40 to 120 min respectively in relation to the degree of comminution of the plant material - intact berries, particle size a) 2.5 > 1 mm and b) < 1 mm. The quality of the oil recovered from the berries by the headspace method was found to be strongly dependent on the conditions of temperature and extraction time applied. The comminution affects greatly the yield of the oil and furthermore the final composition of the aroma.

**EXTRACTION OF PERFUMER'S PRODUCTS BY
1,1,2-TRICHLORO-1,2,2-TRIFLUOROETHANE**

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The study of aromatic plant processes led us to consider the efficiency of various solvents to extract odorous molecules. We especially compared water steam, hexane, cyclohexane, benzene, dichloromethane, ethanol, methyl furan (sylvan) and Forane 113 i.e. 1,1,2-trichloro-1,2,2- trifluoroethane (TTE). This latter led to the best qualitative results in most cases. Therefore, we are presenting here yields obtained with a few perfume plants since Forane 113 cannot be used in foodstuff processes.

Comparison of efficiency of different solvents on lovage roots (*Levisticum officinale*) and carrot seeds (*Daucus carota*) is presented. Quantitative results obtained by 1,1,2-trichloro-1,2,2- trifluoroethane on 7 other plants (*Viola odorata*, *Rosa centifolia*, *Acacia dealbata*, *Robinia pseudoacacia*, *Vetiveria zizanoides*, *Evernia prunastri*, *Pseudoevernia fufuracea*) are presented. Then qualitative results obtained on *Rosa centifolia* are considered. The amount of some main components varied with the extraction method and differences were revealed by the olfactive test. For example GC/MS and GC/FTIR analyses showed that the contents of phenetyl alcohol were 77.28 % (TTE first extraction), 82.64 % (TTE second extraction) and 74.45 % (mixture of TTE/hexane). Those of phenetyl methanol (the same order than above) were 2.08 %, 2.31 % and 1.10 % respectively. The examination of olfactive tests revealed that the second extraction involving TTE approached the most the fresh flower and was the best.

THE CONTENTS AND COMPOSITION OF VOLATILE OIL IN *TANACETUM VULGARE* L. DURING THE ONTOGENETIC DEVELOPMENT

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The contents and composition of volatile oil in various parts of the *Tanacetum vulgare* L. species in 4 stages of ontogenetic development of plants from 12 geonomically different localities were observed. The maxima of essential oils in leaves and flowers were reached in the stage of flower-building. In the drug *Herba tanaceti* maximal oil contents in full flowering plants were achieved. In the essential oil the presence of α - and β -pinene, camphene, limonene, borneol, bornylacetate, artemisiaketone and alcohol, umbellulone, chrysanthenylacetate and further components by means of GLC was assayed. The samples examined could be differentiated in 5 chemotypes. Among the 12 localities followed in only 1 sample the presence of thujone could not be proved.

BIOCHEMICAL AND PHYSIOLOGICAL STUDIES ON *Satureja hortensis* L. GROWN IN SCOTLAND

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Satureja hortensis is native to Mediterranean region. It is used fresh, dried or as oil and oleoresins in food industry, in the making of liqueurs and in perfumery products. The value of oil is in its high carvacrol content (30 to 57% according to US Standards for Herbs and Spices). Savory was grown in Scotland for four consecutive years, dry matter yield and oil quality and quantity were closely monitored.

The oil content increased from 0.5% v/w (seedlings) up to 1.5-2.0% at the beginning of flowering, and this level was maintained up to about two weeks before the end of flowering and the beginning of senescence. A similar pattern was observed over four different seasons. Hot dry season in 1984 did not increase significantly the oil yield, indicating that this type of weather is not the most important factor in volatile oil formation in summer savory.

Analysis of the components by gas chromatography showed that savory oil was of very high quality. Carvacrol level was kept between 40-60%, depending on the environmental conditions of a given year. Consistently higher amounts of carvacrol were observed under greater radiation and higher temperatures which suggests faster enzymatic conversion of γ -terpinene to p-cymene and carvacrol. The highest peak of carvacrol was usually observed at the late vegetative and senescence phase. It was concluded that the optimal time for commercial harvest was during the full flowering period, when high dry matter yield coincides with high oil yield and excellent oil quality.

The experiments with different drying temperatures proved the importance of a low drying temperature, ideally at 36°-40°C. The oil content decreased rapidly at temperature above 55°C, and there was practically no oil left after drying at 85°C and over. Drying in microwave oven did not prove satisfactory, as there was a great loss of volatile oils and marked changes in oil composition.

