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Prof. Dr. K.-H. Kubeczka
Pharmaceutical Biology
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Abstracts

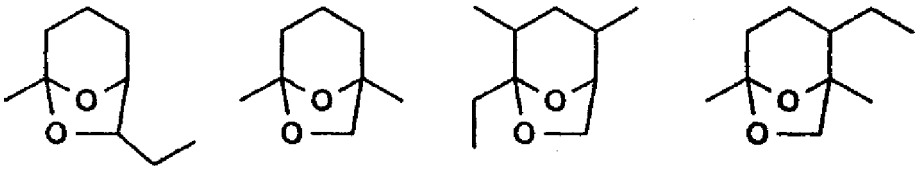
IDENTIFICATION OF VOLATILE BICYCLIC ACETALS FROM INSECTS AND PLANTS

W. Francke, F. Schröder and S. Schulz

Institut für Organische Chemie der Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg

Cryptic ketodiols, represented by bicylic acetals, are widespread among the animal and plant kingdoms. They occur in tiny amounts only and are difficult to separate and to characterize. Structure elucidation by GC/MS, including chiral gas chromatography for the determination of absolute configuration, proved to be the method of choice.

Fragmentation patterns of derivatives of the 6,8-dioxabicylo[3.2.1]octane system (**I-VII**), of the 2,9-dioxabicyclo[3.3.1]nonane system (**VIII-X**) and of spiroacetals showing different ring sizes (**XI-XIII**) will be discussed.



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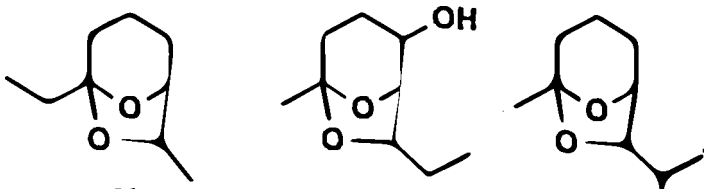

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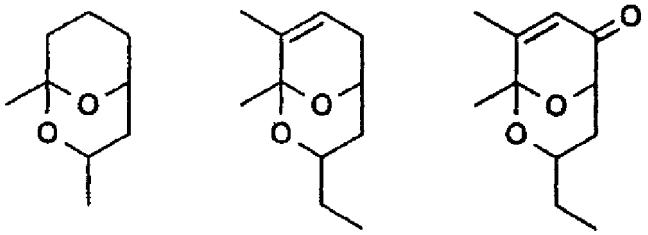

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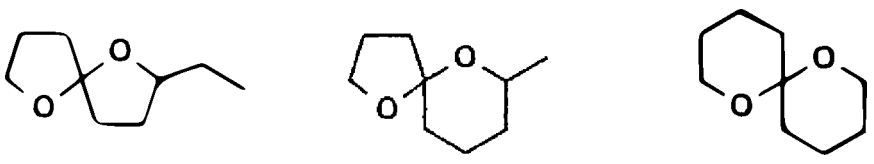

XI XII XIII

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A NOVEL CLASS OF NOR-SESQUITERPENES

V.L.Ferracini[2], M. A. Foglio [3] and A. J. Marsaioli* [1]

1- Instituto de Química , UNICAMP, CP 6154, Campinas 13081-970, SP, Brazil
2- EMBRAPA / Jaguariuna , SP Brazil 3- CPQBA/ UNICAMP, SP Brazil.

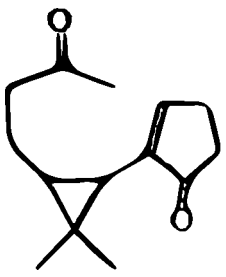
A. de Meijere and K. Rauch
Institut für Organische Chemie der Georg -August Universität- Göttingen- Germany

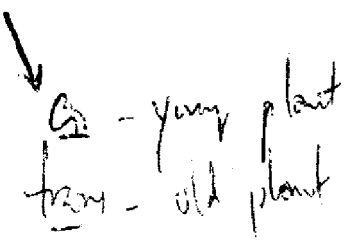
Investigation of volatile mixtures using GC/MS as the main analytical tool restrain the identification of the constituents to known compounds filed in the mass spectra library. Novel structures and novel skeletons can be identified when the compound predominates in the mixture allowing a separation and full characterization. This situation is not true for most essential oils. An alternative approach that allows a better analysis of unknown compounds is to observe the array of identified compounds and to determine wether the biosynthetic pathway leads to oxydations or to oxydative cleavages of standard terpenes. Thus suggestion of new compounds can be made by mass spectra analysis, which must be confirmed by coinjection and mass spectra comparison of the synthetic standard.

Using this approach we have detected several unknown sesquiterpenes present as minor components in the ***Artemisia annua*** essential oil. From the array of volatiles and non-volatiles terpenes present in ***Artemisia annua*** we concluded that the oxydative cleavages of standard terpenes double bond was the preferred biosynthetic pathway to the more oxygenated terpenes.

This rationale led us to the synthesis of some terpenes and compound 1 among them, which was detected in ***Artemisia*** oil in less them 1%. The *cis* and *trans* racemic mixture of 1 was obtained in 5 steps from 6-methyl-5-hepten-2-ol. The key step was the construction of the 5 membered ring applying the Pauson-Khand reaction.

The *cis* and *trans* nor sesquiterpene 1 which we will name **annuone** was present among the hydrodistilled volatiles and among the non volatile components. The presence of 1 can be associated with the age of the plant.





BIOTRANSFORMATION OF TERPENOIDS AND AROMATIC COMPOUNDS BY MICROORGANISMS AND MAMMALS

Asakawa, Y.

Faculty of Pharmaceutical Sciences,Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan

Microbiological functionalization of terpenoids; (+)- and (-)-pinenes, β-pinene, myrcene, camphene, terpinolene, 2- and 3-carenes, *p*-cymene, 1,4- and 1,8-cineoles, (+)- and (-)-menthols, (+)- and (-)-neomenthol, isomenthol, (-)-carvotanacetone, myrtenol, (-)-perillaldehyde, (+)-citronellal, (-)-phellandral, *trans*- and *cis*-carveols, borneol, isoborneol, (+)-germacrane-4,5-epoxide, germacrone, curdione, cryptoporic acids, (-)-β-caryophyllene, (+)- and (-)-cyclocolorenones, (-)-cuparene and aromatic compounds; 6-gingerol, 6-shogaol, dihydroasarone, methyl dihydroeugenol, cuminaldehyde, acetophenone, 34 simple aromatic aldehydes *etc* were carried out using the microorganisms, *Aspergillus* species (*A. niger, A. awamori, A. fumigatus, A. sojae, A. usami, A. cellulosae* and *A. terreus), Euglena gracilis* Z, *Dunaliella tertiolecta, Chlorella ellipsoides, Hansenula anolama* and *Paecilomyces varioti*) and rabbits as mammals.

Euglena gracilis Z, *Dunaliella tertiolecta, Chlorella ellipsoides* easily reduced terpene aldehydes and related aldehydes to the corresponding primary alcohols. Enantio- and diastereoselectivity in the biotransformations of *trans*- and *cis*-carveols, borneol and isoborneol by *Euglena* were observed.

Some metabolites contained biologically active compounds, such as mosquito repellant and plant growth regulatory active 8-hydroxymenthol. Insect pheromones such as (-)-*trans*-verbenol, 8-hydroxy-*p*-cymene and 9-hydroxy-*p*-cymenene. Piscicidal active albicanol. Odoriferant compounds like nogigikualcohol and *cis*- and*trans*-shisools. Antifeedant chamic acid and a mushroom component.

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BIOTRANSFORMATION OF MONOTERPENOIDS BY FUNGAL SPORES

Jan C.R. DEMYTTENAERE & H.L. DE POOTER

Laboratory of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, Coupure Links 653, B-9000 Gent, Belgium

ABSTRACT

During recent years there has been an increasing interest to replace "synthetic" flavouring substances by "natural" ones. These products can be obtained by extraction from plants but they can also be produced by biotechnological processing using microorganisms. *De novo* synthesis of 'flavours', such as volatile esters, and the bioconversion of monoterpenoids are fields of investigation that gain a growing interest: these reactions are performed by bacteria (e.g., *Pseudomonas*), fungi (e.g., *Aspergillus niger*, *Penicillium*, *Cladosporium*, *Botrytis cinerea*), yeasts and even algae. The biotransformation of terpenoids by plant cell cultures has also been reported. Although many articles have been published about biotransformation of monoterpenoids by whole fungal cells (1-3), the bioconversion of these substrates by fungal spores seems a new area.

This paper reports the production of bioflavours using fungal spores. A simple, quick, reproducible and efficient method was developed to carry out biotransformation reactions on volatile terpenoids. Since the transformation of the terpenoid alcohols nerol and geraniol seems to take place through an oxidative pathway (4), we thought that it would be worthwhile to test the biotransformation of citral.

A conical flask, the inside of which was covered with spores of *Penicillium italicum* on a layer of agar, was treated with citral in the vapour phase. The biotransformation reaction was monitored with dynamic headspace techniques. A very efficient transformation of citral to 6-methyl-5-hepten-2-one with a yield of approx. 90% occurred. The same techniques can be applied on a preparative scale.

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Accumulation of Volatiles in Root Cultures, Regenerated from Callus of *Melissa officinalis* L. (Lamiaceae)

T. van den Berg, A. Abou-Mandour, F.-C. Czygan

Department of Pharmaceutical Biology, University of Würzburg,
Mittlerer Dallenbergweg 64, 97082 Würzburg, Germany.

Root cultures regenerated (1) from callus of Lemon Balm (*Melissa officinalis* L., Lamiaceae) were cultivated under in-vitro conditions. Subsequently the volatiles obtained by hydrodistillation were analyzed qualitatively (GC/MS) and quantitatively. During the first 70 days the root cultures exhibit a four-fold increase of dry weight, but a six-fold increase of volatiles. In older roots a slight decrease of the amount of volatiles was observed. Among the 30 identified and quantified components eremophilene shows a special accumulation pattern, i.e. an earlier maximum and significant loss before 70 days. Two isomers of 2,3-dimethylcyclohexanone were detected in a fixed ratio. Concerning amount and composition of volatiles (hexanal, 2-pentylfurane, 2,3-dimethylcyclohexanone, eremophilene, dehydroabietane), the root cultures were very similar to callus cultures (2) of *Melissa*.

Compared with roots of intact plants a significant lack of monoterpenoids in root cultures is evident. Therefore contact to shoot of the plant might be necessary for accumulating monoterpenoids in the roots of this Labiatae.

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THE VOLATILE OIL CONSTITUENTS OF *DIODIA SARMENTOSA*

T.O.E. Ekpendu[1], A.A. Adesomoju[1], O. Ekundayo[1], J.I. Okogun[1,2], I. Laakso[3]

[1] Department of Chemistry, University of Ibadan, Ibadan, Nigeria
[2] National Institute for Pharmaceutical Research and Development, Idu - Industrial Area, P.M.B. 21, Garki, Abuja, Nigeria
[3] Division of Pharmacognosy, University of Helsinki, Finland

The volatile oil of the Medical Plant *Diodia sarmentosa* has been analyzed by GC/MS and found to contain palmitic (51.3%), oleic (6.3%), myristic (4.9%), lauric (4.4%), linoleic (3.4%), palmitoleic (3.4%) and α-linolenic acid, pentadecanoic, and other acids as well as tetradecanal, phytol, cadinols, nerolidol, eudesmol and ß-ionone.

These results will be compared with previous work on *M. scaber* which belong to the same family Rubiaceae.

FT-IR GAS CHROMATOGRAPHIC ANALYSIS OF PERFUMES

J. Seebode[1], K. Krishnan[1]

[1] Bio-Rad Laboratories GmbH, Bischofstr. 86, D-47809 Krefeld, Germany

Perfumes, natural or synthetic, are complex mixtures consisting of numerous components. Gas chromatographic (GC) and gas chromatographic-mass spectrometry (GC-MS) techniques have been extensively utilized for the analysis of perfumes and essential oils. A limited number of perfume samples have also been analyzed by FT-IR gas chromatographic (GC-FTIR) techniques. Most of the latter studies have been performed using the conventional light pipe (LP) based GC-FTIR systems. In recent years, cold-trapping)in a matrix or neat) GC-FTIR systems have become available. The cold-trapping systems are capable of sub-nanogram sensitivities. In this paper, comparison data between the LP and the neat cold-trapping GC-FTIR systems is presented. The neat cold-trapping interface is known as Tracer. The results of GC-FTIR analysis of some commercial perfumes is also presented. For comparison of LP and Tracer GC-FTIR systems, a reference (synthetic) mixture containing 16 major and numerous minor constituents was used. The components of the mixture are the compounds commonly encountered in commercial perfumes. The GC-FTIR spectra of the reference mixture was obtained under identical chromatographic conditions from a LP and a Tracer system. A comparison of the two sets of data thus generated do indeed show the enhanced sensitivity level of the Tracer system. The comparison also shows that some of the major components detected by the Tracer system were absent from the LP data. Closer examination reveals that these compounds undergo thermal decomposition on contact with the hot gold surface that is part of the LP system. GC-FTIR data were obtained for three commercial perfume samples. The major components of these samples could easily be identified by spectra search against a digitized spectral library created using the Tracer data from the reference mixture.

IRMGCMS: A TECHNIQUE FOR ^{13}C AND ^{15}N ISOTOPE DETERMINATION ON COMPLEX GC MIXTURES

A.W. Hilkert[1], J.Oesselmann[1]

1 Finnigan MAT GmbH, Barkhausenstr. 2, 28088 Bremen, Germany

The combination of an isotope ratio mass spectrometer (IRMS) with a gas chromatograph (GC) offers highest precision for isotope abundance determination while maintaining GC resolution. With a dedicated interface between the GC and the IRMS for conversion of the GC eluates into CO_2 and N_2 gases, sample sizes in the pmol range can be measured. The precision for isotope ratio monitoring gas chromatography mass spectrometry (irmGCMS) is 0.0003 at% or better.

This technique can be extended to headspace analysis, trace gas determination in atmosphere and can also be used in parallel with a GCMS system.

A technical overview of the current techniques with a main focus on irmGCMS will be given. Applications with respect to food adulteration, flavors, tracer experiments on plants, authenticity controls on beverages and drugs will be presented.

GC-IRMS and Enantioselective Analysis in the Authenticity Control of Peppermint Oil

Birgit Faber, Armin Mosandl, Institut für Lebensmittelchemie J.W. Goethe Universität Frankfurt, Marie-Curie-Str. 9, 60439 Frankfurt, Germany

Enantioselective analysis using multi-dimensional gas chromatography (MDGC) is reported as an efficient tool in the authenticity control of peppermint oil.

Capillary gas chromatography coupled on-line with isotope ratio mass spectrometry (GC-IRMS) is used as a further method in the analysis of peppermint oil.

Using GC-IRMS, the $\delta^{13}C_{PDB}$-values of some typical peppermint oil constituents are determined. By the method of the internal isotopic standard (i-IST), a characteristic isotopic fingerprint of authentic peppermint oil is established and used for the authenticity control of commercially available peppermint oil.

While the enantioselective analysis conclusively detects the adulteration of synthetic racemates, GC-IRMS furthermore enables to detect the adulteration with non-chiral or enantiopure substances.

Enantioselective capillary gas chromatography coupled on-line with IRMS (enantio-GC-IRMS) combines the efficiency of both methods to a powerful tool in the authenticity control of essential oils.

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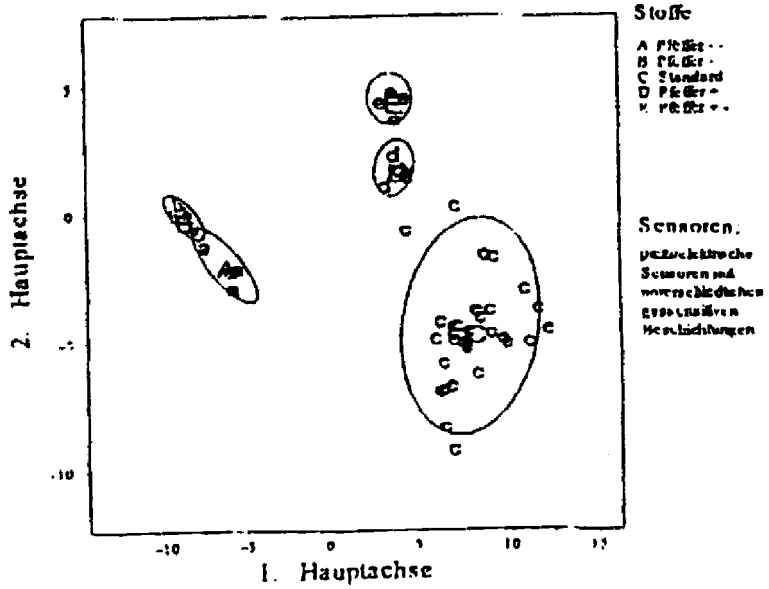
AN INTELLIGENT SENSOR SYSTEM FOR THE CHARACTERIZATION OF AROMA

Vonach, B.
MITU GmbH, Landsberger Str. 13, D-86926 Greifenberg, Germany

Whilst existing analytical techniques rely on the separation of an aroma into its constituents volatile components, new electronic measurement techniques detect the overall aroma. All theses devices use an array of non-sclective, but different sensor elements which provide a signal pattern (fingerprint) characteristic of a particular aroma. This signal pattern is then evaluated, using modern methods of pattern recognition in which complex aroma patterns can be trained-in and identified.

The heart of the sensorsystem described here consists of a chemosensor array based on quartz crystals, coated with different gas-sensitive materials. A special oscillator circuit is built up for each sensor element. Each oscillator has a basic frequency of 10 Mhz with a resolution of ±1 Hz. The adsorption of volatiles on on the gas-sensitive coating of a sensor element causes a frequency change (=sensor signal) of the oscillator. Typical signal levels for essential oils are in the region of 10^3 Hertz.

The potential use range from quality control of foods and cosmetics to the detection of odours in the environment, and of course fast characterization of essential oils. Discrimination of hop- and carrott-varieties due to their different aroma as well as objective aroma measurements for the quality control of spice mixtures has been performed.



OCCURRENCE OF SESQUITERPENE ENANTIOMERS AND THEIR IDENTIFICATION

W. A. König, C. Fricke, I. H. Hardt, S. Melching, A. Rieck, and Y. Saritas

Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

A systematic screening of essential oils of many liverworts (*Hepaticae*) and higher plants by EI gas chromatography-mass spectrometry revealed that a substantial portion of the sesquiterpene hydrocarbons can not easily be identified. This is partly due to the fact that reliable mass spectrometric data, especially of rare sesquiterpene hydrocarbons, are not available, that mass spectra greatly depend on instrumental conditions, that many of the minor components have never been identified, and that isolation of pure compounds from the complex mixtures is extremely difficult. We have started to build up a library of mass spectra correlated to gas chromatographic retention times. In addition, many compounds were isolated by preparative gas chromatography and correlated to their ^{1}H-NMR spectra. In the course of these investigations a number of new structures were identified from vetiver oil and from the volatiles of liverworts.

There is an even greater lack of knowledge about enantiomeric compositions of sesquiterpene hydrocarbons in plant volatiles. After preparative isolation of liverwort constituents the occurrence of enantiomers with configurations opposite to those of higher plants was not only confirmed, but we were able to prepare standard solutions of known enantiomeric composition (1). These mixtures were separated by enantioselective capillary gas chromatography with cyclodextrin derivatives as chiral stationary phases. Two-dimensional gas chromatography was applied to determine the absolute configuration and to study the enantiomeric composition of single sesquiterpene constituents in different plants (2). In some cases, e.g. *Cedrela odorata*, enantiomeric mixtures were found (3), giving new insights to the biosynthesis of these compounds.

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COMPOSITION AND POTENTIAL USES OF THE ESSENTIAL OIL OF SCOTTISH *MYRICA GALE* (L) HARVESTED FROM THE WILD POPULATION

K.P. Svoboda[1], A. Inglis[1], J. Hampson[1], S.G. Deans[2], B. Galambosi[3], and I. Branson[4]

[1] Plant Science Department, SAC, Ayr, U.K.
[2] Biochemical Science Department, SAC, Ayr, U.K.
[3] South Savo Research Station, 50600, Mikkeli, Finland
[4] Port House, Rothesay, Isle of Bute, U.K.

Myrica gale (L) is a shrub native to Scotland and other temperate regions of the world, growing in damp acidic soil, often in shade, in bogs, wet heath, and moors.