The total count of bacteria in the fresh herb was very high, but it decreased considerably after drying in the oven. Coliform bacteria were not found either in fresh or in dried material. The microbiological quality of the herb appeared to be of a high standard in comparison with the products available on the market. Ten bacterial genera were tested against a number of the predominant constituents in the savory oil. The constituents which were most potent were carvacrol, 1,8-cineole, eugenol, linalool, α -pinene, α -terpineol and thymol.

In spite of the fact that the material used was not specifically adapted to the West of Scotland, the results in general indicate that the content and quality of savory oil were as high in the cool, wet lowlands of Scotland, as in warm, dry areas of central and southern Europe.

ANTIMICROBIAL ACTIVITY OF PLANT VOLATILE OILS AND THEIR CONSTITUENTS

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Plant volatile oils have been known since antiquity to possess biological activities: antibacterial and antifungal properties having been most exploited. Only comparatively recently have the oil constituents responsible for these actions been chemically defined. Terpenes and phenolic compounds are responsible for microbial growth inhibition in many cases, with these compounds being active at low concentrations. Bioassays using agar diffusion wells have demonstrated the marked inhibitory powers of small volumes (10 μ l) of volatile oil against relatively high numbers of bacteria ($>10^6$ ml $^{-1}$).

Over 50 plant volatile oils have been screened by this method against a set of 25 test bacteria, several of which are of public health significance (*Enterococcus faecalis*, *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus* and *Yersinia enterocolitica*). Undiluted and a series of diluted oils were evaluated, and ranked according to their overall antibacterial properties. This showed that *Angelica* was the most inhibitory oil in this study, being inhibitory to all the test bacteria. Both Gram positive and Gram negative bacteria were susceptible, suggesting a common mode of action. In the case of Bay (the second most inhibitory oil, which is high in phenolic compounds, this is probably a non-specific cytotoxic reaction. In the case of Thyme oil, all the test bacteria were inhibited with the exception of *Clostridium* and *Flavobacterium*.

More detailed studies have been performed on a number of volatile oils, in particular *Satureja hortensis* (summer savory), *Artemisia dracunculus* (French tarragon) and *Origanum majorana* (marjoram). Carvacrol and other main components of summer savory volatile oil have been tested against the set of test bacteria. They showed extensive inhibition of bacterial growth. In *Origanum* species, Eugenol and Thymol exerted particularly high degrees of inhibition of growth.

In addition to action against pathogenic bacteria, the volatile oils exert considerable inhibitory powers against filamentous fungi. In particular, mycotoxigenic fungi exposed to concentrations of 10 μ l ml $^{-1}$ (1% v/v) *Origanum majorana* volatile oil have exhibited inhibition levels of $>90^\circ$ compared with non-inhibited controls. Even at concentrations of 1 μ l ml $^{-1}$, marked inhibition was recorded. It is significant that plant volatile oils have this ability to prevent the growth of such harmful fungi at these relatively low concentrations. Mycotoxins are secondary metabolites produced after the cessation of active growth. It has been determined that growth inhibition by plant volatile oils does NOT result in the fungi undertaking 2 $^\circ$ metabolism: there is inadequate 1 $^\circ$ metabolic and biochemical commitment for this to occur.

There may well be a future for such natural compounds to be used as fungicides within agriculture. Assessment of their potential is currently being undertaken at the West of Scotland College.

SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC), A NEW TECHNIQUE FOR THE ANALYSIS OF ESSENTIAL OILS.

F.Höfler, O.Villioth and G.Alt

Dionex GmbH, Richard-Klinger-Str. 15, 6270 Idstein

Supercritical Fluid Chromatography as a new chromatographic technique has solved a number of application problems where neither gas nor liquid chromatography deliver satisfying results. Examples for these applications are the analysis of thermolabile and non-chromophoric compounds. Carbon dioxide as a mobile phase gives the advantage to separate components at low temperatures ($T > 31^{\circ}\text{C}$) and to utilize common GC- and HPLC-detectors.

Mono-, di- and triglycerides from hazel nut oil and cold pressed olive oil for example can be analysed without derivatisation. High efficiency separations were achieved using capillary columns and the universal flame ionisation detector (FID).

An important tool for the analysis of biological materials is the combination of supercritical fluid extraction (SFE) as an on-line sample enrichment and sample introduction technique for SFC. Short extraction times of 5 to 30 minutes were achieved which attributes to the high diffusion coefficients of supercritical fluid compared with liquids.

Applications for the use of SFE-SFC-analysis are, for example, the extraction and separation of components from majoram, black pepper and tea leaves.

For identification of components SFC can be combined with spectrometric techniques such as mass spectrometry (MS) and fourier transform ir-spectroscopy (FTIR).

20th International Symposium on Essential Oils

Würzburg / Germany
September 10 - 14th,
1989



Organization:
Prof. Dr. K.-H. Kubeczka
Pharmaceutical Biology
University of Würzburg

Program

Program

Sunday September 10, 1989

- 17.00 Registration
- 19.00 Get-Together-Party

Monday September 11, 1989

- 9.30 - 10.00 Opening Session, Welcome Addresses
- 10.00 - 12.00 Scientific communications
- 14.00 - 16.00 Scientific communications
- 16.00 - 18.00 Poster session
- 20.00 Chamber Concert
(Toscana hall of the Residence)

Tuesday September 12, 1989

- 9.00 - 12.00 Scientific communications
- 14.00 - 17.15 Scientific communications/Posters
- 18.00 Reception by the Lord Mayor of the city
- 20.00 Symposium dinner (Marienberg Castle)

Wednesday September 13, 1989

- 9.00 - 12.00 Scientific communications
- 14.00 - 18.00 Scientific communications/Posters
- 19.30 Barbecue in the Botanical Garden

Thursday September 14, 1989

- Workshops on instrumental analysis of essential oils
- 9.00 - 10.30 Introduction lectures (lecture hall of the
"Industrie u. Handelskammer", Neubastr. 66)
- 10.30 - 12.30 Demonstration of dedicated analytical
equipment (university main building)
- 14.30 - 20.00 Half-day excursion to the surroundings of
Würzburg: landscape, culture, botany,
viniculture

Exhibition

Manufacturers of analytical equipment will show their products during the congress.

Scientific program

Monday September 11, 1989

- 9.30 Opening Session
Welcome Addresses
- 10.00 Opening lecture
A. BAERHEIM SVENDSEN, Leiden, The Netherlands
Essential Oil Research and Essential Oil Symposia 1969-1989
- 10.45 Coffee break
- 11.00 C. BICCHI, Torino, Italy
Some Applications of Multidimensional Gas Chromatography to
Essential Oil Analysis
- 11.30 A. MOSANDL, Frankfurt, Germany
Chirality Evaluation of Essential Oil Compounds Using
Multidimensional Gas Chromatography (MDGC)
- 12.00 Lunch break
- 14.00 F. HÖFLER, Idstein, Germany
Supercritical Fluid Chromatography (SFC), a New Technique for
the Analysis of Essential Oils
- 14.30 D. JOULAIN, Grasse, France
A Stereoselective Synthesis of (±)-Geosmin
- 15.00 H. SPREITZER, Wien, Austria
Syntheses and Odour of Patchoulol-Analogues
- 15.30 G. BUCHBAUER, Wien, Austria
Conformational Calculations on Sandalwood Odour Molecules
- 16.00 Coffee break
- 16.15 Poster session

Tuesday September 12, 1989

- 9.00 P. WEYERSTAHL, Berlin, Germany
Comparative Studies of Various *Artemisia* Oils
- 9.50 D. LAMPARSKY, Dübendorf-Zürich, Switzerland
Bergamot Oil and Mandarin Oil – Two Raw Materials of Special
Interest to the Fragrance and Flavour Industry

- 10.20 C. ZDERO, Berlin, Germany
Geigeria – A Source of Interesting Compounds
- 10.50 Coffee break
- 11.05 H. SURBURG, Holzminden, Germany
Volatile Constituents of Bird Cherry Flowers (*Padus avium* Mill.)
- 11.35 E. STAHL-BISKUP, I. LAAKSO
Essential Oil Polymorphism in Finnish *Thymus* Species
- 12.05 Lunch break
- 14.00 W. BOLAND, Karlsruhe, Germany
Biosynthesis of 1-Alkenes in the Plant and Insect Kingdom
- 14.30 M.H. BOELEN, Sevilla, Spain
The Chemical Composition of Spanish Myrtle Oil
- 15.00 J.L. SORIANO-FERUFINO, Cochabamba, Bolivia
Variations in the Essential Oil Composition of *Minthostachys andina* (Brett) Epling
- 15.30 Coffee break
- 15.45 E.A. ABOUTABL, Cairo, Egypt
A Comparative Study of the Essential Oils from *Melaleuca* Species Growing in Egypt
- 16.15 K.B. YAACOB, Bangi-Selangor, Malaysia
Essential Oil of *Polygonum* spp.
- 16.45 F.-C. CZYGAN, Würzburg, Germany
Fragrances - Subject of Fine Arts and Literature (in German)