The essential oil is a complex mixture of a few major and numerous minor terpenes, with yields highest during the flowering period. Pharmaceutical and Aromatherapy companies are showing a renewed interest in the plants mainly due to the insecticidal properties of the essential oils.

Considerable differences have been reported in the composition of these oils from plants growing in different geographical locations, and even within a localised population. Consequently, for this research project an island population on the west coast of Scotland was chosen for study.

Stems and leaves were collected at random from the population throughout the period of May - September 1994. Provided the clipping is not too severe, plants will regrow readily after harvesting. A similar collection was done in Finland during May - September 1995.

Leaves, flowers and stem were distilled and analysed (GC, GC-MS) separately. Essential oil glands on the leaf surface were recorded by microscope magnification (x500).

Stand characteristics and biomass production were measured. Essential oil yield was quite low, 0.3% for leaves and 0.97% for flowers.
Main oil components were: α-pinene 18.3 - 38.9%; 1,8 cineole 2.5 - 23.9%; germacrone 0.4 - 13.2%; nerolidol 0.5 - 4.9%; L-limonene 3.6 - 6.5%.

PULEGONE-RICH ESSENTIAL OILS OF TURKEY

K.H.C. Baser[1], N. Kirimer[1], and G. Tümen[2]

[1] Medicinal and Aromatic Plant and Drug Research Centre (TBAM), Anadolu University, 26470 Eskisehir, Turkey

[2] Faculty of Education, Department of Biology, Balikesir University, 10100 Balikesir, Turkey

Pulegone is a monoterpene ketone with a refreshing odour reminiscent of mint. This is the reason why pulegone-rich essential oil containing plants are often named in Turkish after mint.

Six Labiatae genera (i. e. *Acinos, Calamintha, Cyclotrichium, Mentha, Micromeria* and *Ziziphora*) have been found to contain pulegone as a major constituents in their respective essential oils.

This study covers the essential oil compositions of pulegone-rich Labiatae species growing wild in Turkey.

DISTILLATION EFFECTS ON THE YIELD AND THE COMPOSITION OF EUCALYPTUS CITRIODORA HOOK ESSENTIAL OIL

RAMZI MEJDOUB and STAVROS Th.KATSIOTIS[1]

International Centre for Advanced Mediterranean Agronomic Studies,
M.A.I.Ch, POB 85, 73100 Chania, Crete, Greece.

[1]Department of Pharmaceutics, School of Pharmacy, Aristotle University,
Thessaloniki, 54006, POB 1589, Greece

The essential oil variation of *Eucalyptus citriodora Hook* (growing in Crete, Greece) from the stantpoind of yield and composition was evaluated among different proceeding methods (Hydrodistillation, Simultaneous Distillation Extraction, of integrals and comminuted leaves and of different proceeding durations). The chemical composition of the essential oil was determined by GC and GC/MS. More than eighty components were detected, forty-four of them were identified representing the 98.63% of the total oil. The main resulted constituent was citronellal (74.2-83.25%) and the main compounds of the oil were isopulegol (3.90-8.21%), β-citronellol (6.63-10.77%) and citronellyl acetate (1.57 - 2.57%). The extraction methods have highly significant effects on the essential oil yields (2.00-4.26%) and on the majority of the studied variables that have different patterns of rank of the distillation methods considering their highest yields. Mainly, citronellal and β-citronellol present their highest value under the Hydrodistillation of the comminuted leaves, the hydrocarbons monoterpenes under the Simultaneous Distillation Extraction of 1 hour, whereas the sesquiterpenes and the oxygenated monoterpenes under the Simultaneous Distillation Extraction of 2 hours which gives also the highest yields of the traces constituents.

CHARACTERISTICS OF THE ESSENTIAL OIL FROM THE FRUITS OF *ZANTHOXYLUM SIMULANS*

C.-C. Chyau[1], J.-L. Mau[2] and C.-M. Wu[3]

[1] Department of Food Nutrition, Hung-Kuang Institute of Nursing and Medical Technology, Taichung, Taiwan, ROC

[2] Department of Food Science, National Chung-Hsing University, Taichung, Taiwan, ROC

[3] Food Industry Research and Development Institute, Hsinchu, Taiwan, ROC

Chinese peppers, *Zanthoxylum simulans* Hance, were commonly cultivated in Guangdong, Henan, Shanxi, Jiangsu and Hebei provinces in mainland China. Normally, the dried fruits were served as a spice for food flavoring, especially in stew meat cooking.

The essential oil was isolated from the fruits using steam distillation and liquid carbon dioxide extraction, respectively, and analyzed by GC, GC-MS (both EI and CI modes) and GC-FTIR. The major components (>10%) found in both oils were β-myrcene, limonene, 1,8-cineole and (Z)-ocimene. As compared with the essential oil from *Zanthoxylum bungeanum* fruits (1), this study prepared the essential oil from *Zanthoxylum simulans* fruits with a higher percentage of 1,8-cineole and a smaller percentage of limonene and β-myrcene.

The yield in steam distillation was 1.69% (w/w), while that in liquid carbon dioxide extraction was 6.38% (w/w). Although the yields of two extraction methods were different, the two essential oils were composed of similar constituents. However, some minor components, including isobutyl acetate, isoamyl acetate and α-terpinene, were not found in the extract of liquid carbon dioxide.

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SEARCH FOR NEW AROMA CHEMICALS FROM GENUS OCIMUM; ITS PAST POTENTIAL AND FUTURE PROSPECTUS

Bansilal Bradu

Department of Botanical Sciences
Regional Research Laboratory
Jammu-Tawi 180001,India

Genus Ocimum(Lamiaceae) comprising of over 160 species has versatile group of aromatic plants with varied combinations of essential oils yielding heterogeneous group of aroma chemicals of perfumery,flavour and pharmaceutical value. They contain terpenes, sesquiterpenes, alcohols, esters, aldehydes, ketones, phenols, ethers etc.

For the last more than two decades, the cytogenetics and plant breeding department of this institute has been commandeering pioneering efforts regarding intensive studies on cytogenetical, cytotaxonomical, cyto-chemotaxonomical investigations bordering on sound breeding programme related to inheritance pattern of physico-chemical characteristics of the essential oils with a view to locate and evolve new improved and useful varieties or cultivars of Ocimum species. A wide array of plant types having essential oils rich in linalool, citral, methyl chavicol, elemicin , thymol, eugenol, iso-eugenol, methyl- iso-eugenol, alpha and beta terpiniol, geraniol etc. have been evolved.

The recent development of genetically upgraded strain of Ocimum gratissimum coined as `Clocimum-3C or RRL-Og-14 having eugenol ranged from 85-90% which formed an economic substitute of clove oil of commerce has been an important contribution of the improvement programme. Efforts are continued to widen the germplasm collection of Ocimum species with view locate and evolve improved strains rich in nerolidol, safrole, farnesol, bisabolol etc.

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RELIABILITY OF ANALYTICAL DATA IN THE ANALYSIS OF ESSENTIAL OILS

Carlo Bicchi[1], Arianna Binello[1], and Monica Fresia[1]

[1] Dipartimento di Scienza e Tecnologia del Farmaco, Via Pietro Giuria 9 - I-10125 Torino

This communication aims to evaluate how far retention indices (RI) can go in identifying an essential oil component at an intra- and intralaboratory level. Three essential oils (*Artemisia roxbourghiana, Tagetes lucida* and *Matricaria chamomilla*) were investigated with different columns and under different analytical conditions. RI of a group of selected test compounds in each oil were determined through the equations of both Kovats and Van Den Dool, by analysing them with several columns coated with a) two different stationary phases (OV-1, Carbowax 20M), b) the same stationary phase supplied by different manufacturers, and c) the same stationary phase but having different inner diameters (0.18, 0.25 and 0.32 mm). The oils were analyzed under different conditions: constant pressure, constant flow, constant average linear carrier gas velocity and different temperature rates. The great number of data obtained were used to determine what other parameters besides stationary phase influence RI reliability, the range of variation of RIs, with different columns and/or under different operative conditions, and the validity of δ-RI.

Some considerations will also be made on how GC data can be used in association with GC-MS data. In particular the limits on the identification of essential oil components done with a mass spectral library and the role of the personal library versus GC retention data will be discussed.

ESSENTIAL OILS: EVALUATION OF SUBSTANCES AND THEIR INFLUENCE ON HUMAN ALERTNESS

J. Ilmberger[1], E. Heuberger[2] and G. Buchbauer[2]

[1] Dept. Physical Medicine, Klinikum Großhadern, Ludwig-Maximilians-University, Marchioninistr. 15, 81366 Munich, Germany

[2] Inst. of Pharmaceutical Chemistry, Center of Pharmacy, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria

A variety of effects of essential oils on human cognitive behavior have been reported. Especially, inhalation of essential oils seems to influence certain aspects of attention (e.g. vigilance). It is unclear, however, whether this influence takes place already at the most basic level of attention, i.e. alertness, or whether effects are observed only when attentional demands are more complex, as for example in selective attention.

Alertness, which can be experimentally defined as speed of information processing, is measured in single reaction time tasks. We used this paradigm to examine the effects of inhalation of the oils of peppermint, menthol, cineol, jasmine and ylang-ylang. Subjects were seated in front of a computer screen and had to hold one of two buttons. Upon appearance of a visual stimulus, this button had to be released (decision time) and the second button had to be pushed (motor time). In one session, two series of trials had to be performed, each series consisting of 220 stimuli (taking approximately 20 minutes). Subjects were wearing surgical masks, to which at the first series water and at the second series the essential oil was applied. In a control group, the second application also consisted of water. At the end of each series, subjects had to rate the hedonic value (pleasant/unpleasant), intensity (strong/weak), effect (stimulating/tiring) and degree of relaxation (I feel relaxed/tense) on a visual analogue scale.

Between-group comparisons (water vs essential oils) showed no effects of inhalation of essential oils on decision times but significant effects on motor time; these effects were based on an improvement of motor times in the control group in the second series; no such improvement was shown in the experimental groups. This effect may be a general sedative one as subjectively experienced intensities were rather high.
Intra-group analysis showed complex, mostly curvilinear relations between subjective ratings and decision and motor times. Some of these relations were also observed in the control group and must be seen as placebo effects. It is concluded that on the level of alertness, performance is influenced in a significant way by subjective ratings of essential oils; essential oils on this level have no specific, generally stimulating or sedating, effects.

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INDUCTION OF PLANT VOLATILES BY HERBIVORES AND JASMONATES

W. Boland, J. Hopke, T. Koch, T. Krumm and J. Piel

Institut für Organische Chemie und Biochemie, Gerhard-Domagk-Str. 1, D-53121 Bonn, Germany.

If plant leaves are damaged by herbivores, this may induce the biosynthesis and emission of (novel) volatiles acting as phytoalexins or even as chemical cues for host- and prey-seeking insects.

The induction process demands for injection of salivary enzymes of the herbivore into the plant leaf; mechanical damage is, in general, not effective. In particular ß-glucosidases, cellulases and pectinases are highly active elicitors of odor induction (e.g. in Lima beans, *Phaseolus lunatus*).

Besides of high molecular elicitors also low molecular compounds like jasmonic acid (JA), methyl jasmonate (JA-Me) and related compounds from the octadecanoid signalling pathway are involved (1). Odor induction by JA appears to be a common, probably archetype aspect of the plant's defense and is observed from primitive ferns to modern dicotyledonous plants (2).

The qualitative and/or quantitative composition of the induced volatiles depends on the type and the concentration of the elicitor. Structural analogues of JA or, the even more active, phytotoxin Coronatin can be used for a „selective" induction of different biosynthetic pathways in *P. lunatus*. The very high elicitor activity of conjugates of JA or JA-analogues with the amino acids Ile and Leu, but not Val or Phe, for example, suggests that such conjugates may represent further intermediates in the signal transduction pathway *en route* to volatile production.

Some plants convert JA into the volatile methylester (JA-Me) which may act as a volatile signal inducing a prophylactic build-up of chemical defenses in still uninfested plants. Ca. 20 nmol JA-Me are sufficient to enhance the level of furanocoumarins in leaves of celery plants by a factor 40-70 within four days (3).

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DEVELOPMENTAL CHANGES IN ACTIVITY OF ENZYMES INVOLVED IN CARVONE BIOSYNTHESIS IN CARAWAY

H.J. Bouwmeester[1], J. Gershenzon[2], M.C.J.M. Konings[1] and R. Croteau[2]

[1] Institute for Agrobiological and Soil Fertility Research (AB-DLO), P.O. Box 14, 6700 AA Wageningen, The Netherlands

[2] Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, USA

(+)-Carvone and (+)-limonene are the main components of caraway essential oil. Recently carvone has been introduced as a sprouting inhibitor for potatoes and for a higher profitability of caraway culture, it would be interesting to increase the carvone content of caraway seed. Biosynthesis of (+)-carvone in caraway might occur via a pathway similar to that described for (-)-carvone in *Mentha spicata* (Gershenzon et al., 1987; Plant Physiol. 89: 1351-1357). That is, a cyclase converts geranyl pyrophosphate (GPP) to limonene. Limonene is hydroxylated to *trans*-carveol, which is subsequently dehydrogenated to carvone. For caraway this pathway would imply that limonene is both an end product (which is stored in the essential oil ducts) and an intermediate in the biosynthesis of carvone. In earlier work it was shown that accumulation of carvone in seeds of caraway starts a few weeks later than accumulation of limonene. Formation of both compounds stops several weeks before seed maturation. The aim of our work was to demonstrate the involvement of the three enzymes mentioned above and to relate their activity patterns to the changes in accumulation rates of limonene and carvone during seed development.

In caraway seed extracts the activities of all three enzymes were observed. Cyclase and dehydrogenase activities were found in the cytosolic fraction, whereas hydroxylase activity was located in the microsomal fraction. The cyclase highly specifically converted GPP to (+)-limonene. As in *M. spicata*, the hydroxylase had the characteristics of a cytochrome-P450 enzyme. Just as the (-)-limonene hydroxylase from *M. spicata* (Karp et al., 1990; Arch. Biochem. Biophys. 276: 219-226), the (+)-limonene hydroxylase from caraway accepted also the other enantiomer of limonene as a substrate. For the dehydrogenase (+)-*trans*-carveol seemed the preferred substrate, although also both *cis*-carveol enantiomers but not (-)-*trans*-carveol were oxidized to carvone. Already in the earliest stages of seed development GPP:(+)-limonene cyclase and (+)-*trans*-carveol dehydrogenase were active, but (+)-limonene hydroxylase was not, permitting only formation of limonene and not carvone. In later stages (+)-limonene hydroxylase activity increased, which would be in agreement with the onset of carvone accumulation at these stages. The activity of all three enzymes decreased towards seed maturation. The results suggest that carvone biosynthesis and accumulation in caraway are mainly limited by limonene hydroxylase activity.

THE ROLE OF VERNALIZATION IN GENERATIVE DEVELOPMENT OF CARAWAY (CARUM CARVI f. biennis)

É. Németh, J. Bernáth

Department of Medicinal Plant Production, University of Horticulture and Food Industry
Villanyi str. 29. H-1114 Budapest, Hungary

At our department several physio-ecological, genetical and analytical investigations are carried out for the improvement of biological basis of caraway production. Physiological difference between annual and biennial caraway is still not satisfactory cleared up (1,2,3).

In the present work we studied the effect of vernalization period on the development of the biennial caraway *(Carum carvi f. biennis)*. The experiments were carried out in phytotron chambers, using four genotypes of different origin (Swiss, Finnish, Rumanian and Hungarian) with both more and less developed plant individuals.

It was found, that the applied 5-8^{o}C cold treatment was not a prediction for flowering, if the plants had a root stock thicker than 8 mm.

However, vernalization significantly increased the umbels' number per plant as well as stem diameter. Some other morphological characteristics (umbel size, plant height) were affected by the cold treatment in interaction with genotype. Genotype differences were greater under not vernalized circumstances.

Plants without vernalization showed a sudden growth, while those after the treatment had a slower growth. In the contrary, vernalization had not any effect on the dinamics of flowering.

The correlation system of the studied morphological characteristics was hardly changed by the vernalization.

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ESSENTIAL OILS AND THEIR CONSTITUENTS AS EFFECTIVE FUMIGANTS FOR THE CONTROL OF STORED-PRODUCT INSECTS

E. Shaaya[1], M. Kostjukovsky[1]

[1] Department of Stored Product, ARO, The Volcani Center, Bet Dagan 50250, Israel

The fumigant toxicity of a large number of essential oils extracted from various spices and herb plants and some of their major constituents was assessed against a number of major stored-product insects. Three groups of active materials were distinguished according to their activity against the various insects. Recently we have isolated a compound which was found most active of all the essential oils and their constituents which were tested against stored-product insects. A concentration of 1.8 g/m^3 air caused 100% mortality of all adult insects tested after 24 h exposure.

In semi field experiments using columns filled with 75% wheat, 92 h exposure time, 100% mortality of adult *Sitophilus oryzae* and *Tribolium castaneum* was recorded at a concentration of 10 and 40g/m^3 respectively.

Tribolium castaneum was found most resistant, of all insects tested, to essential oils and their monoterpenes. The activities of these compounds are insect stage specific. The possible involvement of the enzyme acetylcholinesterase in the suspectibility of the insects to these compounds was examined.

COMPARATIVE ULTRASTRUCTURE OF GLANDULAR TRICHOMES OF *DRACOCEPHALUM MOLDAVICA* L., *NEPETA CATARIA* L. and *NEPETA CATARIA* var. *CITRIODORA* BALB. IN CONNECTION WITH ESSENTIAL OILS BIOSYNTHESIS

M.R. Kolalyte[1]

[1] Department of Plant Resources, V.L. Komarov Botanical Institute, Prof. Popov str. 2, 197376 St. Petersburg, Russia

Dracocephalum moldavica L., *Nepeta cataria* L. and *Nepeta cataria* var. *citriodora* Balb. are aromatic plants from Lamiaceae family. Geraniol predominates the essential oils of *Nepeta cataria* while essential oils of *Dracocephalum moldavica* and *Nepeta cataria* var. *citriodora* contain significant amounts of citral (1).

The data from literature shows that essential oils of Lamiaceae are produced in all types of glandular trichomes (2). The study by scanning electron microscope showed that leaves of *Dracocephalum moldavica* and *Nepeta cataria* bares peltate glands and capitate glandular hairs. Leaves of *Nepeta cataria* var. *citriodora* bare peltate glands and two types of glandular hairs.

The ultrastructural study showed that capitate glandular hairs of investigated species have well developed rough endoplasmic reticulum (RER) and dictyosomes. They are supposed to secrete not terpenes but, probably, glycoproteins.

Peltate glands of *Nepeta cataria* and *Nepeta cataria* var. *citriodora* are characterized by presence of leucoplasts sheathed by reticular tubules and smooth endoplasmic reticulum (SER). Peltate glands of *Dracocephalum moldavica* had unsheathed leucoplasts, both RER and SER and unusual associations of SER which are proposed to be the site of oxygenating of terpenes. Peltate glands of investigated species and some of the two types of capitate glandular hairs of *Nepeta cataria* var. *citriodora* are proposed to produce terpenes.

Accumulation of terpenes in all investigated species takes place in subcuticular cavity. The possible sites of terpene biosynthesis and intercellular transport and correlation between ultrastructure of secretory cells and essential oils secretion will be discussed.

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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF ESSENTIAL OIL OF *AGERATUM CONYZOIDES* L.

A.A. Abena[1], J.M. Quamba[2], and A. Keita[3]

1 Départment des Sciences Physiologiques Faculté des Sciences de la Santé. Universite'Marien Ngouabi, B.P. 69, Brazzaville, Congo.

2 Laboratoire d'Etuedes Physico. chimiques. Faculté des Sciences. Université Marien Ngouabi, B.P. 69, Brazzaville, Congo.

3 Département de Médicine Traditionelle. Institut National de Recherche en Santé Publique, B.P. 1746, Bamako, Mali.

Ageratum conyzoides L. is an Asteracea widely used in African traditional medicine in various diseases.

Among these indications, are mentioned fever, enteralgia, cephalgia, occular infection and inflammatory process.

This plant contains an essential oil which could support some indications of *Ageratum conyzoides* L..

The aim of this work is to study the oral analgesic and anti-inflammatory activities of *Ageratum conyzoides* L. using the classical tests in the mice.

Tail-flick and writhing tests were used for the evaluation of analgesic effect and cotton pellet granulation for the anti-inflammatory activity. Concomitantly gastric tolerability was tested by macroscopic observation of stomach.

Essential oil of *Ageratum conyzoides* possess an analgesic activity at the doses of 2, 3 and 4 ml/kg. Doses of 3 and 4 ml/kg per os were found to have a significant anti-inflammatory (cotton pellet granuloma) activity. The daily oral administration for 7 days failed to show gastric toxicity.

These results are in accordance with the folk use of *Ageratum conyzoides* L.

AROMATHERAPY - PLANT MEDICINE OR ESOTERIC HOAX. EXAMINING THE SCIENTIFIC BASIS OF A POPULAR PHENOMENON

Kurt Schnaubelt[1]

[1] Pacific Institute of Aromatherapy, P.O.Box 6723, San Rafael, CA 94903, USA

Much confusion has arisen in the recent past over the validity of the concept of aromatherapy. In addition there is even disagreement about the meaning of the term aromatherapy itself. Authors of popular as well as scientific works on the topic tend to include their individual interpretation of what actually constitutes aromatherapy to them. A review of the literature shows that aromatherapy has its origin in clear scientific thought and experimentation. The evolution of aromatherapy will be discussed starting with its origin as strictly medical use of essential oils to the current state of aromamedicine which is practised in conjunction with phytotherapy and other wholistic modalities. The apparent difficulty to assess the complex actions of essential oils which combine antimicrobial effects with profound interaction in the psycho-neuro-endocrine complex will be discussed. Some of the clinically most effective aromatherapy treatments for common ailments will be summarized.

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ESSENTIAL OIL COMPOSITION OF *JUNIPERUS BREVIFOLIA*, AN ENDEMIC SPECIES OF THE AZORES

José G. Barroso[1], A. Christina Figueiredo[1], Luís G. Pedro[1], Carlos A. Ribeiro[2], Johannes J.C. Scheffer[3]

[1] Departamento de Biological Vegetal, Faculdade de Ciencias de Lisboa, Bloco C2, Campo Grande, 1780 Lisbon, Portugal

[2] Departamento de Biologia, Universidade dos Acores, Rua da Mae de Deus 58, 9502 Ponta Delgada Codex, Acores, Portugal

[3] Jardim Botanico da Madeira, Caminho do Meio, Bom Sucesso, 9000 Funchal, Madeira, Portugal

[4] Division of Pharmacognosy, LACDR, Leiden University, Gorlaeus Laboratories, PO Box 9502, 2300 RA Leiden, The Netherlands

Juniperus brevivolia (Seub.) Antoine (= *J. oxycedrus* L. var. *brevifolia*), locally known as cedro-das-ilhas, cedro-do-mato, cedro-da-terra or zimbro, is an endemic species of the Azores that occurs on all the islands, except St. Maria and Graciosa.[1,2] Being the only conifer tree that was not introduced in the Azorean archipelago, *J. brevifolia* is a typical component of the laurelwood, the so-called cloud-zone forest. This species generally occurs above 500m, on Pico it is found up to 1500m, and in the west Azores, such as on Corvo, even down to 200m.[2] Due to the high quality wood of this species, it has been continuously cut and old stands of some size are now rarely seen in the archipelago.[2]

The essential oil was isolated by hydrodistillation and distillation-extraction, either from twigs or from fruits, and analyzed by GC and GC-MS. Both oils isolated by hydrodistillation were obtained in the same yield (0.3%, v/w). Thirty-three components of the oil from the twigs were identified and thirty-eight of that of the fruits, amounting to almost 100% and 98% of the total oils, respectively.

Both oils were dominated by the monoterpene fraction (93% for the twigs and 87% for the fruits), limonene being its main component (74% for the twigs and 57% for the fruits). The oxygen-containing monoterpenes represented only 0.3% and 1.2% of the twig and fruit oils, respectively.

The sesquiterpene fraction occurred in low amounts (1.7% for the twigs and 6.0% for the fruits). α-Humulene and cedrol (0.4% each) were the main sesquiterpenes of the twig oil, and germacrene-D (2.1%) was the main one in the fruit oil.

Apart from mono- and sesqui-terpenes, the oils contained also a diterpene fraction, *epi*-13-manool being its most abundant component (5.2% for the twigs and 3.3% for the fruits).

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ESSENTIAL OIL COMPOSITION OF *MELANOSELINUM DECIPIENS*, AN ENDEMIC SPECIES OF THE MADEIRA AND AZORES ARCHIPELAGOS

Jaime A.T. da Silva[1], A. Christina Figueiredo[1], José G. Barroso[1], Luís G. Pedro[1], M. Avila Gomes[2], Susana S. Fontinha[3], Johannes J.C. Scheffer[4]

[1] Departamento de Biological Vegetal, Faculdade de Ciencias de Lisboa, Bloco C2, Campo Grande, 1780 Lisbon, Portugal

[2] Jardim Botanico do Faial, 9900 Horta, Acores, Portugal

[3] Jardim Botanico da Madeira, Caminho do Meio, Bom Sucesso, 9000 Funchal, Madeira, Portugal

[4] Division of Pharmacognosy, LACDR, Leiden University, Gorlaeus Laboratories, PO Box 9502, 2300 RA Leiden, The Netherlands

Melanoselinum decipiens (Schrad. et Wendl.) Hoffm. [*Selinum decipiens* Schrad. et Wendl.; *Trapsia decipiens* (Schrad. et Wendl.) Hook. fil.], commonly known as aipo-da-serra or aipo-do-gado,[1,2] is an endemic species of the Madeira and Azores archipelagos. On Madeira, *M. decipiens* grows at low altitudes (0-800m),[2] between shady rocks and banks in the northern laurelwood zone.[1,2] Ne in this archipelago, the species can also withstand long summers without requiring shady habitats. The Azorean *M. decipiens* always grows above an altitude of 400m, preferably in shady habitats with a constant water supply, and in an organic-rich soil. The Madeiran plants have longer stems and shorter leaves than the Azorean ones. In the Madeira archipelago this species is frequently utilized as both cattle fodder and as an ornamental plant.[1]

The essential oil of *M. decipiens*, from both Madeira and the Azores, was isolated by hydrodistillation and distillation-extraction from the aerial parts of the plant, collected during the vegetative phase, and the samples obtained were subsequently analyzed by GC and GC-MS. The same yield (0.2%, v/w) was obtained for the oils of both origins. Thirty components of the Madeiran essential oil were identified, and fourteen of that of the Azorean oil, amounting to 95% and 99% of the total oils, respectively. Both oils were dominated by the monoterpene fraction (87% for the Madeiran *M. decipiens* and 86% for the Azorean sample), ß-pinene (72%) and limonene (82%) being the main components of the Madeiran and Azorean oils, respectively), the former being dominated by n-nonane (4%).

Differences between the Madeiran and the Azorean *M. decipiens* exist in both the morphology and the environmental tolerance. Moreover, both specimens can be further differentiated by their essential oil composition.

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ACCUMULATION OF VOLATILES IN CELL SUSPENSION CULTURES OF *GERANIUM ROBERTIANUM* var. *PURPUREUM*

Luís G. Pedro[1], M. Salomé Pais[1], Johannes J.C. Scheffer[2]

[1] Departamento de Biological Vegetal, Faculdade de Ciencias de Lisboa, Bloco C2, Campo Grande, 1780 Lisbon, Portugal

[2] Division of Pharmacognosy, LACDR, Leiden University, Gorlaeus Laboratories, PO Box 9502, 2300 RA Leiden, The Netherlands

A the late growth phase and the early stationary phase of cell suspension cultures of *Geranium robertianum* var. *purpureum*, maintained in liquid MS medium, some suspended cells showed cytoplasm inclusions. Histochemical tests suggested that these inclusions contained fatty acids, some of them unsaturated.

The essential oil isolated from the suspended cells of *G. robertianum* var. *purpureum*, after 8 days of culture, was obtained in a yield lower than 0.005% (v/w). The oil consisted almost totally of fatty acids; they represented 95% of the total oil, hexadecanoic (40%), octadecadienoic (36%) and octadecatrienoic (10%) acids being the most important representatives. Among the other identified compounds were four oxygen-containing monoterpenes: 1,8-cineole (0.1%), borneol (0.1%), terpinen-4-ol (0.6%) and α-terpineol (0.6%).

One of the terpenoids detected as volatile component of the suspended cells, *i.e.* 1,8-cineole, was not detected in the oil isolated from the intact plant. Borneol and α-terpineol were present in similar percentage amounts in the oils from the suspended cells and from the plant. Terpinen-4-ol, constituting only 0.6% of the oil from the cells, amounted to 5% of the oil from the plant. The volatile oil from the suspended cells did not contain any monoterpene hydrocarbon, although these compounds represented the major fraction of the oil from the plant, and sesquiterpenes were not detected either. The fatty acids detected in the essential oil from the suspended cells, were also present in the oil from the plant. The most striking differences were those related to hexadecanoic and octadecadienoic acids, as their concentrations in the oil from the plant were 4 and 8 times lower than in the mixture of volatiles from the suspended cells.

Terpinen-4-ol and α-terpineol were the only oxygen-containing monoterpenes showing marked fluctuations in their concentrations during the growth cycle of the suspension cultures. All fatty acids detected in the essential oil showed fluctuations in their concentrations during the growth cycle of the suspension cultures, hexadecatrienoic (0.4-5%), heptadecanoic (0.2-2.5%), octadecadienoic (7-40%) and octadecatrienoic (traces-6%) acids being noteworthy. The total fatty acids concentration increased at the end of the stationary phase, which can be related to cell death and consequent membrane degradation.

The Scent of Orchids

Chirospecific Analysis, Structure and Properties of 4-Methyl-5-decanolides

Dietmar Bartschat, Detmar Lehmann, Armin Dietrich and Armin Mosandl*
Institut für Lebensmittelchemie, Biozentrum, Johann Wolfgang Goethe-Universität Frankfurt, Marie-Curie-Str. 9, 60439 Frankfurt/Main, Germany

Roman Kaiser, Givaudan-Roure Forschung AG, CH-8600 Dübendorf, Switzerland

Racemic mixtures of synthetic *cis*- or *trans*- 4-methyl-5-decanolide were seperated by enantioselective HPLC with Chiraspher-RT to yield all four stereoisomers as enantiopure compounds of distinct odour activities.
In order to elucidate stereochemical features the isolated stereoisomers were reduced to their corresponding 4-methyl-1,5-decandiols by reductive ester cleavage with $LiAlH_4$. Absolute configurations were derived from ^{1}H-NMR studies of diastereomeric di-(R)-MTPA esters of these 1,5-diols.
Using enantioselective multidimensional capillary gas chromatography (column combination SE 52 / heptakis-(2,3-di-O-methyl-6-O-tert.butyldimethylsilyl)-ß-cyclodextrin), the direct enantioselective analysis of all four lactone stereoisomers was achieved. The application of this method to the scent of living, white flowering orchids (*Aerangis confusa*) proves *cis* -(4S)-methyl-(5S)-decanolide as the unique and genuine stereoisomer of *Aerangis* lactone.

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Recent Developments in the Authenticity Control of Essential Oils

Birgit Faber, Claudia Frank, Armin Mosandl, Institut für Lebensmittelchemie J.W. Goethe Universität Frankfurt, Marie-Curie-Str. 9, 60439 Frankfurt, Germany

Enantioselective capillary gas chromatography as well as isotope ratio mass spectrometry coupled on-line with capillary gas chromatography (GC-IRMS) are efficient methods in the origin assignment of flavor and fragrances. The application of both methods is demonstrated on peppermint oil (1,3) and coriander (2) oil.

Some chiral compounds are analyzed by enantio-GC-IRMS, a combination of both methods.

The essential oils of authentic samples of different origin are compared with commercially available oils and spices.

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Chiral compounds of essential oils: XX
Elution order of the furanoid linalol oxides on common gas chromatographic phases and modified cyclodextrin phases

P. Kreis, A. Dietrich and A. Mosandl
Institut für Lebensmittelchemie, Johann Wolfgang Goethe-Universität
Marie-Curie-Str. 9
D-60439 Frankfurt/M, Germany

Keywords:

Elution order and Kovats Indices of *cis*-linalol oxides and *trans*-linalol oxides on SPB-1, Supelcowax-10, SE-52
Gas chromatographic resolution and elution order of linalol oxide stereoisomers

Summary

The furanoid *trans*- and *cis*-linalol oxides are detectable in many essential oils, which contain linalol in considerable amounts.
But unfortunately, the gas chromatographic elution order of *trans*- and *cis*-linalol oxides is incorrectly given in the literature up to now.
This investigation reports the corrected elution order of the diastereomeric linalol oxides using common nonchiral gas chromatographic phases.
Furthermore the separation and elution order of all four linalol oxide stereoisomers on differently modified ß-cyclodextrin phases is presented.

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CLASSIFICATION OF SIXTY STRAWBERRY GENOTYPES BASED ON THEIR ESSENTIAL OIL COMPOSITION AND DEGREE OF MITE SUSCEPTIBILITY

A. Bélanger[1] and S. Khanidzadeh[1]

1 Centre de R & D en horticulture, Agriculture and Agroalimentaire Canada, St-Jean-sur-Richelieu, Québec, Canada J3B 3E6

We have compared the natural concentration of folair essential oils in more than sixty strawberry cultivar with different degree of susceptibility toward TSSM. Two-spotted spider mite (TSSM) *Tetranychus urticae* Koch. is one of the most important strawberry pest. The essential oil compositions ware analyzed by capillary GC and identified by GC/MS.

'Chandler', 'Fern' and 'Lester' which were highly susceptible and intermediate to susceptible had lower linalool, α-terpineol, ß-cyclocitral composition than 'Kent', Honeoye' land 'Bounty' which were classified as resistant or intermediate to resistant cultivars. Susceptible in their essential oil than resistant cultivars ('Kent', 'Honeoye' and 'Bounty'). Arking, Cruz, Chambly, Glooscap, Jewel, Midway, Oka, Pandora and Robinson are among the cultivars studied.

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SOME APPLICATIONS OF CYCLODEXTRIN DERIVATIVES IN GC ENANTIOSEPARATIONS OF ESSENTIAL OILS

C. Bicchi, Angela D'Amato, Valeria Manzin

Dipartimento di Scienza e Tecnologia del Farmaco - Università di Torino - Via Pietro Giuria 9 - I-10125 Torino

Cyclodextrin derivatives (CDDs) are now well established stationary phases for GC enantioseparation of volatile racemates. Biosynthetic and geographical origins, technological treatments and/or genuineness of most of the essential oils can now be evaluated through the enantiomeric composition of their optically active components without derivatizing them. Although a CDD of general use has not yet been found, the most recent derivatives are able to separate increasingly high numbers of racemates. Generally, the first CDDs for use as GC stationary phases were applied to the analysis of a single racemate characterising one or, more often, several essential oils. With the "last generation" CDDs, in many cases, an essential oil can be evaluated through several of its optically active components in a single GC run. This affords crossed and complementary comparisons between the enantiomeric component abundances giving, as a consequence, a more reliable determination of origin and genuineness of the essential oil under investigation. Some applications are discussed in this light.

PRELIMINARY RESULTS IN THE STUDY OF THE COMPOSITION OF *ARTEMISIA ROXBURGHIANA* ESSENTIAL OIL

Carlo Bicchi, Monica Fresia, Patriczia Rubiolo and Ombretta Panero

Dipartimento di Scienza e Tecnologia del Farmaco - Università di Torino - Via Pietro Giuria 9 - I-10125 Torino

Artemisia roxburghiana Besser is a Compositae growing wild in Nepal. The oil was obtained by hydrodistillation of the aerial parts and was analyzed by GC with two different stationary phases (OV-1 and CW-20M) and GC/MS with different ionization techniques, both as such and after fractionation. Fractionation was carried out both by distillation under vacuum and by flash-chromatography on silica with solvents of different polarities.

Thirty-nine compounds have been identified; the principal ones are 1,8-cineole (17.4%), α-thujone (9.9%) and camphor (15.9%).

VARIABILITY OF THE ESSENTIAL OILS OF *TAGETES lucida CAV.* FROM GUATEMALA

I. Goehler[1], Ch. Franz[1], P. Rubiolo[2] and C. Bicchi[2]

[1] Institut für Botanik und Lebensmittelkunde
der Veterinärmedizinischen Universität Wien
Linke Bahngasse 11, A-1030 Wien, Austria

[2] Dipartimento di Scienza e Tecnologia del Farmaco, Univ. degli Studi di Torino
Via P. Giuria 9, I-10125 Torino, Italy

Pericón (*Tagetes lucida* CAV., Asteraceae) is an endemic species of Centroamerica, in large amount collected from the wild and used as medicinal plant especially for herbal teas. The essential oil consists of more than thirty already identified compounds (BICCHI et al. 1994), characterized mainly by estragole, methyleugenol and anethole.

Sixteen distribution areas of this species had been identified in Guatemala, all of them located in the „Altiplano" region between 1.300 and 2.600 m a.s.l. Within and especially between the populations a large variation in the composition of the essential oils (concentration of the main compounds) could be observed. Mixed types but also monosubstance individuals were detected, and one accession has shown a divergent oil composition consisting mainly of sesquiterpenoids.

The data up to now obtained offer the possibility to select specific lines according to chemotype and intended use.

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Regenerated Plants of *Melissa officinalis* L. (Lamiaceae): Influence of Selected Phytohormones on Growth and Essential Oil Accumulation.

G. Binder, T. van den Berg, A. Abou-Mandour, F.-C. Czygan

Department of Pharmaceutical Biology, University of Würzburg,
Mittlerer Dallenbergweg 64, 97082 Würzburg, Germany.

Lemon balm (*Melissa officinalis* L., Lamiaceae) has been regenerated from callus cultures (1) and cultivated under in-vitro conditions. The influence of various phytohormones (IBA, BAP, NAA and others) on morphology and essential oil accumulation was studied.

Growth and morphology were significantly affected by NAA (enhanced root formation, light green leaves) and BAP (suppressed root formation, enhanced shoot formation with suppression of shoot elongation). Despite even drastic morphological changes the treated plants accumulate an essential leaf oil typical for in-vitro cultured *Melissa officinalis* (1) with only minor changes.

In general the total amount of essential oil was not affected by the applied phytohormones whereas the formation of single compounds was enhanced or reduced with statistical significance. Changes in relative amounts of single substances (i.e. geraniol) were especially pronounced for BAP.

Special interest deserves that the effects of BAP on morphology and essential oil composition can be reduced or eliminated by addition of appropriate amounts of IBA.

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BAP: 6-Benzylaminopurin, NAA: (a)-1-Naphtylacetic acid, IBA: Indole-3-butyric acid

The Essential Oil Composition of Natural and Cultivated *Achillea collina* L. (Asteraceae) during Ontogeny

M. Orth and F.-C. Czygan

Department of Pharmaceutical Biology, University of Würzburg,
Mittlerer Dallenbergweg 64, 97082 Würzburg, Germany.

The essential oil of *Achillea collina* L. was investigated to compare the qualitative and quantitative composition during ontogeny and under different growth conditions. The essential oils were obtained from plants growing under natural conditions (Guttenberg Forest near Würzburg) and those cultivated in the Botanical Garden of Würzburg but originated from the a.m. population.

GC and GC-MS analyses yielded great amounts of sesquiterpene hydrocarbons, including the guaiane derived chamazulene, formed from sesquiterpene lactones during hydrodistillation, and lower proportions of monoterpenes (as shown by 1). The main components of sesquiterpenes were germacrene D, caryophyllene and chamazulene.

The qualitative composition of the identified sesquiterpenes from the cultivated population is corresponding to the natural population. In contrast the composition of monoterpene hydrocarbons (β-pinene and sabinene) differs between these populations. Moreover *Achillea* plants growing under controlled conditions (humidity and fertilizing) yielded lower amounts of essential oil.

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THE ESSENTIAL OIL COMPOSITION OF FRUITS FROM *SMYRNIUM GALATICUM* CZECHOTT (APIACEAE)

K.-H. Kubeczka, Ulrike Mölleken, and W. Schultze

Department of Pharmaceutical Biology, University of Hamburg,
Bundesstraße 43, D-20146 Hamburg, Germany

Smyrnium galaticum is a biennial umbellifer growing in central Turkey, mentioned in "Flora of Turkey" as a "species imperfectly known" (1). In the course of our investigations of essential oils from different *Smyrnium* species, the fruit oil of *S. galaticum*, obtained by hydrodistillation, has been analyzed by means of GC, GC-MS, IR and NMR.