Wednesday September 13, 1989

- 9.00 F. BOHLMANN, Berlin, Germany
Possibilities and Limits of Structure Elucidation by Modern NMR-Techniques
- 9.50 W. SCHULTZE, Würzburg, Germany
New Developments and Applications of Mass Spectrometry in Essential Oil Research
- 10.20 G. LANGE, Würzburg, Germany
Chemical Ionization Mass Spectrometry of Sesquiterpenoids
- 10.50 Coffee break

- 11.05 F. TATEO, Milan, Italy
Criteria for Application of Computerized Methods to the Study of
Flavouring Mixture Composition
- 11.35 W.R. ABRAHAM, Braunschweig, Germany
Chiral Intermediates by Microbial Epoxidations
- 12.05 Lunch break
- 14.00 K. KNOBLOCH, Erlangen, Germany
Physiology and Biochemistry of the Alliinase System from Garlic
(*Allium sativum* L.)
- 14.30 B. GALAMBOSI, Mikkeli, Finland
Agrobotanical Study on Different Basil Varieties Grown Indoors
in Finland
- 15.00 E.H. GRAVEN, King Williams Town, South Africa
Effect of Soil Type and Nutrient Status on the Yield and
Composition of *Tagetes minuta* Oil
- 15.30 Coffee break
- 15.45 K.P. SVOBODA, Ayrshire, Scotland
Biochemical and Physiological Studies on *Satureja hortensis* L.
Grown in Scotland
- 16.15 S.G. DEANS, Ayrshire, Scotland
Antimicrobial Activity of Plant Volatile Oils and their
Constituents
- 16.45 G. PETRI, Budapest, Hungary
Essential Oil Plant Preparations Against Rheumatism
(Development and Standardisation)
- 17.15 F. PERINEAU, Toulouse, France
Extraction of Perfumer's Products by 1,1,2-Trichloro-1,2,2-tri-
fluoroethane

Poster

- P-1 S.P. ANGADI, T. VASANTHA KUMAR
Breeding Strategies in Scented Geranium
- P-2 V. ANTONOVA ANTONOVA, R. TSVETKOV RAEV
Common relations on Development of Essential Oil Plant Production in Bulgaria
- P-3 J. BARROSO, L. PEDRO, M.S. PAIS
Analysis of the Essential Oil of *Crithmum maritimum* L.
- P-4 T.A. VAN BEEK, M.A. POSTHUMUS, G.P. LELYVELD, A. SHEAK, S.R. ADHIKARY
Investigation of Nepalese Essential Oils I. The Oil of *Cinnamomum cieciodaphne* (Sugandha kokila)
- P-5 I. BOHN, K.-H. KUBECZKA, W. SCHULTZE, V. FORMACEK
Two Chemotypes of *Chaerophyllum hirsutum* L. and New Constituents of the Respective Essential Oils
- P-6 R. BOS, J. KLOOSTERMAN, H. HENDRIKS
Study on the Composition of some Dutch Tansy Oils by Means of GC-MS and GC-FTIR
- P-7 E.-J. BRUNKE, E.J. GRAVEN, F.-H. HAMMERSCHMIDT, G. SCHMAUS
On the Essential Oil of *Lippia javanica* (Spreng.)
- P-8 S. BURKHARDT-SISCHKA, M. ÖZGÜVEN, H.S.Z. AKSZU
Antimicrobial Activity and Chemical Composition of Essential Oil of *Thymbra spicata* Cultivated in Turkey
- P-9 M.M. CARMO, E.J. RAPOSO, R. SEABRA, F. VENANCIO, S. FRAZAO
Results of the first Cultures of the *Ocimum basilicum* L. in Portugal
- P-10 C. DEIGELE, K. LOHWASSER, M. KLOOS, R. SCHÖNWITZ

Annual Variation of Monoterpene Concentrations in Needles and Buds of *Picea abies* (L.) Karst.