The essential oil of *S. galaticum* is characterized by a high content of oxygenated compounds (87%) with a remarkable proportion of furanosesquiterpenoids (82%). Furanodiene and its Cope rearrangement product isofuranogermacrene are the main constituents (together 69%).

Additionally a new furanosesquiterpene has been isolated (9%). Structural elucidation by means of ^{13}C and ^{1}H NMR combined with IR and MS led to the structure of furanoelema-1,3-diene with an ether linkage between C-2 and C-3. Comparison of the spectral data with published data of smyrnicordiolide, which has been found in different *Smyrnium* species (1-4), suggested the new component to be the furan analog of smyrnicordiolide. It is therefore named **smyrnicordifuran**.

The fraction of hydrocarbons (13%) is dominated by sesquiterpenes (11%), above all germacrene-d (6%), germacrene-b (1%) and β-elemene (1%).

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THE ESSENTIAL OIL OF *HAPLOPHYLLUM LINIFOLIUM* (RUTACEAE)

K.-H. Kubeczka[1], W. Schultze[1], P. Torres[2], and M. Grande[3]

[1] Department of Pharmaceutical Biology, University of Hamburg, Institute of Pharmacy, Bundesstrasse 43, 20146 Hamburg, Germany

[2] Instituto La Torreta, c/Eduardo Ferrández s/n, 03203 Elche, Alicante, Spain

[3] Departamento de Química Orgánica, Universidad de Salamanca, plaza de los caídos 1, 37008 Salamanca, Spain

The Rutaceous shrub *Haplophyllum linifolium* (L.) G. Do. (*H. hispanicum* (L.) Spach = *Ruta linifolia* L. var. *rosmarinifolia*) is an Iberian endemism rich in essential oil which has been used in folk medicine for treatment of vitiligo and leucodermia (1).

Previous articles on the secondary metabolites of this plant reported the isolation of several coumarins (2) and cytostatic lignans (3) but we have not found any reference concerning the essential oil. In this communication we report upon the essential oil composition of the fruits and aerial parts of *H. linifolium* collected near La Mata (Alicante, Spain).

The essential oils from the fruits and the aerial parts of *H. linifolium* were isolated by hydrodistillation, fractionated according to our standard procedure (4), and analyzed by GC/MS. Main components of both oils are monoterpene hydrocarbons with ß-phellandrene (25.5% and 42.6%, respectively) and ß-pinene (23.7%; 19.8%) as dominating constituents. The more interesting characteristic is the presence of relatively high amounts of bornyl formiate, a compound rarely found in essential oils. Both oils exhibit a very similar qualitative composition differing only in the percentual portions of the individual components.

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INVESTIGATION OF THE COMPOSITION OF TEA INFUSIONS FROM MEDICINAL PLANT DRUGS CONTAINING ESSENTIAL OILS

K.-H. Kubeczka[1] and B. Frank[2]

[1] Department of Pharmaceutical Biology, University of Hamburg, Bundesstrasse 43, D-20146 Hamburg, FRG

[2] Kneipp Werke Würzburg, Am Grundbach 5, D-97271 Kleinrinderfeld, FRG

Investigations concerning the composition of crude plant drugs, which are used to prepare medicinal tea infusions, are often found in the literature, whereas analyses dealing with the compositions of tea infusions are relatively rare. Therefore, we wish to report on the composition of tea infusions from medicinal plant drugs containing essential oils. For our experiments we have selected commercial tea-bag preparations of peppermint (Fol. Menthae piperitae), sage (Fol. Salviae), and fennel (Fruct. Foeniculi).

After preparation of the respective tea infusions, the lipophilic volatile constituents were separated and concentrated by solid phase extraction. The tea infusions were pulled through BondElut LRC C18 columns (Varian 1211-3027) using a vacuum pump. Elution of the absorbed volatiles was performed with n-pentane. This solution (2 ml) was concentrated to 100 μl under nitrogen and reduced pressure, yielding the purified sample for gc analysis.

The gas chromatogram of the volatiles from the tea infusion exhibited numerous constituents that can also be found in the essential oil from peppermint leaves obtained by conventional hydrodistillation. However, in contrast to the distillation product, the tea infusion volatiles consisted nearly exclusively of oxygenated compounds, whereas hydrocarbons, representing xx,x% of the hydrodistillation product, were lacking. The absence of hydrocarbons in the tea preparation may be due to their low solubility in water. Therefore, it may be assumed that the composition of tea infusions from medicinal plant drugs containing essential oils is determined by the partition coefficients of the individual oil constituents.

In order to confirm this presumption, commercial peppermint oil was shaken with water according the prescription of the pharmacopoea for preparing aromatic waters. After separation of the aqueous layer, the obtained oil-saturated aqueous solution was submitted to hydrodistillation. GC analysis of the respective essential oil revealed that, comparable to the tea infusion volatiles, only oxygenated oil constituents are found in the aqueous solution of the aromatic water.

In summary, the composition of the volatiles of a tea infusion from medicinal plant drugs containing essential oil can be considered a result of a partitioning process of the individual oil constituents. Therefore, the qualitative and quantitative composition of the volatiles from a tea infusion is in good agreement with those from the respective aromatic water. These observations are confirmed by further investigations with sage and fennel, which yielded comparable results.

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COMPARISON OF TWO CGC CARBOWAX COLUMNS

C. Blum, W. Schultze and K.-H. Kubeczka

Department of Pharmaceutical Biology, University of Hamburg,
Bundesstraße 43, D-20146 Hamburg, Germany

Polyethylene glycol is a very important stationary phase in the field of GC of essential oils, perfumes and similar multicomponent mixtures.

A widely used type of such columns is DB-Wax from J&W Scientific, which is characterized by a long life time. However, a disadvantage is the relatively low final temperature, which to our experience should not exceed 220-230°C in essential oil analysis.

In contrast, Supelco offers a similar column (Supelcowax-10) for which an upper temperature limit of 280°C is specified.

	DB-Wax	Supelcowax-10
phase (bonded)	poly ethylene glycol	poly ethylene glycol
column length, film thickness	30 m, 0.25 µm	30 m, 0.25 µm
temperature limits	20-250°C	35-280°C
theor. plates/meter	4275	4287
program	45°C, 3°C/min, 220°C, isotherm	45°C, 3°C/min, 260°C, isotherm
Flow, split	1 ml/min N_2, 1:15	1 ml/min N_2, 1:15

A comparison of these two columns using mixtures of alkanes, fatty acids as well as nutmeg and marjoram essential oils gave the following results:

- Elution order of the individual compounds often remains unchanged. Separating power is similar with some differences, e.g. the monoterpenes α-pinene and α-thujene could better be separated on Supelcowax-10, whereas the separation of myrcene and α-phellandrene as well as of cis-sabinene hydrate and linalol could only be performed on DB-Wax (at this temp. program).

- The broader range of temperature programming and the higher final temperature of the Supelco column resulted in clearly shorter retention times of late eluting compounds (e.g. hexatriacontane (C_{36}): 180 min / 102 min, not methylated behenic acid (C_{22}): - / 99 min).

- Retention index calculation in program mode is possible up to pentacosane (C_{25}) on DB-Wax and triacontane (C_{30}) on Supelcowax-10, respectively.

To summarize, Supelcowax-10 column seems to be more suited for essential oils analysis when components of high retention times occur. Moreover, it is advantageous in case of a dual column system with an apolar column (with high final temperature) parallel.

THE ESSENTIAL OIL AND VALEPOTRIATES FROM ROOTS OF *VALERIANA JAVANICA* BL. GROWN IN INDONESIA

R. Bos[1], H. J. Woerdenbag[1], H. Hendriks[1], Sidik[2], H. V. Wikström[3] and J. J. C. Scheffer[4]

[1] Department of Pharmaceutical Biology, University Centre for Pharmacy, Groningen Institute for Drug Studies (GIDS), University of Groningen, Ant. Deusinglaan 2, 9713 AW Groningen, The Netherlands

[2] Laboratory of Pharmacognosy, Department of Pharmacy, Padjadjaran University, P.O. Box 6571, Cihapit, Bangdung 40114 A, Indonesia

[3] Department of Medicinal Chemistry, University Centre for Pharmacie, Groningen Institute for Drug Studies (GIDS), University of Groningen, Ant. Deusinglaan 2, 9713 AW Groningen, The Netherlands

[4] Division of Pharmacognosy, Leiden/Amsterdam Center for Drug Research (LACDR), Leiden University, Gorlaeus Loboratories, P.O. Box 9502, 2300 RA Leiden, The Netherlands

In Indonesia, the roots of *Valeriana javanica* Blume (synonym: *V. hardwickii* Wall. (1); local name: Akar Valerian) (Valerianaceae) are used as a mild tranquilizer (2). This is the same indication as for *V. officinalis* L. s.l., *V. wallichii* DC. and *V. edulis* Nutt. ssp. *procera* F.G. Meyer in Western phytotherapy. In addition, the roots of *V. javanica* are traditionally used in Indonesia as insect repellant and as "devil repellant", after birth (2). So far, no phytochemical data are available on this species.

The essential oil from *V. javanica* roots originating from Tawangmangu, Solo, was investigated by GC and GC-MS. The roots contained 0.36% (V/w) essential oil on a dry weight (DW) basis. Main constituents of the oil were α-kessyl acetate (36.2%), α-cedrene (14.8), patchouli alcohol (10.3%), kessane (6.8%), valeranone 3.2%), and bornyl acetate (2.6%).

Root extracts were analysed for valepotriates and valerenic acid derivatives by HPLC. The valepotriates included valtrate (2.24%), isovaltrate (0.60%) and acevaltrate (0.11%). Valerenic acid derivatives were not detected. From a phytochemical point of view, *V. javanica* does not resemble with the *Valeriana* species used in Western countries. However, some similarity exists with the Japanese *V. fauriei* Briq..

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THE ESSENTIAL OIL OF *TANACETUM PARTHENIUM* (L.) Schultz-Bib.

H. Hendriks, R. Bos and H.J. Woerdenbag

Department of Pharmaceutical Biology, University Centre for Pharmacy, Groningen Institute for Drug Studies (GIDS), Antonius Deusinglaan 2, 9713 AW Groningen, The Netherlands.

The composition of the essential oil of *Tanacetum parthenium* (L.) Schultz-Bip. (feverfew; Asteraceae) of various origins was investigated using GC and GC-MS. Camphor and chrysanthenyl acetate were the main costituents of samples originating from Belgium, Egypt, England and the Netherlands. No infraspecific variation in the composition of the oil was found.

Furthermore the essential oil content and composition of Dutch feverfew during a vegetation period was studied. The young herb, before the formation of the stems, had a relatively high percentage of essential oil (0.53% V/m), calculated on the dry weight. After a sharp decline at the beginning of the formation of the stems (0.30% V/m), the percentage of the essential oil increased until full bloom (0.83% V/m).

During the development of the plant the percentage of camphor rose from 28.0 to 47.5%, whereas the amount of chrysanthenyl acetate decreased from 30.2 to 22.1%.

In none of the essential oils investigated, the toxic monoterpenes α- or β-thujone could be detected. This is an important fact, because feverfew is used over longer periods of time as a migraine prophylactic agent. The sesquiterpene lactone parthenolide is held responsible for this biological activity. In addition, chrysanthenyl acetate may display an analgetic effect by inhibiting the enzyme prostaglandin synthetase.

Based on the results of our investigations and on literature data, a number of recommandations are proposed with respect to the essential oil of *T. parthenium*.

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AMMIMAJANES - NOVEL VOLATILE DITERPENES FROM *AMMI MAJUS* L.

W.-R. Abraham[1], C. Löwenstein[2], H.-P. Hanssen[3], V. Sinnwell[4] and E. Stahl-Biskup[2]

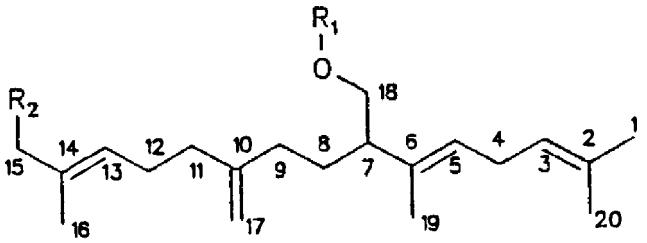
1 GBF - Gesellschaft für Biotechnologische Forschung mbH, Dept. Microbiology, Mascheroder Weg 1, D-38124 Braunschweig, Germany

2 University of Hamburg, Dept. Pharmaceutical Biology, Bundesstrasse 43, D-20146 Hamburg, Germany

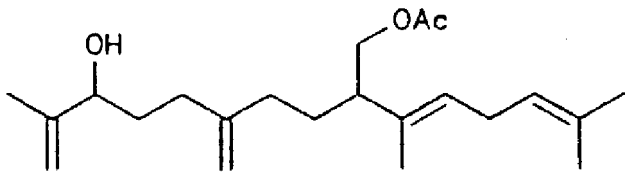
3 IMB - Industrielle Mikrobiologie, Lurup 4, D-22927 Grosshansdorf, Germany

4 University of Hamburg, Institute of Organic Chemistry, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

Seven diterpenes **1-7** were isolated from the higher boiling fraction of the essential oil from the aerial parts of *Ammi majus* L. (Apiaceae). Their structure proved to base on a novel irregular acyclic hydrocarbon skeleton which was named ammimajane. Structure elucidation was accomplished by ^{1}H NMR, ^{13}C NMR, IR, and MS. Aside from the ammimajanes phytol, isophytol and isoelemicin were identified.



	R_1	R_2
1	Ac	H
2	Ac	OAc
3	H	H
5	H	OAc
6	Ac	OH



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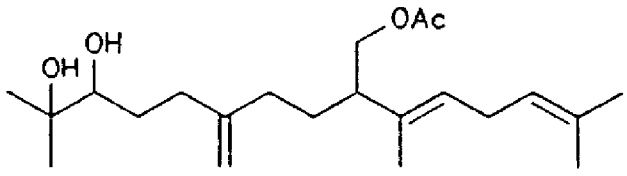

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GC ANALYSIS OF ESSENTIAL OILS AND THEIR MIXTURES IN THE DRUG CONTROL

I. Buben[1] and I. Kvetkova[2]

1 State Institute for Durg Control, Srobarova 48, 10041 Prague 10, Czech Republic

2 Faculty of Pharmacy, Charles University, Heyrovskeho 1203, 50165 Hradec Kralove, Czech Republic

There are only two Pharmacopoeias which include, on large scale analyses of essential oils (AB-DDR 2, from the year 1975 and Ph. It. 1991). In order to gain more information about the chromatographic behaviour of various kinds of essential oils and of their mixtures used in pharmaceutical preparations, we studied 18 different essential oils with eight chromatographic columns of various diameters and polarity.

It is apparent that for the proper evaluation of results the correct approach must be taken. If an exhaustive information about all the components is needed (even minorite or spots), a long capillary column with a small diameter (usually 0.20 - 0.32 mm) must be used. Generally, as we demonstrate in the analysis of essential oils and their mixtures presented in this study, the application of wide-bore columns is of an advantage. This type of columns shows an effeciency about 10-times better than the one seen in packed columns, and about 5-times worse than the one obtained with conventional capillary columns. Taking the economical point of view into account, which is currently of particular importance in the Czech Republic, it is possible to use wide-bore capillary columns with the simple older types of gas chromatographs. The separation ability of these columns satisfies most of the demands encountered in our profession (e.g. analysis of the main components of essential oils or their mixtures). The validation of analytical methods is a useful way of evaluating the suitability of a given chromatographic system for specific task. This selection is illustrated by the means of pictures, graphs and tables.

TCD: A NEW METHOD IN AROMATHERAPY RESEARCH

B. Našel[1], Ch. Našel[2], P. Samec[3] and G. Buchbauer[1]

[1] Institute of Pharmaceutical Chemistry, University of Vienna, Centre of Pharmacy, A-1090 Vienna, Althanstrasse 14, Austria

[2] Department of Radiology, Division Neuroradiology, University of Vienna, AKH-Wien, A-1090 Vienna, Währingergürtel 18-20, Austria

[3] Institute of Radiology, Neurological Hospital Rosenhügel, A-1130 Vienna, Riedelgasse 5 Austria

In continuation of our studies on physiological effects of fragrance compounds and essential oils [1-4] we introduce a new method in aromatherapy research: *Trans craniell doppler* sonography (TCD) [5] proved to be a very valuable tool to gain more knowledge about the alterations of cerebral blood flow (CBF) caused by the inhalation of the camphoraceous smelling compound 1,8-cineol, also known as eucalyptol. After an adequate period for adaption to the situation human volunteers inhaled this fragrance - given via a breathing system - for 20 minutes. After removing it, TCD was performed for another 30 minutes to evaluate alterations of blood flow velocity in the exhalation period. Carefully interpretation of the results of these experiments showed that the reduction of blood flow velocity amplitude can correlate with reactive dilatation of vessel diameter, which can lead to an increase of the CBF as well. These results seem to be in accordance to earlier CBF-studies obtained by Xe-CT [3, 4].

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SYNTHESIS OF THE ENANTIOMERES OF MADROL®

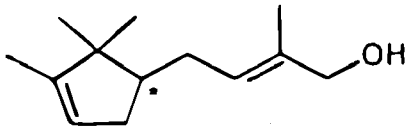
Ph. Lebada[a], L. Wiesinger[a], G. Buchbauer[a] and P. Wolschann[b]

[a] Institute of Pharmaceutical Chemistry, University of Vienna, A-1090 Vienna, Austria
[b] Institute of Theoretical Chemistry, University of Vienna, A-1090 Vienna, Austria

East Indian Sandelwood oil is an important essential oil which is widely used in fragrance compositions, on account of its warm and woody odour. Among the group of synthetic sandelwood chemicals which were created to satisfy the world's demand for this odour we will find also the champholenyl derivates [1,2], like Madrol® (formerly Madranol®), Brahmanol®, Sandacore® and many others.

Madrol® is described to exert a powerful Sandelwood odour with sweet note. Madrol®, 2-Methyl-4-[2',2',3'-trimethyl-cyclopenten-(3')-yl-(1')-]-but-2-enol (figure 1), possesses only one asymmetric carbon atom at C 1'. In connection with our studies about structure-activity-relationships of fragrance compounds with Sandelwood odour [3,4] it seemed to be very interesting to know which enantiomere shows this precious odour.
Because of difficulties to separate the antipodes via their diastereomeres we decided in favour of an asymmetric synthesis using (+)/(-)-α-pinene as starting material. Thus in four steps the desired enantiomeres could be obtained [5,6] and only the (-)-Madrol® showed the desired odour properties.

figure 1



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CORRELATIONS of ODOR ATTRIBUTES for COMPLEX MIXTURES

A.Nikiforov[1], G.Buchbauer[2], L.Jirovetz[2], Barbara Remberg[2] and S.Körnig[3]

[1] Inst. Org. Chem., Univ.Vienna, Währingerstr.38, 1090 Vienna, Austria, Fax: 011-43-1--31367-2280, e-mail: Niki@Felix.orc.univie.ac.at.
[2] Inst. of Pharmaceutical Chemistry, Univ.Vienna, Althanstr. 14, 1090 Vienna, Austria,
[3] Chemical Concepts GmbH, Boschstr.10, Weinheim, Germany

The odor attributes of complex odor systems, which exhibit distinct effects in medical and aroma applications (aromatherapy, perfumery), could not be predicted until today. Thus it was the aim of this work to investigate, whether there is a correlation between the concentration displacement of the volatiles and the odor attributes of such complex systems.

In the first part of this work, the selection of appropriate odor systems, the preparation of olfactorically representative samples and the instrumental analysis were performed. The dynamic headspace method was used for the preparation of 25 samples of the following odor systems: flowers: apple, grape, Exacum affine, different plant parts of Aesculus hippocastanum, leaves of Plectranthus coleoides, different mushrooms, sunflower stems, peels of Juglans nigra Chenopodium botrys plant, SCF-extract of shellac, needles and twigs of Douglas Fir, as well as old books and fragrance candles with different notes. Sensoric evaluation was performed by professional perfumers who classified the samples in terms of "flowery", "fruity", "fresh", "green" and "mushroom-like" odor notes. Investigation by means of GC-FID, GC-FTIR-MS and GC-Sniffing technique allowed the identification of more than 350 different components in total, mostly terpenes, ketones, lactones and esters.

In the second part, the structures of 61 796 components of NIST/EPA/NIH mass spectra library(R) as MOL-files were used in the commercially availably structure and sub-structure based "ISIS/BASE (R)" of MDL Information Systems for the the selection of mass spectra in the development of classifiers for semiautomatic preselection of groups of target compounds in instrumental data (GC-MS) of the odor systems investigated. The used substructure based form of NIS/EPA/NIH library proved also to be a very effective tool in a variety of further applications (structure pool etc.).

In the third part of this work, multivariate evaluations, via PCA (projection in plane of the 1st and 2nd eigenvector), of 350 selected components, which were identified in investigated odor samples, based on a 350 x 25 (volatiles identified x odor systems) data matrix, were performed in order to find out if the displacement of the components concentrations of the samples correlates with their odor notes, as defined by professional perfumers. As a result of this, it became clear that the odor notes "flowery", "fruity", "fresh", "green" and "mushroom-like" may be correlated with a certain distribution of terpenes, alcohols, aldehydes and ketones, but not with the group of terpenes alone. These results indicate the principal possibility of odor notes prediction based on instrumental data via multivariate analysis of the volatiles displacement in complex mixtures.

Acknowledgement: The support of the „**Hochschuljubiläumstiftung der Stadt Wien**" **(Project H-78/94)** is gratefully acknowledged.

INFLUENCE OF BLOSSOMING DEGREE OF LAVENDER ON THE ESSENTIAL OIL YIELD AND QUALITY

Evg.V. Georgiev[1] and A.D. Balinova-Tzvetkova[2]

[1] Higher Institute of Food and Flavour Industry, Dept. of Fatty and Essential Oils Technology, 26 Maritza Boul., 4002-Plovdiv, Bulgaria

[2] Institute for Roses, Aromatic and Medicinal Plants, 6100-Kazanlik, Bulgaria

The influence of the stage of lavender blossoming degree on the changes of essential oil during 4 vegetation periods has been determined. The greatest content of essential oil has been established under 50-80 % of degree of blossoming. The essential oil quantity in one inflorescence has been greatest during the same period, as well. After that it has decreased simultaneously with mass of inflorenscence decrease. Because of that for obtaining 1 kg of lavender oil from completely blossomed and from faded lavender inflorescences more raw material, respectively lavender plantations are necessary.

There are no any substantial quality indices changes of essential oils during the period of maximum essential oil content. Then they possess also the highest smell estimation.

ESSENTIAL OIL *LABIATAE* IN BULGARIA

J. Cherneva[1] and A. Balinova[2]

[1] Institute of Botany BAS, Sofia, Bulgaria

[2] NIRELK - Kazanlik, Bulgaria

A brief review on the distribution and the investigations on the plants of *Labiatae* (*Lamiaceae*) in Bulgaria is represented. The list of the cultivated essential oil plants and investigated genera and species are given. Traditional crops (plants) are *Lavandula*, *Mentha*, *Salvia*. Cultivars from *Lavandula*, *Melissa*, *Mentha*, *Salvia*, *Thymus* are created in Bulgaria.

Labiatae family in Bulgaria includes 31 genera and 145 species. Most of them are used as remedies in the popular medicine and in the culinary.

The biggest genera are: *Stachys* - 22 species, *Thymus* - 19, *Salvia* - 18 and *Mentha* 6, *Satureja* - 5, *Sideritis* - 4, *Nepeta* - 4, but *Hyssopus*, *Melissa*, *Origanum* - 1 species.

The essential oils of *Hyssopus*, *Melissa*, *Origanum* are discussed.

IMPROVED GAS CHROMATGRAPHIC SEPARATION OF CHIRAL COMPOUNDS OF INTEREST IN FLAVOUR AND FRAGRANCE APPLICATIONS

S. Claude[1], R. Tabacchi[1], A. Saxer[2] and D. Joulain[3]

[1] Institut de Chimie, Université de Neuchatel, avenue de Bellevaux 51, CH-2000 Neuchatel, Switzerland

[2] Institut für Organische Chemie der Universität Bern, Freiestrasse 3, C-3012 Bern, Switzerland

[3] Reserach Division, Robertet S.A., B.P. 100, 06333 Grasse, France

Today, the natural irone isomers are obtained industrially from *Iris pallida* or *Iris germanica* rhizomes by either traditional treatment (aging, followed by steam distillatin), or biotechnology processing. Whatever the origin of the plant material and the type of processing, this natural product ist always a mixture of several geometric isomers with different enantiomeric compositions. Therefore, an appropriate analytical method is very desirable to ensure quality control of this precious raw material. For this purpose, we have developed a chiral column based on substituted ß-Cyclodextrin which allows for the first time the total gas chromatographic separation of all natural irone stereoisomers (*cis*- and *trans*-α-irone, *cis*- and *trans*-γ-irone and ß-irone) in a single run within less than two hours.

The effectiveness of this material has been a successfully tested on other natural aroma compounds. Some results will be presented.

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ON THE "LAWANG" ESSENTIAL OILS

D. Joulain[1], R. Laurent[1]

[1] Robertet S.A., Research Division, B.P. 100, 06333 Grasse, France

The essential oil of Lawang has been known for a long time as originating from bark of various *Cinnamomum* species.

We could examine commercial essential oils of Lawang, distilled in Irian Jaya (Indonesia) under out control, from bark not fully identified, and essential oils distilled in our laboratory from a single bark type.

We confirm that the variation in the eugenol contents in the oils is related to the lack of definition of the starting raw material.

We report in detail on the composition of these oils.

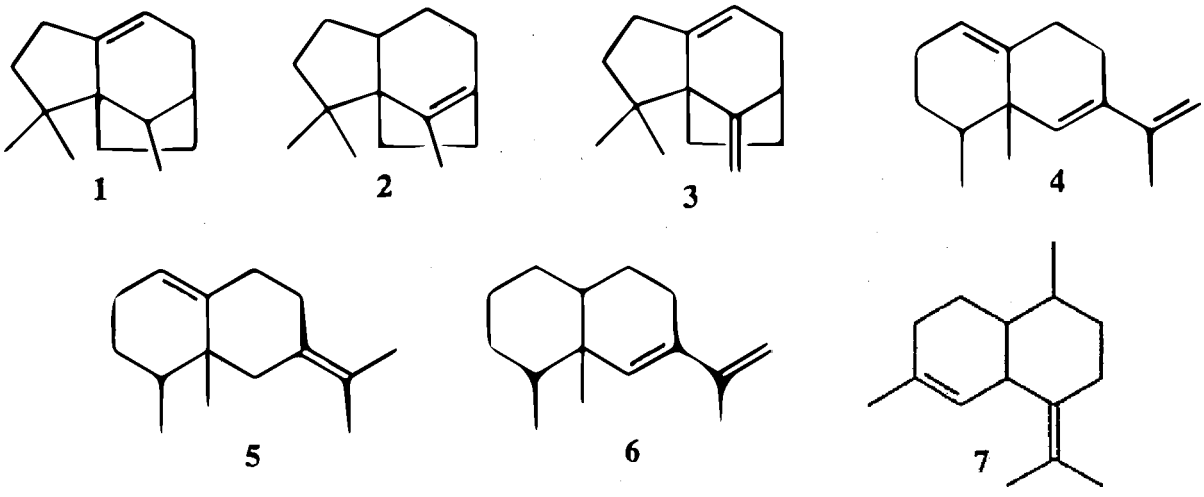
NEW SESQUITERPENE COMPONENTS FROM VETIVER OIL

S. Jung, W.A. König*

Department of Organic Chemistry, University of Hamburg
Martin-Luther-King-Platz 13, D-20146 Hamburg, Germany

The main components of vetiver oil, which is prepared from the roots of Vetiveria zizanioides by steam distillation, are sesquiterpenes. Smadja reported 102, partly not identified, sesquiterpene components in vetiver oil. These are hydrocarbons, alcohols, acids, epoxides, aldehydes and ketones of cadinane, eudesmane, valencane, elemane, spirovetivane and other skeletons.The goal of this work was to isolate some of the already known sesquiterpenes for use as chiral reference compounds and to isolate and identify new components from vetiver oil.

Because of the complexity of vetiver oil we had to perform the separation in several steps. At first we used a simple silica gel column with changing solvent mixtures to separate the oil into several fractions. This immediately showed that the vetiver oil is much more complex than presumed. Only a few main components were obtainable from the hydrocarbon fractions via preparative gas chromatography. An additional silica gel column was used, now with pentane cooled at approx. 240K. In this way we now achieved a separation into fifteen instead of five fractions. From these fractions, several new sesquiterpenes were isolated via preparative gas chromatography using various stationary phases. Although the isolated compounds were not completely pure, the investigation by one and two dimensional NMR experiments was feasible.The following structures were assigned:



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IDENTIFICATION OF (+)-β–CARYOPHYLLENE IN ESSENTIAL OILS OF LIVERWORTS BY ENANTIOSELECTIVE GAS CHROMATOGRAPHY

C. Fricke, A. Rieck, I.H. Hardt, W.A. König

Institute of Organic Chemistry, University of Hamburg,
Martin-Luther-King-Platz 6, 20146 Hamburg, Germany

The enantiomeric composition of β–caryophyllene in several liverworts and higher plants was investigated by comparison with a synthetic standard of racemic β–caryophyllene (1). The synthesis of the racemic standard proceeded according to a procedure of J.M. Greenwood et al. (2).
A combination of capillary columns with conventional (here a nonpolar CpSil 5) and chiral stationary phase in a double oven gas chromatograph (2D-GC) was employed for the investigation of essential oils (3). As chiral stationary phase heptakis(2,6-di-O-methyl-3-O-pentyl)-β–cyclodextrin was used (4).
The investigation by two-dimensional gas chromatography revealed the presence of (+)-β–caryophyllene in several essential oils.
The unusual (+)-enantiomer was found in different amounts in the essential oils of the liverworts *Pellia endiviifolia*, *Pellia epiphylla* and *Metzgeria conjugata*.
The enantiomeric composition of β–caryophyllene of a given liverwort also depends on the site of collection. In *Pellia endiviifolia*, collected in Trittau near Hamburg, we found the largest proportion (65% ee) of the (+)-enantiomer.
Remarkable is also the occurrence of (+)-β–caryophyllene in the essential oil of *Cedrela odorata*, a Brazilian tree (*Meliacee*).

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NEW CHEMOTYPES OF THE LIVERWORT *CONOCEPHALUM CONICUM*

S.Melching and W.A. König

Institute of Organic Chemistry, University of Hamburg,
Martin-Luther-King-Platz 6, 20146 Hamburg, Germany

Sesquiterpenes are an interesting group of natural compounds with a variety of biological and pharmacological activities. They are found in higher plants and also in bryophytes, here first of all in liverworts (1). By examination of several samples of the liverwort *Conocephalum conicum*, that were collected in different locations in Europe and the US, we found distinct differences of the constituents of the distillates. After distillation and analysis of about 12 samples of phenotypically identical species of *Conocephalum conicum*, we were able to classify the samples into 3 types. Type I is the most prevailing type; 7 of 13 examined samples were of type I, which was found all over Germany and in the US. Type II was only found in Northern Germany and type III was found in Southern Germany and in Wales. These latter two types are more seldom. No relationship between the type and the season of collection was found. The three types seem to be really different chemotypes.

Analysis of the steam distillates of *Conocephalum conicum*, type I, revealed mainly cadinanes, as it is described in earlier literature (2). In all cases the main component was (+)-cubebol. Beside well-known compounds, like δ–cadinene, β–cubebene, β–elemene, we found bicyclosesquiphellandrene in the steam distillates of type I. All analyzed sesquiterpenes were found to be the unusual enantiomeres, as it is described in several publications (3). The number of constituents in type II is lower. We found (+)-aristolene, (-)-calarene and dimethoxystyrene, that was not yet described as a component of an essential oil. Very interesting is the occurrence of two sesquiterpene hydrocarbons with the molecular weight of 202, one of them is the main component. In type III we found mainly eudesmanes, like selina-4,11-diene, β–selinene, eremophilene and as the main component 11-selinene-4-ol (Kongol). It is remarkable, that two of the isolated sesquiterpenes, selina-4,11-diene and eremophilene are of the usual enantiomeric configuration.

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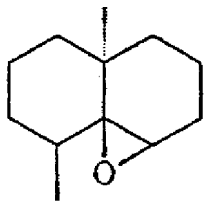
NEW INVESTIGATIONS ON THE LIVERWORT *LOPHOCOLEA BIDENTATA*

A. Rieck, N. Bülow and W. A. König

Institute of Organic Chemistry, University of Hamburg,
Martin-Luther-King-Platz 6, 20146 Hamburg, Germany

Chemo-variations of liverworts (*Hepaticae*) are well-known for quite a long time (1). As a more recent example *Scapania undulata* is mentioned (2). Our investigations on the liverwort *Lophocolea bidentata* indicate the presence of two different chemotypes.
The essential oils of samples collected from several sites all over Germany were compared by GC- and GC-MS-investigations. At two sites, Sachsenwald (northern Germany) and Rhön (central Germany), an epoxy-trinoreudesmane sesquiterpene 1 (3) was found to be the main component of the essential oil whereas in all other samples no trace of this new compound could be detected. Furthermore this genus additionally contained α-cuparenone and dihydro-α-cuparenone. The composition of the other chemotype is in accordance with recent work of Tabacchi, Joulain, Huneck and Herout (4).
Structural elucidation and stereochemical analysis of 1 was performed by mass spectrometry, NMR-investigations, chemical conversion and synthesis. The highly fragrant epoxide 1 was proved not to be an artefact by using mild extraction methods like cold solvent extraction and supercritical solvent extraction (SFE).



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SEASONAL VARIATION OF BUCHU (*Agathosma betulina*) ESSENTIAL OIL

N.F. Collins[1]

[1] Grassroots Natural Products, P.O.Box 16, Gouda 6821, South Africa

Agathosma betulina (Berg.) Pillans (syn. *Barosma betulina*) is a shrub endemic to the Western Cape mountains of South Africa. The leaves and stems are harvested from wild and cultivated plants, and the essential oil extracted by standard hydrodistillation. The oil is pale yellow in colour and possesses a strong, sweetish, mint-like, unpleasant rubbery odour (1). The oil is used in flavours and fragrances, and particularly in flavouring black currant products (2,3).

Though the chemistry of buchu leaf oil has been extensively studied (2), very little is known about the seasonal variation of the oil in terms of the quantity and quality. To determine the optimum time of harvest, plants were harvested regularly over a year to ascertain the variation.

This paper shows that yield is lowest in winter (1.04%) then weather is cold and wet and is highest in summer (2.00%)when hot dry conditions prevail. Variation in the oil composition was small. Consequently, harvesting of buchu is depended on oil yield and therefore the optimum time of harvest is in summer.

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MICROSPOROGENESIS IN MALE STERILE PLANTS OF *SALVIA SCLAREA* L. (LAMIACEAE)

C. Daskalova[1]

[1] Institute of Botany, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Salvia sclarea is a well-known aromatic plant. It is wild grown on limestone places all over Bulgaria. From the air dried leaves it has been obtained 1.5-2.5% essential oil.

The finding out of more perspective cultivars led the necessity of the cytoembryological study of this species.

During the last few years at the Institute of Botany male sterile plants in *S. sclarea* were established in the available collection. The elucidation of the genetic nature of this biological phenomenon involves the study of the microsporogenesis of the male sterile plants.

The sporogenous tissue in the anthes of *S. sclarea* comprises of several layers of polygonal to slightly rounded uninuclear cells. After a period of differentiation they function directly as microspore mother cells (MMC).

The microsporogenesis in the MMC runs normally. Only in some microsporangia some insignificant deviations have been registered as 1-2 lagging behind chromosomes or ones out of the achromatic spindle. As a result of the simultaneous microsporogenesis tetrahedral and isobilateral tetrads are formed in the anther loculi.

The microsporogenesis is accompanied by strongly expressed degeneration process occupying a great percentage of MMC, microspores and tetrads which cause at least the male sterility in *S. sclarea*.

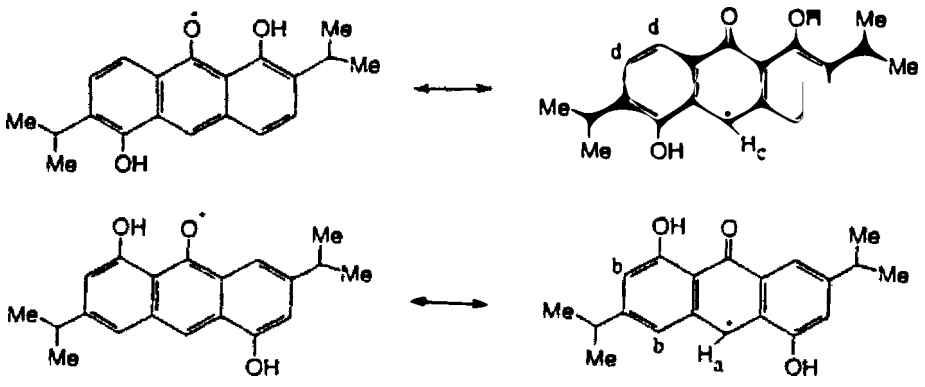
Electron Paramagnetic Resonance Studies on the Antioxidant Compounds Carvacrol and Thymol during Oxidative Stress

S G Deans[1], B A Goodman[2], N Deighton[2] and S M Glidewell[2]
[1]Aromatic & Medicinal Plant Group, SAC Auchincruive, Ayr KA6 5HW, Scotland, UK;
[2]Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK

Aromatic and medicinal plants have, for milennia, been a rich source of antioxidative compounds. In particular, the volatile oil from thyme [*Thymus vulgaris*] and oregano [*Origanum vulgare*] are rich in thymol and carvacrol, both of which have pronounced antioxidant activies [Deans *et al.*, 1993]

There have been indications that one of the common free radicals in living organisms, the superoxide radical anion, has been implicated in a number of disease conditions as well as being a contributor to the degenerative processes associated with ageing. By using the technique of electron paramagnetic resonance [EPR], Deighton *et al.*, 1993 showed that the free radicals induced by the addition of potassium superoxide to volatile oils of thyme and oregano, were chemically identical to those of thymol and carvacrol.

The EPR spectra obtained for thymol and carvacrol showed several changes with increasing temperature : as the temperature increased, the spectrum of carvacrol changed from a broad triplet at RT to a doublet which by 345°K was resolved into a doublet of quintets. In the case of thymol, the 368°K spectra showed a doublet which was subsequently resolved into doublets and a triplet. A pair of doublets on either side of of the main resonances indicated the presence of additional species. These EPR spectra do not represent simple phenoxy radical species, and the following structures are suggested as being the free radical reaction product of thymol and superoxide and carvacrol and superoxide.



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SLOVAKIA AS A PRODUCER OF ESSENTIAL OILS FROM HERBS.

I. Salamon[1], K.P.Svoboda[2] and J Hampson[2]

[1] Research Institute of Agroecology, Michalovce, Slovakia
[2] Plant Science Department, SAC, Ayr, Scotland, U.K.

The essential oils from herbs of varying origins and different aroma- and phyto-therapeutic characteristics are to be found on the world market.
Sovakia, a country in central Europe, has traditionally been the home of producers of medicinal, aromatic and spice plants. Good cultivation methods, harvesting and processing, produce plants with high quality essential oils. This poster presents our study of key compounds of essential oils from plants grown and processed in Slovakia. The oils were donated by successful, established growers.
Analysis of the essential oils was carried out using a Phillips PU4550 Gas Chromatograph, connected to a Spectrophysics SP4000 integrator. The compounds were identified by their G.C. retention times, and the resulting values were comparable to those of the literature. Oil component standards for comparison were supplied by Fluorochem Ltd, Roth, Sigma and Aldrich.

The composition of essential oils from Slovakia

	% of compound in essential oil							
Chemical compound	Scots pine	Fir needle	Coriander	Angelica	Hyssop	Lavender	Peppermint	Chamomile
α-bisabolol								31.6
α-pinene	56.7	18.7	0.3	10.8				
β-pinene		10.5			14.5			
camphene		16.0						
δ-3-carene	16.7							
chamazulene								7.7
ρ-cymene			5.2					
farnesene								45.0
limonene	15.7	21.5		47.8			6.7	
linalool			58.3			46.5		
linalyl acetate						7.0		
menthol							38.3	
menthone							30.1	
iso-menthone							5.7	
ocimene				2.4				
α-phellandrene				4.3				
pinocamphone					32.8			
iso-pinocamphone					13.4			
iso-pinocampheol					2.6			
γ-terpinene			6.9					
terpinen-4-ol				6.7				

In regard to our GC- analysis of the essential oils, it is possible to state that the essential oils from Slovakia are comparable to the same products which are purchased on the world market.