- P-11 N.M. DUKIC, S.Z. HUSAIN, R. JANCIC, O. GASIC, A. LUKAS
Distribution and Composition of Essential Oil Constituents as Chemotaxonomic Markers in three *Mentha* L. Species in Yugoslavia
- P-12 S. EBEL, K.-H. KUBECZKA, P. MÜLLER, M. WEYANDT-SPANGENBERG
Application of GC/UV-Coupling to the Analysis of Essential Oils
- P-13 P. EVERS, R. KREBBER, W.A. KÖNIG
The Investigation of the Stereochemistry of Flavour Compounds using Chiral Capillary Gas Chromatography with Cyclodextrin Stationary Phases
- P-14 C. FRANZ, M. AUER
Crossing Results of Marjoram (*Majorana hortensis* Moench.) with Different Odour and Flavour Profile
- P-15 A. GÄBLER, W. BOLAND
Biosynthesis of Homoterpenes in Higher Plants; Stereochemical Implications
- P-16 B. GALAMBOSI, Y. HOLM, SZ. ZS. GALAMBOSINE
Yield and Volatile Oil of Four Perennial Herbs Grown in Finland
- P-17 B. GALAMBOSI, I. KUMPULAINEN
Heavy metal content of herbs grown in Finland and Central Europe
- P-18 M.L. GOSH
Growth and Developmental Physiology in Relation to Essential Oil Synthesis of *Mentha piperita* L. under Varying Fertilizer, Photoperiod and Gibberellic acid treatments in the Gangetic Plains of West Bengal, India
- P-19 Y. HOLM, A.-R. SIIRA, R. HILTUNEN
A Chemical Study on Various Basil Chemotypes Cultivated in Finland

- P-20 M. KARMAZIN, D. MASTNA, I. BUBEN
The Contents and Composition of Volatile Oil in *Tanacetum vulgare* L.
During the Ontogenetic Development
- P-21 S.T. KATSIOTIS, C.R. LANGEZAAL, J.J.C. SCHEFFER
Composition of the Leaf Essential Oils of Various Hop Cultivars
- P-22 S.T. KATSIOTIS, C.R. LANGEZAAL, J.J.C. SCHEFFER
Analysis of the Volatile Compounds from Cones of Ten *Humulus lupulus* L. Cultivars
- P-23 N. KIRILOV NEDKOV, G. VELINOV GEORGIEV
Study on the Effect of Irrigation of Mint (*Mentha piperita*) Variety
"Sofia" by Means of Various Irrigation Techniques
- P-24 K.-H. KUBECZKA, V. LEITERER, W. SCHULTZE
Composition and Accumulation Sites of the Essential Oil from
Different Parts of *Poncirus trifoliata* Raf.
- P-25 K.-H. KUBECZKA, O. MOTL, V. FORMACEK
The Structure of Cyprotene, a Novel C₁₄-Hydrocarbon Related to
Sesquiterpenoids
- P-26 O. MADOJEMU-AGU, M.C. CANTORIA
Some Constituents from Philippine Cultivars of *Allium cepa* L. and
their Oral Hypoglycemic Effect in Rabbits
- P-27 P. MANNINEN, S. CARLSON, Y. HOLM, R. HILTUNEN, M.-L. RIEKKOLA
SFE/SFC for the Analysis of Aromatic Plants
- P-28 J. MICHAILOVA
Micropropagation of Lavender
- P-29 C. NEUMANN, W. BOLAND
Biosynthesis of Algal Pheromones: Stereochemical Course of Fatty Acid
Cyclization

- P-30 I. NYKÄNEN, L. NYKÄNEN, M. ALKIO
The Compositions of *Angelica* Root Oils Obtained by Supercritical CO₂ Extraction and Steam Distillation
- P-31 O.A. ONAYADE, A. LOOMAN, J.J.C. SCHEFFER, A. BAERHEIM SVENDSEN
Composition of the Herb Essential Oil of *Hyptis spicigera*
- P-32 O.A. ONAYADE, L. NTEZURUBANZA, J.J.C. SCHEFFER, A. BAERHEIM SVENDSEN
Composition of the Essential Oil of *Hoslundia opposita* Collected in Nigeria and Rwanda
- P-33 A. PAULI, K.-H. KUBECZKA
A Simple and Effective Synthesis of Phenylpropane Derivatives Occurring in Essential Oils
- P-34 R. PICCAGLIA, M. MAROTTI, G.C. GALLETTI
HRGC of Essential Oil from a *Satureja montana* L. Ecotype grown in Northern Italy
- P-35 J. POHJOLA, H. VUORELA, R. HILTUNEN
A Headspace Gas Chromatographic Screening Method for the Volatiles in Scots Pine (*Pinus sylvestris* L.) Needles
- P-36 E. SARER
Essential Oil from *Salvia potentillifolia* Boiss. et Heldr.
- P-37 C. SCHMID, W. SCHÜRMANN, R. SCHÖNWITZ
Partition Coefficients of Plant Cuticles for d-Limonene
- P-38 W. SCHULTZE, S. HOSE, J. SCHULZ, F.-J. HAMMERSCHMIDT, A. ZÄNGLEIN, K.-H. KUBECZKA
Investigations on the Underground Parts of Balm (*Melissa officinalis* L.). Essential Oil Composition and Microscopical Studies
- P-39 R.P. SRIVASTAVA, M. RANDRIAMINAHY, P. PROKSCH