TOWARDS A MODEL OF TECHNICAL AND ECONOMIC OPTIMIZATION OF SPECIALIST MINOR CROPS

N. Verlet[1], J. Collins[2], K.P.Svoboda[2] and S.G.Deans[3]

[1] Ministère de L'Agriculture, CFPPA, 2 Avenue de Venterol, Nyons. BP36
[2] Plant Science Department, SAC, Ayr, U.K.
[3] Biochemical Sciences Department, SAC, Ayr, U.K.

The development of most specialised crops, particularly medicinal and aromatic plants, is hindered by the lack of technical and economic data. This sector is very complex, competition is fierce and the markets are rapidly satisfied. In Europe, competitive production is dependant upon research, which is faced with the diversity of the species concerned, specialist cultivation techniques and the diverse industries utilizing the products.

In response to the this, the EC has initiated a Concerted Action Programme involving eight European countries (France, UK, Italy, Ireland, Greece, Germany, Austria, Finland). The aims of this study (which will be undertaken over a two year period) are to compile a technical and economic database on aromatic and medicinal plant research being undertaken in each country, to make available and distribute the data, and thus promote exchanges between the research teams. Developing markets in Europe will be identified and evaluated, by establishing priority research area, and by recognising any technical points hindering development.

Preliminary results in the UK have indicated that very little research is being undertaken into the adoption of medicinal and aromatic plants as alternative crops for agriculture despite the increasing demand for high quality oils. It has been established that many temperate climate plant species can be grown in the UK and produce oils of a high standard (1, 2).

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2) Svoboda, K. P., Hay, R. K. M. and Waterman, P. G.: Journal of the Science of Food and Agriculture **53**, 193-202 (1990)

A Comparison of the Protective Effects of Thyme and Lovage Volatile Oils on the PUFA Status of Neonates.

K A Youdim[1], E Simpson[1], H J D Dorman[1], S G Deans[1] and H J Finlayson[2]

[1] Aromatic & Medicinal Plant Group, SAC Auchincruive, Ayr KA6 5HW, Scotland, UK;

[2]Department of Biochemical and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK

The importance of polyunsaturated fatty acids (PUFAs) in tissues is associated with an extensive range of nutritional and metabolic features. Protection of such PUFAs in the heart of rat neonates was studied by supplementing the mothers diet with 360μg/day of either Thyme (*Thymus vulgaris*) or Lovage (*Levisticum officinalsi*) volatile oil, over a 6 week period immediately prior to conception and throughout the pregnancy.

Analysis of the *free fatty acid* (FFA) content in the neonate hearts showed significant increases from the controls in the percentages of arachidonic acid (20:4) and docosahexaenoic acid (22:6) for both Thyme and Lovage treatments. Arachidonic acid contents (13.43±0.272) increased by 23% using Thyme (16.56±1.529) and 14% using Lovage (15.39±0.428). In the case of docosahexaenoic acid (4.40±0.77), protection was greatest using Lovage with a 41% increase (6.25±0.488) compared to the 30% increase using Thyme (5.73±0.146).

Analysis of the *phospholipid* (PL) fraction of the neonate hearts treated with Thyme and Lovage, also showed increases in the content of arachidonic and docosahexaenoic acid. For arachidonic acid (19.05±0.483), Lovage (22.48±0.452) had the greater effect with an 18% increase compared to a 12% increase using Thyme (21.33±0.452), and for docosahexaenoic acid (5.02±0.146) Lovage (7.28±.590) once again had the greater effect, with a 45% increase than Thyme (6.17±0.299), which only had a 23% increase.

The lipid content of arachidonic and docosahexaenoic acid in the FFA and PL fractions of neonate hearts increased using both oils, with Lovage showing the greatest effect especially on docosahexaenoic acid levels.

KAY gratefully acknowledges financial support from Scotia Pharmaceuticals.

ANTIOXIDANT-RICH PLANT VOLATILE OILS: *IN VITRO* ASSESSMENT OF ACTIVITY

H.J.D. Dorman[1], K.A. Youdim[1], S.G. Deans[1] and M. Lis-Balchin[2]

1 Aromatic & Medicinal Plant Group, SAC Auchincruive, Ayr KA6 5HW, Scotland UK.

2 South Bank University, London UK

Plant volatile (essential) oils have been recognized since antiquity to possess biological activities (1). Chief amoungst these are their antibacterial (2), antifungal (3), and antioxidant properties (4). Five volatile oils, geranium, monarda, nutmeg, oregano and thyme ware evaluated for their antioxidant properties at concentrations 0.75 to 100 parts per million (ppm). Nine components, α-terpinene, carvacrol, γ-terpinene, thymol, myrcene, linalool, thujone, ß-caryophyllene, p-cymene were tested in the concentration range 100 to 300 ppm in the egg yolk and the chicken muscle assays. This was done by measuring Thiobarbituric Acid Reactive Species (TBARS), primarily Malondialdehyde (MDA), in three avian assay systems: chicken egg yolk, one-day old chick livers and muscle from mature chickens.

All the oils and a number of the oil constituents demonstrated extensive antioxidant capacities. Monarda, nutmeg and thyme oils were shown to be the most effective in the egg yolk assay system; nutmeg oil was the most potent in the chick liver. Monarda, nutmeg, oregano and thyme appeared to possess equal activity in the muscle assay. α-terpinene, carvacrol, γ-terpinene, thymol manifested very close antioxidant values in the muscle (in the region of 80% inhibition) but less so in the egg yolk system.
The differences in the quantitative aspects of the assays reflects the qualitative and quantitative aspects of the lipids present in the tissues, therefore, all three would be useful as *in vitro* primary tier antioxidant test.

HJDD gratefully acknowledges financial support from the Ministry of Agriculture, Fisheries and Food.

DIFFERENCES IN BIOACTIVITY BETWEEN THE ENANTIOMERS OF α-PINENE

M. Lis-Balchin[1], R.J. Ochocka[2], S. Deans[3], M. Asztemborska[4], S. Hart[5]

[1] School of Applied Science, South Bank University of London, UK
[2] Medical Academy, Faculty of Pharmacy, Gdansk, Poland
[3] Scottish Agricultural College, Biochem. Sciences Dept., Auchincruive, UK
[4] Inst. of Physical Chemistry, Polish Acad. of Sciences, Warsaw, Poland
[5] Pharmacology Department, Kings College, London, UK

Many terpenes used therapeutically or as flavour and fragrance materials are chiral compounds and are used as components of essential oils, which when produced commercially differences in the enantiomer composition of different components in different plant essential oils and the use of chiral separations has been advocated for the identification of adulteration (Schultze et al. 1994) or source (Ochocka et al., 1991, Sybilska et al., 1994). Reports of studies of biological activity of enantiomers have been very scant, but as stereospecificity of drug action is related to the molecular asymmetry of the receptors, differences in biological activity are suspected.

The biological action of the enantiomers of α-pinene were studied using 25 different bacteria, 20 different strains of *Listeria monocytogenes* and 3 filamentous fungi; the pharmacological activity was studied on the guinea-pig ileum in vitro. The results showed differences in activity of the enantiomers both against microorganisms and pharmacologically: 18 out of 25 different L. monocytogenes strains were affected more by the (+) isomer. Two of three filamentous fungi were affected more by the (+) enantiomer. The (-) enantiomer was more spasmogenic on smooth muscle than the (+) enantiomer; changes in mechanism of action were however not detected.

COMPOSTION OF THE ESSENTIAL OIL OF *Erigeron canadensis* L.

J. Góra[1], A. Lis[1], and A. Kalemba[1]

1 Institute of General Food Chemistry, Technical University of Lódz, Stefanowskiego 4/10, 90-924 Lódz, Poland

Erigeron canadensis L., Compositae, is a strong expansive weed. It was introduced into Europe from North America.

Essential oil of plants growing wildly in Poland has been investigated. Flowering top parts of the plants were harvested, air-dried and hydrodistilled for 5 h to produce an essential oil with intensive, spicy odor in 0.80 % yield.

Components of the oil were separated by vacuum distillation and repeated flash chromatography. They were identified by GC, GC/MS and NMR spectroscopy.

About forty components were identified. The main constituents were limonene (78.02 %) and α-trans-bergamotene (5.98 %).

The large difference was observed in quantitative composition of the oils which had been published in literature before (1,2). Our oil is more similar to American oil than to Japanese one.

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COMPOSTION OF THE ESSENTIAL OIL OF CULTIVATED SUMMER SAVORY "SATURN"

J. Góra[1], A. Lis[1], and A. Lewandoswki[2]

1 Institute of General Food Chemistry, Technical University of Lódz, Stefanowskiego 4/10, 90-924 Lódz, Polnad

2 Research Centre of Cultivars Testing, 63-022 Slupia, Poland

Summer savory (*Satureja hortensis* L.) is a seasonal plant.

Essential oil of a new cultivar of summer savory has been investigated. This cultivar called "Saturn" was bred in Poland by directional crossing and selection of Yugoslav strain of *Satureja hortensis* L. with the population cultivated commonly in Poland.

Flowering plants were harvested, air-dried and hydrostilled for 5 h to produce an essential oil in 4.46 % yield.

Thirty components representing 99.97 % of the oil were identified by GC and GC/MS. The main components were γ-terpinene (40.93 %), carvacrol (39.28%) and p-cymene (6.24 %).

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *SOLIDAGO VIRGAUREA* L.

D. Kalemba[1], J. Góra[1]

Institut of General Food Chemistry, Technical University of Lódz, 90-924 Lódz, ul. Stefanowskiego 4/10, Poland

Solidago virgaurea L. (Compositae) is a medicinal plant with diuretic ans spasmolytic activity. The essential oils of some subspecies of *S. virgaurea* growing in Asia have previously been studied by Fujita (1).

The essential oil of *S. virgaurea* growing wild in Poland was obtained by hydrodistillation of the aerial parts and was analysed by GC, GC/MS and ^{1}H-NMR, both as such and after fractionation. Fractionating was carried out both by vacuum distillation and by flash-chromatography.

More than forty compounds were identified. Main constituents are monoterpene hydrocarbons, which represent 85 % of the oil with α-pinene, sabinene and limonene dominating.

1) Fujita, S.: Nippon Nogeikagaku Kaishi **64**, 1729-1732 (1990).

A CHEMOTAXONOMIC STUDY OF *LARIX* SPECIES

Y. Holm, T. Söderholm, I. Laakso and R. Hiltunen

Pharmacognosy Division, Department of Pharmacy, P. O. Box 56,
FIN-00014 University of Helsinki, Finland

Three species of larch, *Larix decidua* Mill., *L. sibirica* (Münchh.) Ledeb. and *L. leptolepis* (Sieb. et Zucc.) as well as two hybrids, *L. sibirica* x *leptolepis* and *L. leptolepis* x *sibirica* were analysed for their leaf volatile oil composition on a β-cyclodextrin phase.

Samples were obtained from *Larix* collection at Ruotsinkylä Field Station (Forest Research Institute), 20 km north of Helsinki, Finland, in June 1994. The crushed leaves were hydro-distilled and the oils were dried. For enantiomeric analysis the monoterpene hydrocarbon fraction was separated from the oxygen-containing components on a Sep-Pak® silica column (Waters Associates, USA).

GC analyses were carried out on a non-chiral fused silica NB-351 column or a on chiral heptakis-β-cyclodextrin column. In the GC-MS analyses the same columns were used. The identification was based on GC retention times of authentic samples, GC-MS spectra and retention data obtained on β-cyclodextrin columns.

The leaf oil of *L. leptolepis* was characterized by high contents of (-)- and (+)-α-pinene and myrcene, while *L. sibirica* had a high content of 3-carene. *L. decidua* showed about equal amounts of (-)- and (+)-α-pinene and a high content of 3-carene. When *L. leptolepis* and *L. sibirica* were crossed the progeny inherited either a high myrcene from *L. leptolepis* or a high content of 3-carene from *L. sibirica* in a certain ratio. It is important to note that the high content of (+)-α-pinene in *L. leptolepis* parent trees was not inherited by the progeny.

Earlier it has been claimed that only the larch twig oil is suitable for chemotaxonomic investigations (1). This study showed that also the leaf oil provides equally good information, when analysed on a chiral column and using cluster analysis to classify the material. The cluster analysis divided the investigated larch trees into two main groups. The first group consisted mostly of *L. sibirica* and hybrids with high 3-carene and the other group of *L. decidua* and *L. leptolepis* and hybrids with low 3-carene. In the second group *L. decidua* trees formed a distinct subgroup.

1) Rudloff, E.: J. Nat. Prod., 50 (2), 317-321 (1987).

THE ESSENTIAL OILS OF *ARTEMISIA ANNUA* L.: COMPARISON OF SEED MATERIALS OF DIFFERENT ORIGIN CULTIVATED IN FINLAND

Y. Holm, I. Laakso and R. Hiltunen

Pharmacognosy Division, Department of Pharmacy, P. O. Box 56,
FIN-00014 University of Helsinki, Finland

The essential oil compositions of leaf and flowers of *Artemisia annua* L. from Yugoslavia, France, China and Vietnam have been recently reviewed by Lawrence (1). The materials used in this study were from the following sources: 1) Seeds of Chinese origin from Purdue University, USA and Tasmania, Australia, 2) seeds from two varieties of Hungarian (Budakalasz) origin from Rennes, France, 3) seeds of Yugoslavian origin from Tasmania, 4) seeds of Italian origin (Pisa) and 5) of Hungarian origin from Szeged University. The plants were cultivated in experimental fields in 1994 in Puumala, Finland.

In each case, the essential oils were isolated by hydrodistillation from 50 grams of aerial parts at the stage of full flowering. Oil yields ranged from 0.25 - 0.90 µl g (dw).

GC-MS analyses were made on an HP 5890 GC coupled to an HP 5970 quadrupole mass selective detector. The columns used were a non-chiral fused silica NB-351 column (25 m x 0.32 mm i.d., Nordion, Finland) and a chiral heptakis-β-cyclodextrin column (30 m x 0.25 mm i.d., J & W Scientific, USA).

Artemisia annua oils of Chinese origin were characterized by germacrene-D (12-13 %), β-caryophyllene (8 %), artemisia ketone and 1,8-cineol (7-8 %) and camphor (4-7%). Monoterpene hydrocarbon components were found in small amounts only (0.2-2 %). Oils of Hungarian origin (Bukalasz, Szeged) showed high levels of camphor (23-39 %), artemisia ketone (12-24 %) and germacrene-D (10-13 %). Low 1,8-cineol (1-3 %) and monoterperpene hydrocarbon (0.1-6 %) contents were typical for these oils. In the oil of Italian origin, camphor (31 %) was combined with rather high content of 1,8-cineol (12 %) and germacrene-D (11 %) but low levels of monoterpene hydrocarbons (0.4-6 %). In this material, the Yugoslavian oil had the highest proportion of germacrene-D (19 %) and an exceptionally high level of (+)-α-pinene (12 %).

Despite the low levels of monoterpene hydrocarbons, the enantiomeric proportions of α- and β-pinene, camphene, limonene and terpinen-4-ol were determined from all the oils studied. Considerable variation in the ratio was found for all enantiomeric pairs exept for (+) / (-)-terpinen-4-ol, where it was approximately 1:1.

1) Lawrence, B.M.: *Artemisia annua* oil, In Progress in Essential Oils, Perf.Flav., 20, (March / April), 52-54 (1995).

GC/MS ANALYSIS OF ESSENTIAL OILS OF *ROSMARINUS OFFICINALIS*

St. Ilieva[1], K. Koleva[1] and M. Markova[1]

[1] Institute for Roses, Aromatic and Medicinal Plants, 6100 Kazanlik, Bulgaria

This work is carried out to study the composition of the essential oils of two origins of *Rosmarinus officinalis*. The essential oils were obtained by steam distillation from fresh plants.

The chemical composition was studied by GC and GC/MS.

The main components of the investigated oils are: α-pinene, camphene, ß-pinene, limonene, cineole, bornylacetate, camphore. The quantity of cineole in the oil of *Rosmarinus officinalis* - Ochrid origin - is more than 30 %. In the oil of Varna origin it is less than 20 %. In the oil of the second origin α-pinene is the main component (25-32 %).

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.,1, 249-259 (1989)

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USE OF ESSENTIAL OILS FOR SANATION OF INTRAHOSPITAL INFECTIONS AND PATIENTS WITH CHRONIC NONSPECIFIC LUNG DISEASES - CARRIER OF MUSHROOMS SP. CANDIDA

N.V. Kazarinova[1], L.M. Muzychenko[1], K.G. Tkachenko[1], A.M. Snurgaya[1], V.N. Brejnev[1] and A. O.M. Usov[1]

[1] Institut of General Pathology and Human Ecology SB RAMS (Novosibirsk); Botanical Institute after Komarov V.L., RAS (St. Petersburg); Institute of Chemical Kinetics and Combustion, SB RAS; JS "Rakurs" (Novosibirsk)

The problem of intrahospital infections (IHI) is one of the urgent problems of modern medicine. The purpose of the present work, is search of new effective sanative means of natural origin for elemination of intrahospital infections. The tests, conducted on the museum culture, have demonstrated that the essential oils (EO) from Labiata family completely inhibit the following species of conditionally pathogenic microflora: Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Candida albicans 1, Ostreptococcus faecium, Streptococcus faecalis, Pseudomonas aerginosa, Klebsiella pneumoniae, Salmonella enteritidis, Salmonella typhimurium, Citrobacter freundii, Shigella flexneri, E. coli 25983, Proteus mirabilis, Proteus morganii. It was shown decrease of quantity of colonies in medical institutions conditionally pathogenic and pathogenic microflora (dressing rooms of a burn centre, hospital rooms). Cleansing of rooms with EO was condueted by made aerosol with help of ultrasonic inhalers. Work of the apparatus at frequency of megacycle per second range and at densities of ultesonic active power IY/sg sm makes possible to get aerosol with drops less than 0.5 mc in diameter. After cleansing rooms by EM quantity of colonies was decreased in 2-3 times and reached the Russian standard. Taking in to condideration sanative effect of essential oil to Candida albicans and absence of highly effective drugs in treatment of candidiasis examiniations on influence of EO on yeast mushrooms of SP Candida in patients with CHNSLD were conducted. Fungicidal activity has been revaeled. Thus use of essential oils as sanative agents for intrahospital infections assumes the introduction of new methods of sanation, that, of great importance for medical institutions (operative-dressing rooms, puerperal homes). The EO are the most promissing ones in treatment of candidiasis.

ESSENTIAL OIL PLANTS AS SOURCES OF ANTIBACTERIAL SUBSTANCES FOR DIFFERENT PREMISES OR LODGINGS

K.G.Tkachenko

Botanical Garden of the Komarov Botanical Institute of RAS,
2, prof. Popova str., 197376, St.Petersburg, Russia

It is known that essential oils and their separated components have antibacterial activity relative to wide spectrum of microorganisms, bacteria, viruses and fungi.

New ways of using essential oil plants and essential oils are suggested. Using of certain assortment of essential oil plants in interior allows to solve two important problems - 1) to make green an interior to obtain positive psychological factor; and 2) to achieve medicinal effect - decreasing number of microb cells in air (in phytorecreation halls, industrial shops, offises, clinics and children institutions). It allows to decrease 20 - 300 times a microbal pollution of air. The basis of assortment for direct phytodesign includes species from the following families: *Cupressaceae, Pinaceae, Myrthaceae, Lauraceae, Lamiaceae* and many other. The whole number of plants necessary for achieving desirable effect should be 100 - 120 for appartment of 100 - 120 m^2 volume.

Another way is to use essential oils themself or their complex compositions. Quantity of sprayed essential oils or their compositions should be not more than 0.1 ml for appartment of 100 - 120 m^3. Duration of spraying of essential should be not more than 20 - 25 min. Number of microb cells during spryaing decreases 2 - 4 times. When using essential oils in compositions or seperated oils their allergic properties, individual endurance by patients, also psychotropic effects of essential oils on the nervous system.

The way of using essential oils more cheap and economical comparing with creation of phytorecreation zones in different appartments. Nevertheless the both ways of using essential oil plants, essential oils and their compositions must develop thus improve the air of appartments and normalisation emotional-psychological state of workes (in offices), patients (in clinics), children (in schools and kinder-gardens).

EFFECT OF STEAM DISTILLATION, SOLVENT- AND SUPERCRITICAL FLUID EXTRACTIONS ON THE COMPOSITION AND PHYTOTHERAPEUTICAL VALUE OF THYME EXTRACTS

A. Kery[1], M. Oszagyán[2], B. Simándi[2], K. Horváth[3], E. Lemberkovics[1] and E. Rónyai[2]

1 Institute of Pharmacognosy, Semmelweis University of Medicine, H-1085 Budapest, Hungary
2 Department of Chemical Engineering, Technical University of Budapest, H-1521 Budapest, Hungary
3 Department of General and Analytical Chemistry, Technical University of Budapest, H-1521 Budapest, Hungary

Thyme (*Thymus vulgaris* L. Lamiaceae) besides being a familiar culinary herb, is one of the oldest medicinal plants we know. Its mucus-dissolving and expectorant effect in the treatment of cough and bronhitis has been proved by modern science. As various chemical substances are involved both in the culinary value and the bioactivity to obtain high quality extracts and concentrates of thyme has a great value.

Conventional steam distillation and solvent extraction were compared with supercritical fluid extraction (SFE) using carbon dioxide. As starting material the intact crude drug and previously carbon dioxide extracted plant material were used respectively in order to clarify the significance of monoterpene glycosides and stability in thyme plant and extracts.

The SFE oil extract of thyme contained 10-15 % thymol and 30-35 % carvacrol while steam distillation produced an oil containing 48-50 % thymol and only 8-10 % carvacrol. The SFE extracts were collected in separate samples and significant change was observed in compositions of the samples during the extraction. Some thymol was also found in form of monoglucoside both in the native and carbon dioxide preextracted plant material using enzymatic and acidic hydrolysis followed by steam distillation and conventional extraction methods. Preextracted plant material with carbon dioxide was found to be a good raw material for the preparation of thyme flavonoids and depsides with valuable antioxidant activity.

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2) Czygan, F.C., Hänsel, R.: Zeitschrift für Phytotherapie 14, 104-110 (1993).
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PRODUCTION BIOLOGICAL EVALUATION OF LAMIACEAE SPECIES IN HUNGARY

Máthé I.[1,2], Lemberkovics É.[4], Dános B.[3], Nyiredy Sz.[3], Simándi B.[5], Máthé Á.[2]

[1]Institute of Pharmacognosy of the A. Szent-Györgyi Medical University, H-6720 Szeged, Hungary,
[2]Lamiaceae Research Team at the Institute of Ecology and Botany of the Hungarian Academy of Sciences, Vácrátót,
[3]Medicinal Plant Research Institute, Budakalász,
[4]Institute of Pharmacognosy of the Semmelweis Medical University, Budapest
[5]Department of Chemical Engineering of Technical University, Budapest

The production biological evaluation of medicinal plants, among them that of the *Lamiaceae* species is a common research objective in a number of institutes in Hungary. The 92 *Lamiaceae* species native or introduced to Hungary are the object of our present work focusing only on the cultivated and certain introduced species.

The methods used are as follows: the plants were grown in experimental fields, from seeds either collected in Hungary or obtained via international seed exchange. The populations of the same species established at the same location provided a possibility for obtaining information on diversity of these species.

The phytomass of plant organs was determined separately, whereas their chemical ingredients, predominantly their triterpene content: oleanolic, ursolic acids and some phenolic components like rosmarinic, caffeic acids, herniarin, etc. content were measured together with essential oil content and composition. TLC, HPLC, GC-MS, Densitometry, UV-VIS, IR spectroscopy were used for the identification and the comparative evaluation of chemical constituents.

Salvia officinalis L., *Lavandula angustifolia* L., *Ocimum basilicum* L., etc. were the main species involved in this study also serving as model plants. The variation of their phytomass, essential oil content, together with the polaric components from methanol extracts were investigated. The oil fractions gained by supercritic CO_2 fluid extraction of Salvia officinalis, Lavandula angustifolia and Melissa officinalis were also introduced.

The time dependent variation (e.g. in the course of the growing season) of the populations of the same species shows great diversity both in the phytomass and in the concentration of chemical ingredients, regarding also their active principle production. It could be established that in the case of all species the range of production parameters is rather wide so that the presently cultivated cultivars should be replaced with newly selected ones.

ESSENTIAL OIL FORMATION IN CHAMOMILE HAIRY ROOT CULTURES.

É. Szõke, É. Lemberkovics, J. Troilina and I. László

Semmelweis University of Medicine, Institute of Pharmacognosy, 1085 Budapest, Hungary

During our experiments we studied the influence of Mg ions on the hairy root cultures genetically transformed with Agrobacterium rhizogenes strains A 4, R 1601, and 15834. As $MgSO_4$ has an exceptionally positive effect on rooting of *Matricaria recutita* L. (Asteraceae) organized cultures, it is interesting to demonstrate its effect on the growth of isolated roots and transformed hairy roots(1,2). We observed the biomass production and essential oil formation in the cultures with respect to the therapeutically important compounds.

The cultures grew well on the Murashige - Skoog medium suplemented with $MgSO_4$. It was established that the fresh weight of the root is parallel to the concentration of $MgSO_4$ salt. Similar tendency was observed in the hairy root cultures genetically transformed with Agrobacterium rhizogenes strains A 4, 15834, and R 1601.

The essential oil content increased by adding $MgSO_4$ in 740 mg/l concentration. Some of the important essential oil components (t-β-farnesene, t-α-farnesene, α-bisabolol,spatulenol, spiroethers) increased considerably. These were identified by GC and GC-MS methods (3).

Summarising our results, it was found that $MgSO_4$ positively influences both the biomass rate and the essential oil production. Furthermore it also has a significant effect on its composition.

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3) Lemberkovics, É. : J. Chromatogr., 318, 125-131 (1985)

ESSENTIAL OIL PRODUCTION OF *CHAMOMILLA RECUTITA* (L.) RAUSCHERT IN GERMANY

S. Kistler[1]

[1] Kistler & Co.GmbH, Lindenstraße 5, D-85254 Sulzemoos/Orthofen

Tody very high demands are made by the pharmaceutical companies regarding the essential oils of the chamomile plant.

Besides the absolute purity of the product a high concentration of *Chamazulen* and (-)-α-*Bisabolol* is required, whilst at the same time freedom from *Bisabololoxids*.

The original genetic material of the plant heavily influences the quality of the product, as does the cultivation technique used, the harvest and also the manufacturing process.

Continual improvements in the cultivation processes, as well as homogenisational water vapor distillation put us in the position of being able to produce oils to the highest quality standards.

Because of this we would like to represent our business in its entirely.

ESSENTIAL OILS AND ECOLOGICAL AGRICULTURE

A. Mateeva[1] and H. Sermann[2]
1 Department of Entomology, Higher Institute of Agriculture, Mendeleevstr. 12, BG 4000 Plovdiv, Bulgaria
2 Department of Entomology, Humboldt University, Berlin, Germany

It is clear today more than any time that the ecological agriculture is the unique chance for soil's survival and preservation. The conception the soil is a living organism, a living system in which all must be balanced takes a central place in it.

The aim of the present investigation is to establish the essential oil's role in the ecological agriculture's context. A number of observation is carried out about the directs toxical effect of a set of essential oils active substances on economically important diseases and enemies.

On the base of the investigations it has been fixed that some of the essential oils grown in Bulgaria can be used successfully as:

- a predecessor culture acting an important role in the density reduction and the wrecking activities of the peach greenfly, etc.;
- a means of acaricide toward insects with sucking and nibbling oral organs;
- producing a high economical effect when used in the ecological farm's crop-rotation.

Seasonal Variation of the Essential Oil Composition of various Central-European *Thymus*-species

Ch. Mechtler, R. Länger, J. Jurenitsch

Institute of Pharmacognosy, University of Vienna, Centre of Pharmacy,
Althanstraße 14, A-1090 Wien

Chemical polymorphism as well as intraindividual differences in the composition of the essential oil are well-known phenomena of Central European *Thymus*-species (1, 2, 3, 4).The aim of our work was to clarify the influence of ecological factors or the stage of development within a vegetation period on the composition of the essential oil.

About 60 individuals of *Thymus kosteleckyanus* OPIZ, *Th. pulegioides* L., *Th. praecox* OPIZ and *Th. glabrescens* WILLD., collected in Slovakia, Czech Republic and Austria have been cultivated in the botanical garden of the institute. In the period from May to September 1994 four samples were taken from each individuum. To avoid uncertainty caused by intraindividual differences several stems were extracted with dichloromethane.

The qualitative composition of the essential oil of each individuum remained unchanged during the vegetation period, whereas within all four species about the half of the plants showed a considerable variation of the ratio of the main compounds (examples in table 1).

The comparison of flowering stems of individual plants prior to transfer to the garden and after one year of cultivation led to the conclusion that different ecological factors seem to have no significant influence on the composition in our material.

Table 1: Examples for the variation of the percentage of main components of the essential oil during a vegetation period.

	Th. kosteleckyanus				Th. praecox				Th. pulegioides			
Month of harvest	5	6	7	8/9	5	6	7	8/9	5	6	7	8/9
p-cymene	4.1	39.4	9.2	38.6	3.4	41.0	6.7	9.6	34.4	45.2	9.3	7.9
γ-terpinene	24.1	11.5	21.8	14.8	39.5	24.3	31.9	38.7	10.5	10.7	13.7	20.8
thymol	38.3	10.7	35.2	13.4	36.8	8.4	36.1	25.6	11.1	8.2	21.1	28.6
β-caryophyllene	0.8	0.3	0.5	0.3	1.2	0.2	1.9	1.2	13.7	10.5	20.7	11.0
β-bisabolene	10.1	4.5	8.4	4.6	4.9	1.7	7.4	5.7	2.5	1.9	24.2	16.0

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ESSENTIAL OILS OF *THYMUS* SSP. FROM PORTUGAL: SECTION *MASTICHINA*

L. Salgueiro[1], R. Vila[2], S. Cañigueral[2], O. Roque[1], A. Proença da Cunha[1] and T. Adzet[2]

[1] Laboratório de Farmacognosia, Faculdade de Farmácia, Universidade de Coimbra. Rua do Norte, 3000 Coimbra, Portugal

[2] Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona, Av. Diagonal, 643. E - 08028 Barcelona, Espanha

Continuing our investigation on the essential oils of *Thymus* ssp. from Portugal, we now report on the qualitative and quantitative composition of the volatile oils of the plants of the Section *Mastichina*. This Section includes three Iberian taxa: *Th. mastichina* L.(L.) ssp. *mastichina*, *Th. mastichina* L. (L.) ssp. *donyanae* R. Morales and *Th. albicans* Hoffmanns. & Link. The second one had not been previously indicated in the Portuguese Flora, and the composition of its essential oil is investigated for the first time.

The composition of the volatile oils of several populations of different localities of these three taxa was investigated by GC-FID and GC-MS using two fused silica capillary columns of different stationary phases, and when necessary by ^{13}C-NMR. The analytical conditions are reported in (1).

In total 93 compounds were identified, meaning a percentage of the essential oils ranging from 96,0% to 98,5%. All samples were characterized by their high 1,8-cineole content. Another important constituent of the essential oils of two samples of *Th. mastichina* ssp. *mastichina* from Estremadura and three samples of *Th. albicans* from Algarve was linalool. Borneol was also found as a major compound of the essential oil of *Th. mastichina* ssp. *donyanae*.

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ESSENTIAL OILS OF THREE *THYMUS KOTSCHYANUS* VARIETIES FROM TURKEY

G. Tümen[1], K.H.C. Başer[2] , N. Kırımer[2]

[1] Faculty of Education, Department of Biology, Balıkesir University, 10100 Balıkesir, Turkey

[2] Medicinal and Aromatic Plant and Drug Research Centre (TBAM), Anadolu University, 26470 Eskişehir, Turkey

Water-distilled essential oils from three varietes of *Thymus kotschyanus* : var. *kotschyanus*, var. *eriophorus* and var. *glabrescens* were analysed by GC and GC/MS.

Carvacrol was found as major constituent in all oil samples but one. The only oil containing thymol as the major compound was obtained from a sample of var. *glabrescens*.

FLAVOUR COMPONENTS OF JAPANESE TRADITIONAL FOOD, DRIED AND ROASTED SHEET "NORI" (*PORHYLA YEZOENSIS* F. NARAWAENSIS)

M. Miyazawa[1], A. Takahata[2] and T. Urano[3]

1 Department of Applied Chemistry, Kinki University, Higashiosaka, Osaka, 55, Japan
2 Fuso Chemical Industry Co., Ltd. 2-6-6, Nitaka Yodogawa, Osaka, 532, Japan
3 U-MAC Ltd. 421, Kamiyuge, Yuge-cho, Ochigun, Ehime-ken, 794-25, Japan

Two kind of volatile oils (dried sheet (D) and roasted sheet (R) were obtained from dried and roasted sheet "Nori" (*Porhyla yezoensis* f. *narawaensis*) by steam distillation. More than 70 kind of flavor components from these oils were identified by capillary GC, GC-MS. The main constituents were palmitic acid (D: 37.8% /R: 56.1%), phytol (3.8% / 0%), 1Z, 3Z-cyclooctaziene (3.7% / 0%), heptadecene (3.6% / 4.2%), tetradecanoic acid (3.5% / 4.0%), ß-ionone (2.4% / 0.66%), 6-undecanone (2.4% / 0%), α-ionone (2.1% / 0.7%), dihydroactinidiolide (2.0% / 4.1%) and 9Z,10Z-octadecadienoic acid (0% / 5.2%).

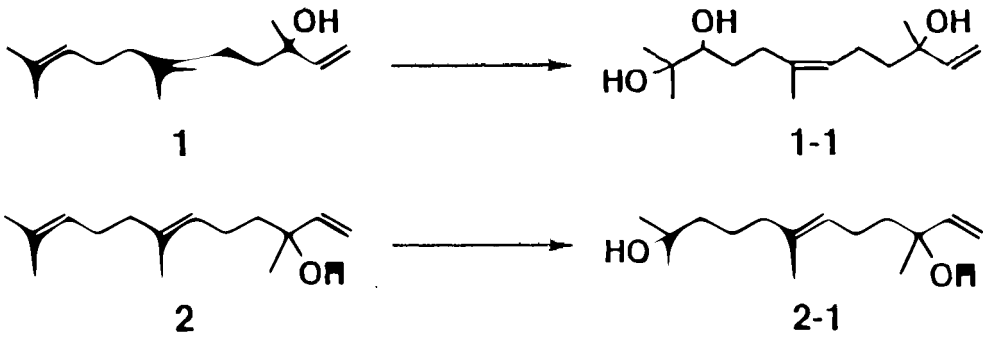
MICROBIAL TRANSFORMATIONS OF ACYCLIC TERPENOIDS BY *GLOMERELLA CINGULATA*

M. Miyazawa, H. Nankai and H. Kameoka

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Higashiosaka-shi, Osaka 577, Japan

Acyclic terpenoids are widely distributed in nature, and they are important biosynthetic precursor of cyclic terpenoids in living organisms such as higher plants. If it is possible to produce new cyclic terpenoids from acyclic terpenoids by microbial transformation, it will be one of the valuable way for synthetic organic chemistry. Therefore, we have tried the biotransformation of two acyclic terpenoids.

Microbial transformations of two acyclic sesquiterpene alcohols, *cis*-nerolidol(**1**) and *trans*-nerolidol(**2**) by *Glomerella cingulata* were investigated. On the microbial transformation of **1**, epoxydation of remote double bond and subsequent hydrolysis of the epoxide was mainly proceeded and to give (*Z*)-3,7,11-trimethyl-1,6-dodecadien-3,10,11-triol(**1-1**) as major metabolite. While on the case of **2**, hydration at remote double bond was mainly proceeded and to give (*E*)-3,7,11-trimethyl-1,6-dodecadien-3,11-diol(**2-1**) as major metabolite. This difference in product formation by *G. cingulata* with **1** and **2** maybe explained by influence of the *cis*/*trans* configuration of these substrates.



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TRANS, 2,TRANS, 4-DECADIENOIC ACID ISOBUTYL ESTER AND OTHER CONSTITUENTS OF THE ROOT ESSENTIAL OIL OF *ZANTHOXYLUM ZANTHOXYLOIDES* LAM. (WATERM.) FAMILY

O.A. Onayade[1]

[1] Department of Pharmacognosy, College of Health Sciences, Obafemi Awolowo University, Ile-IFe, Nigeria

- abstract not available -

EFFECT OF PLANTING DENSITY AND HARVEST DATE ON CHEMICAL COMPOSITION OF SAGE ESSENTIAL OIL

R. Piccaglia[1], M. Marotti[1], V. Dellacecca[1]

[1] Dipartimento di Agronomia, Università di Bologna, Via Filippo Re,8 40126 Bologna, Italy

Sage (*Salvia officinalis* L.) is a typical aromatic plant of the Mediterranean area. It is used in the food industry as flavouring and preserving agent and in the manifacture of perfumes and cosmetics. As a medicinal, it has antispasmodic, astringent and tonic effects.

Sage essential oil possesses good antibacterial and antioxidant properties (1) and some its compounds such as 1,8-cineole, camphor and thujone have a marked biological activity (2).

The sage at full flowering stage is generally harvested to obtain essential oil whereas sage at vegetative stage is used as herbalistic product. Sage oil yield and composition are determined by genotype but are also influenced by environmental factors and cultural methods (3,4). Aim of our work was to study quali-quantitative composition of essential oil of sage grown at three planting densities and subjected to successive cuts a different development stages.

The date of harvest significantly affected the oil composition. In particularly differences were found in sage cut at vegetative stage in April in comparison with sage harvested at full flowering stage in June. This latter had higher contents of ß-pinene, 1,8-cineole and lower amount of sesquiterpenes. Very important differences occurred between sage harvested at vegetative stage in spring and sage cutted at the same stage in autumn. The oil obtained from autumn sage was very rich in thujones, camphor and 1,8-cineole and showed low amounts of sesquiterpenes.

The different planting densities (about 4, 6 and 8 plants/m^2) significantly affected the ß-thujone content of sage harvested at the vegetative stage; the highest density produced oils with the highest levels of ß-thujone.

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EFFECTS OF POLLUTANTS ON THE VOLATILE OIL OF *MENTHA PIPERITA* L. LEAVES. INHIBITION OF MUTAGEN-INDUCED REVERTANTS IN *SALMONELLA TYPHIMURIUM*

M.M. Saleh[1] and F.A. Hashem[1]

[1] Pharmaceutical Science Department, National Research Centre, Cairo, Egypt

The percentage yield of volatile oil of *Mentha piperita* L. leaves decreased in cases of lead, cement and limestone dust pollution while it increased in cases of sulphate pollution, where sulphur is an essential element activates several enzymes (1). Hydrochloric acid pollution gave a very mild effect. Lead, sulphate, cement and limestone pollution showed a great decrease in menthone and menthol concentrations and an increase in the percentage of menthofuran concentration. Hydrochloric acid pollution showed a mild decrease in menthone and menthol and no increase in menthofuran. The alcoholic extract of polluted *Mentha piperita* L. exhibited more desmutagenic and antimutagenic activities against *Salmonella typhimurium* TA100 strain using EMS and RL induced reversion than the untreated sample (2).

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ANALYSIS OF ESSENTIAL OIL OF *NEPETA NUDA SSP.GLANDULIFERA* DAVIS.

E.Sarer, B.Konuklugil

Ankara University, Faculty of Pharmacy, Department of Pharmacognosy 06100 Tandogan, Ankara, Turkey

The genus *Nepata* (Lamiaceae) comprises some 250 species which are spread out over Europe, central and southern Asia, the southwest Russia and Turkey. The genus *Nepeta* is represented in Turkey more than 30 species, some of them are endemic (1). *Nepeta nuda ssp. glandulifera* is an endemic one and its essential oil has not been analyzed until now.

Due to their diuretic, antiseptic, antitussive, antispasmodic, antiasthmatic, febrifuge and astringent activities (2,3). Plants were collected on June 1994 from south Anatolia. The essential oil was obtained by hydrodistillation and the means yield of the oil was 0.4 % vol / wt. A light yellow volatile oil with a pleasant odor has been investigated by GC-MS. Nerolidol was proved to be main component together with caryophyllene.

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CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM CALAMINTHA TAURICOLA

E. Şarer. S. Solakel Pançalı

Department of Pharmacognosy, Faculty of Pharmacy, Ankara University,

06100, Tandoğan, Ankara - Turkey.