Deterrent and Insecticidal Chromenes and Sesquiterpenes from the Asteraceae

- P-40 G. STANIC, N. BLAZEVIC, J. PETRICIC
Study of the Essential Oils of *Satureja montana* and *Satureja subspicata*
- P-41 T. TALOU, M. DELMAS, A. GASET
Comparative Analysis by Dynamic Headspace and Direct Injection of Black Truffle Alcoholic Extract
- P-42 H. ZIEGLER, G. SPITELLER
Aromatic Acids from the Leaves of *Citrus limon*

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P.Kaders GmbH, Hamburg	

General Information

Location and time

The conference and poster session will be held at the university main building, Sanderring 2, in the center of the city. The introductory lectures of the workshop on instrumental analysis of essential oils will be held in the lecture hall of the "Industrie und Handelskammer", Neubaustrasse 66, close to the university main building. The symposium will commence on Monday, September 11th at 9.30 and close at 12.30, Thursday, September 14th.

Registration

On Sunday, September 10th from 17.00-20.00 a registration desk will be located in the ground floor of the restaurant "Hofkellerei-Weinstuben", Residenzplatz 1. From Monday to Thursday, September 11-13, the registration desk will be located in the first floor of the university main building, Sanderring 2, and will be open from 8.30 onwards; phone: 31202.

Slides

Slide size should be 5x5 cm (2"x2"). The speakers are requested to label the slides adequately and hand them over to the operator before the beginning of each session. An overhead projector will be also available.

Poster session

The poster boards are located in the entrance hall of the university building. They will be labelled; the number corresponds to the number listed in the program. Please prepare your poster during the noon on Monday, September 11th. You are required to be present at your poster during the indicated poster session for discussion of your data.

An exhibition will be held during the meeting from Monday to Thursday, September 11-14, in the basement of the university building. Manufacturers and distributors of analytical equipment will show their products.

Lunch

The registration does not include meals. In the vicinity of the conference building are several restaurants. Furthermore, lunches will be offered during the conference in the near student's dining hall ("Burse").

Social events

Sunday September 10, 1989

19.00 Get-Together-Party

An informal welcome with a typical Franconian lunch "Häcker-Brotzeit" will be arranged in the restaurant "Hofkellerei Weinstuben" Residenzplatz 1 (free, beverages not included).

The registration desk will be located there from 17.00 - 20.00.

Monday September 11, 1989

20.00 Chamber Concert

In the Toscana-Hall of the Residence Palace a Chamber Concert will be performed by amateur musicians (partly participants of the symposium) with compositions of Bach, Händel, Haydn and Telemann.

Tuesday September 12, 1989

18.00 Welcome by the Lord Mayor of the city of Würzburg at the city hall (Beim Grafeneckart).

20.00 Symposium dinner in the restaurant of the Fortress Marienberg, offered by Kneipp-Werke/Würzburg (free, beverages not included).

Ascent to the fortress with cars or by foot (walking time about 15 min.).

Wednesday September 13, 1989

19.30 A barbecue (free) will be arranged in the Botanical Garden of the University, Mittlerer Dallenbergweg 64. You can reach the Botanical Garden by tramway line 3 (direction "Heidingsfeld"). Leave at station "Dallenbergbad".

Thursday September 14, 1989

14.30 Trip to the monastery Tüchelhausen and the medieval villages Sulzfeld and Volkach with the chapel "Maria im Weingarten" and its famous wood-carving by Tilman Riemenschneider. Closing in a typical Franconian wine tavern. Return at approx. 20.30.



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- 1 Restaurant *Hofkellerei Weinstuben* (informal welcome)
- 2 University main building (conference, exhibition)
- 3 Student's dining hall
- 4 via Botanical Garden (barbecue)
- 5 Marienberg Castle (congress dinner)