Calamintha species (family Lamiaceae) are the most widely used plants in traditional medicine mainly as stimulant, stomachic, antispasmodic and antiseptic. This species are also known as spices (1,2).

There are eight species of Calamintha growing in Turkey (3). In this research we examined the essential oil from Calamintha tauricola P.H. Davis, which is endemic in southern Turkey.

The plant material was collected on July 1992, from Içel at 1010 m. The essential oil was obtained from the plant material by hydrodistillation in a yield of 1.29 %.

The analyses of the essential oil were done by using LSC and capillary GLC techniques (4). Twenty four compounds has been assumed in the oil. It was seen that, pulegone (50.30 %) and isomenthone (23.92 %) were the main components of the oil.

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THE ESSENTIAL OIL OF *EUPATORIUM ADENOPHORUM* SPRENG.

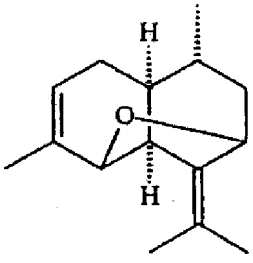
Ingo Seelmann, Peter Weyerstahl and Helga Marschall

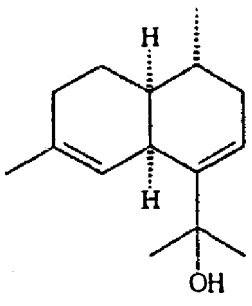
Institute of Organic Chemistry, Technical University of Berlin
Strasse des 17. Juni 135, D-10623 Berlin, Germany

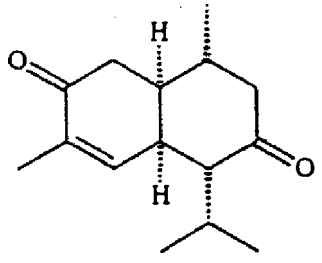
The essential oil of *Eupatorium adenophorum* syn. *Ageratina adenophora* (Compositae) was prepared from blossoms collected in the north of India. Its fragrance is described as parsley, leather-like, terpenoid, distilled lime oil.

The constituents of 70 g of the essential oil of *Eupatorium adenophorum* were carefully analyzed by a combination of GC using Kováts-Indices, GC-MS and NMR techniques after they had been separated by distillation and flash chromatography.

62 components representing more than 93% of the oil could be identified, primarily mono- and sesquiterpene hydrocarbons. Main constituents are camphene (12%), α-phellandrene (13%), p-cymene (9%), bornyl acetate (11%), caryophyllene (3%), γ-curcumene (5%), α-*trans*-bergamotene (1%) and bicyclogermacrene (2%). An astonishing variety of cadinanes with a muurolane type stereochemistry also were perceived. Some of them are new or scarcely known:







Remarkable muurolane structures are emphasized with specific spectral data. A complete table of the components of the *Eupatorium* oil and a discussion of their identification are presented.

CHEMICAL COMPOSITION OF THE *HELICHRYSUM PLICATUM* D.C. SSP. *PLICATUM* ESSENTIAL OIL

E. Sezik[1], and M. Aslan[1]

[1] Gazi Universitesi. Faculty of Pharmacy, Dept. of Pharmacognosy
Ankara 06330, Türkiye

Helichrysum plicatum D.C. ssp. *plicatum* is commonly used as folk medicine in Turkey. Either flowers or aerial parts are used for the treatment dysurea, kidneystones, wounds, burns and ear pains (1-3).

The capitulums used in our research were collected from the Palandöken Mountain (Erzurum) in 1993. The yield of the essential oil was determined as % 0.1 (v/w) by volumetric method (BP 1988). Fiftyfour compounds identified by GC and GC/MS were listed below.

α-Pinene, α-fenchene, camphene, hexanal, undecane, ß-pinene, limonene, 1,8-cineol, (E)-2-hexenal, 2-pentylfuran, γ-terpinene, p-cymene, terpinolene, n-hexanol, nonanal, tetradecane, 1-octen-3-ol, champholene aldehyde, α-copaene, decanal, camphor, (Z)-2-nonenal, linalool, 1-octanol, fenchylalcohol, ß-caryophyllene, α-humulene, α-terpineol, borneol, undecanal, α-muurolene, δ-cadinene, (E,E)-2,4-decadienal, cis-geranylaceton, α-calacorene, caryophyllene oxyde, nerolidol, octanoic acid, hexahydrofarnecyl aceton, torreyol, nonanoic acid (% 3.13), carvacrol, ß-eudesmol, T-muurolol, decanoic acid (% 6.72), tricosan, lauric acid (% 3.99), tetracosan (% 4.1), isobutylphthalate, butylphthalate, tetradecanoic acid (% 9.3), heptacosane, palmitic acid (% 11.76), octacosane.

In contrast to the essential oil contents of the other *Helichrysum* species reported before, the essential oil of *H. plicatum* ssp. *plicatum* contains high percentage of volatile fatty acids.

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INTER- AND INFRA-POPULATION VARIATION OF THE ESSENTIAL OIL YIELD AND COMPOSITION OF *SALVIA FRUTICOSA* MILL. IN CRETE, GREECE

Skoula, M. , Fournaraki, Ch. and Panetsos, K.

Mediterranean Agronomic Institute of Chania, Greece

Salvia fruticosa Mill. is an endemic species of the Eastern Mediterranean region. It is one of the most commercially exploited sage plants referred as Greek or Mediterranean or wild sage. In the island of Crete (Greece) *S. fruticosa* is very abundant especially in the phryganic ecosystems and it is collected in summer by the villagers for additional income. The species is used traditionally all over the Mediterranean Region (1). In Crete, the essential oil or the infusion of the aerial part of the plant is used, against cold, cough, gingivitis, toothache, throatache, stomachache, abdominal pains, diarrhoea, diabetes, hypertension, rheumatism and skin diseases.

Study of the leaf essential oil yield and composition from approx. sixty plants belonging to three populations of *S. fruticosa*, revealed that a considerable variation exist between populations as well as within populations. Although variable, all plants examined, were very rich in essential oil ranging from 2.5% to 6.0% ml/g dw. Of the 26 monoterpenes and sesquiterpenes identified β-pinene, L-phellandrene, 1,8-cineole, α-terpineol and terpinyl acetate differ significantly among the three populations while camphene, sabinene, α-thujone, β-thujone, camphor and borneol are highly variable within each population. Similar variation has been observed in samples collected from Cyprus and other parts of Greece (2). All essential oils were characterised by high 1,8-cineole content (30% to 54%). Detailed investigation of the essential oil composition, however, has shown three distinct chemotypes: a 1,8-cineole rich type, a 1,8-cineole/thujones and a 1,8-cineole/camphor rich type.

The results of this study show that in case of commercial exploitation of *S. fruticosa*, selected clones (based on chemical criteria) should be cultivated in order to obtain crops with homogeneous essential oil production.

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AROMA TYPES IN ESSENTIAL OILS OF THE BULGARIAN REPRESENTATIVES OF THE *MENTHA SPICATA* GROUP

T. Stoeva[1], E. Molle[1], B. Konovska[2]

Institute of Botany, Bulgarian Academy of Sciences
[1] Faculty of Biology, University of Sofia
[2] "Bulgarska Roza", Sofia

In the period between 1992 and 1994 the research was carried out on the wide spectrum of aroma of essential oil extracted from wild representatives of *Mentha spicata* group.

The object of the study were 51 samples taken from 13 habitats in 3 floristic regions of Bulgaria.

As a result of organoleptic analysis 4 basic types of essential oil were established: carvon (C), linalool (L), menthol (M) and mixed (X). The ratio between these types of oil differs depending on the taxonomic status of the species within the *Mentha spicata* group. The carvon type (C) is the dominant one.

The results of the study show that there is a wide range of possibilities for the collection of valuable initial material with special aroma serving the needs of pharmaceutical, food-processing, wine-producing and tobacco industries.

COMPOSITION OF THE ESSENTIAL OIL OF *ARTEMISIA MARITIMA* L. FROM FANØ

H. Surburg[1], M. Güntert[1]

[1] Haarmann & Reimer GmbH, Corporate Research, D-37601 Holzminden, Germany

Artemisia maritima L. ("beach wormwood") is a small shrub which grows in maritime regions especially on sandy and salty soils. On Fanø, a Danish island in the North Sea, the plant (local name: "strandmalurt") occurs abundantly on the north-east coast where the beach region meets the marsh and the land is sometimes flooded by the tide. The aerial parts of the plant have a fresh-spicy herbaceous odour and a characteristic strongly bitter taste. The locals use the dried herb as an ingredients for preparing alcoholic infusions which are consumed as bitter drinks ("morning bitter").

We have investigated an essential oil which we have obtained by a Likens-Nickerson distillation of the blooming herb. By means of GC and GC/MS we have identified about 45 constituents, mainly monoterpenoids. Main constituents of the oil are chrysanthenone (33%) which is mainly responsible for the fresh herbaceous note and davanone (11%). Other characteristic constituents comprise α-thujone (3%), filifolone (2%), ascaridol (4%) and germacrene-D (3.5%). Eugenol (0.4%) and cis-jasmone (1.4%) are organoleptically important minor constituents.

ANALYTICAL CHARACTERIZATION OF TEA FLAVOURINGS USED IN TEA SOFT DRINKS

F. Tateo[1], G. Cantele[1], M. Bononi[1]

[1] Dipartimento di Fisiologia delle Piante Coltivate e Chimica Agraria. Sezione di Chimica Analitica Agroalimentare ed Ambientale, Facolta di Agraria - Universita degli Studi di Milano, via Celoria 2, 20133 Milano, Italia

In some European countries different fruit flavoured "tea soft drinks" has turned out to be of large use. The analytical control of such products can have different purposes. One of the most important is to follow the formulation trend of the "tea flavouring" employed for the flavouring of such beverages.

Therefore different tea flavouring among the most important on the market have been taken into account and have been quite sufficiently analytically characterized by GC/MS. At the same time the fraction of volatile compounds extractable from commercial tea soft-drinks has been examined and the efficiency of the extraction has been verified with different comparative methods. The results have pointed out the existence of quantitative data substantially different for what concerns the composition of volatile compounds extracted with various methods.

The results have pointed out that tea flavourings tend to reproduce at least qualitatively the composition of the flavoured fraction of natural tea. Some interesting considerations have come out as regards some components such as linalool, (Z) and (E) linalool oxide and others studies from the point of view of the chiral analysis.

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SYNTHESIS AND ODOR CHARACTERISTICS OF SOME ISOPRENOIDS AND THEIR SILA ANALOGUES

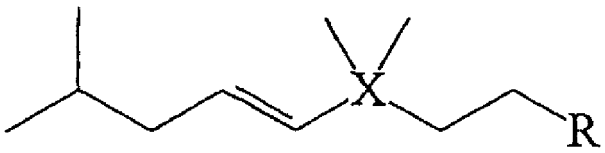
Robert OBARA [1], *Stanisław LOCHYŃSKI* [2], *Czesław WAWRZEŃCZYK* [3]

[1] Institute of Chemistry, Pedagogical University, Kielce, Poland

[2] Institute of Organic Chemistry, Biochemistry and Biotechnology, Technical University, Wroclaw, Poland

[3] Institute of Fundamental Chemistry, Agricultural University, Norwida 25, 50-375 Wroclaw, Poland

The synthesis of (E)-3,3,7-trimethyl-4-octen-1-ol (**1**) and its 3-sila analogue (**2**) is presented. Alcohol **1** was obtained from 3,3-dimethyl-acrylaldehyde in four step synthesis. Its 3-sila analogue **2** was synthesized from 4-methyl-1-pentyne. These alcohols were starting materials for syntheses of acetates **3**, **4** and acetals **5**, **6**, **7**, **8**.



1. X=C; R=OH
2. X=Si; R=OH
3. X=C; R=OAc
4. X=Si; R=OAc
5. X=C; R= -O-CH(CH_3)-O-CH_3
6. X=Si; R= -O-CH(CH_3)-O-CH_3
7. X=C; R= -O-CH(CH_3)-O-*n*Bu
8. X=Si; R= -O-CH(CH_3)-O-*n*Bu

All new derivatives possessed interesting olfactory properties. Syntheses and odor characteristics of compounds obtained are also presented.

26th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

Hamburg / Germany

September 10 - 13th, 1995

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

Program

Scientific program

Monday	**September 11, 1995**	
9.30	Opening Session Welcome Addresses	
10.00	*Chairmen: A. Baerheim Svendsen and J.J.C. Scheffer* Opening lecture P. WEYERSTAHL, Berlin, Germany Structure elucidation of novel compounds exemplified on the essential oil of *Lantana camara*	L-01
10.50	Coffee break	
11.10	W. FRANCKE, Hamburg, Germany Identification of bicyclic acetales from insects and plants	L-02
11.35	A.J. MARSAIOLI, Campinas, Brazil A novel class of nor-sesquiterpenes	L-03
12.00	Lunch break	
14.00	*Chairmen: C. Bicchi and R. Näf* Plenary lecture Y. ASAKAWA, Tokushima, Japan Biotransformation of terpenoids and aromatic compounds by microorganisms and mammals	L-04
14.50	J.C.R. DEMYTTENAERE, Gent, Belgium Biotransformation of monoterpenoids by fungal spores	L-05
15.15	T. VAN DEN BERG, Würzburg, Germany Accumulation of volatiles in root cultures, regenerated from callus of *Melissa officinalis* L. (Lamiaceae)	L-06
15.40	J.I. OKOGUN, Ibadan, Nigeria The volatile oil constituents of *Diodia sarmentosa*	L-07
16.05	Coffee break	
16.30	J. SEEBODE, Krefeld, Germany FT-IR gas chromatographic analysis of perfumes	L-08
16.55	J. OESSELMANN, Bremen, Germany IRMGCMS: A technique for ^{13}C and ^{16}N isotope determination on complex GC mixtures	L-09
17.20	B. FABER, Frankfurt, Germany GC-IRMS and enantioselective analysis in the authenticity control of Peppermint oil	L-10
17.45	B. VONACH, Greifenberg, Germany An intelligent sensor system for the characterization of aroma	L-11

Tuesday	September 12, 1995	
	Chairmen: S.G. Deans and R. Hiltunen	
9.00	Plenary lecture W.A. KÖNIG, Hamburg, Germany Occurrence of sesquiterpene enantiomers and their identification	L-12
9.50	K.P. SVOBODA, Auchincruive, Scotland Composition and potential uses of the essential oil of Scottish *Myrica gale* (L.) harvested from the wild population	L-13
10.15	K.H.C. BASER, Eskisehir, Turkey Pulgegone-rich essential oils of Turkey	L-14
10.40	Coffee break	
11.00	S.T. KATSIOTIS, Chania, Greece Distillation effects on the yield and the composition of *Eucalyptus citriodora* Hook essential oil	L-15
11.25	C.-C. CHYAU, Taichung, Taiwan Characteristics of the essential oil from the fruits of *Zanthoxylum simulans*	L-16
11.50	B.L. BRADU, Jammu Tawi, India Search for new aroma chemicals from genus Ocimum: Its past potential and future prospectus	L-17
12.15	Lunch break	
	Chairmen: P. Weyerstahl and B.L. Bradu	
14.10	Plenary lecture C. BICCHI, Turin, Italy Reliability of gas chromatographic data in the analysis of essential oils	L-18
15.00	J. ILMBERGER, München, Germany Essential Oils: evaluation of substances and their influence on human alertness	L-19
15.25	Coffee break	
15.45 - 18.30	Formal poster sessions	
20.00	Symposium dinner	

Wednesday September 13, 1995

Chairmen: G. Buchbauer and Ch. Franz

9.00	Plenary lecture W. BOLAND, Bonn, Germany Induction of plant volatiles by herbivores and jasmonates	L-20
9.50	H.J. BOUWMEESTER, Wageningen, The Netherlands Developmental changes in activity of enzymes involved in carvone biosynthesis in caraway	L-21
10.15	E. NEMETH, Budapest, Hungary The role of vernalization in generative development of caraway (*Carum carvi* f. *biennis*)	L-22
10.40	Coffee break	
11.00	E. SHAAYA, Bet-Dagan, Israel Essential oils and their constituents as effective fumigants for the control of stored-product insects	L-23
11.25	M. KOLALYTE, St. Petersburg, Russia Comparative ultrastructure of glandular trichomes of *Dracocephalum moldavia* L., *Nepeta cataria* L. and *Nepeta cataria* var. *citriodora* Balb. in connection with essential oils biosynthesis	L-24
11.50	A.A. ABENA, Brazzaville, Congo Analgesic and anti-inflammatory activities of essential oil of *Ageratum conyzoides* L.	L-25
12.15	K. SCHNAUBELT, San Rafael, USA Aromatherapy - plant medicine or esoteric hoax. Examining the scientific basis of a popular phenomenon	L-26
12.40	Closing session / forthcoming meetings	
13.00	Lunch break	
15.00	Excursion	

Lectures

L-01 P. WEYERSTAHL, C. CHRISTIANSEN, A. ECKHARDT, H. MARSCHALL
Structure elucidation of novel compounds exemplified on the essential oil of *Lantana camara*

L-02 W. FRANCKE, F. SCHRÖDER, S. SCHULZ
Identification of bicyclic acetals from insects and plants

L-03 V.L. FERRACINI, M.A. FOGLIO, A.J. MARSAIOLI, A. DE MEIJERE, K. RAUCH
A novel class of nor-sesquiterpenes

L-04 Y. ASAKAWA
Biotransformation of terpenoids and aromatic compounds by microorganisms and mammals

L-05 J.C.R. DEMYTTENAERE, H.L. DE POOTER
Biotransformation of monoterpenoids by fungal spores

L-06 T. VAN DEN BERG, A.A. ABOU-MANDOUR, F.C. CZYGAN
Accumulation of volatiles in root cultures, regenerated from callus of *Melissa officinalis* L. (Lamiaceae)

L-07 T.O.E. EKPENDU, A.A. ADESOMOJU, O. EKUNDAYO, J.I. OKOGUN, I. LAAKSO
The volatile oil constituents of *Diodia sarmentosa*

L-08 J. SEEBODE, K. KRISHNAN
FT-IR gas chromatographic analysis of perfumes

L-09 A.W. HILKERT, J. OESSELMANN
IrmGCMS: A technique for ^{13}C and ^{15}N isotope determination on complex GC mixtures

L-10 B. FABER, A. MOSANDL
GC-IRMS and enantioselective analysis in the authenticity control of peppermint oil

L-11 B. VONACH
An intelligent sensor system for the characterization of aroma

L-12 W.A. KÖNIG, C. FRICKE, I.H. HARDT, S. MELCHING, A. RIECK, Y. SARITAS
Occurrence of sesquiterpene enantiomers and their identification

L-13 K.P. SVOBODA, A. INGLIS, J. HAMPSON, S.G. DEANS, B. GALAMBOSI, I. BRANSON
Composition and potential uses of the essential oil of Scottish *Myrica gale* (L.) harvested from the wild population

L-14 K.H.C. BASER, N. KIRIMER, G. TÜMEN
Pulegone-rich essential oils of Turkey

L-15 R. MEJDOUB, S.T. KATSIOTIS
Distillation effects on the yield and the composition of *Eucalyptus citriodora* Hook essential oil

L-16 C.C. CHYAU, J.L. MAU, C.M. WU
Characteristics of the essential oil from the fruits of *Zanthoxylum simulans*

L-17 B.L. BRADU
Search for new aroma chemicals from genus ocimum: Its past potential and future prospectus

L-18 C. BICCHI, A. BINELLO, M. FRESIA
Reliability of analytical data in the analysis of essential oils

L-19 J. ILMBERGER, E. HEUBERGER, G. BUCHBAUER
Essential oils: Evaluation of substances and their influence on human alertness

L-20 W. BOLAND, J. HOPKE, T. KOCH, T. KRUMM, J. PIEL
Induction on plant volatiles by herbivores and jasmonates

L-21 H.J. BOUWMEESTER, J. GERSHENZON, M.C.J.M. KONINGS, R. CROTEAU
Developmental changes in activity of enzymes involved in carvone biosynthesis in caraway

L-22 E. NEMETH, J. BERNATH
The role of vernalization in generative development of caraway (*Carum carvi* f. *biennis*)

L-23 E. SHAAYA, M. KOSTJUKOVSKY
Essential oils and their constituents as effective fumigants for the control of stored-product insects

L-24 M. KOLALYTE
Comparative ultrastructure of glandular trichomes of *Dracocephalum moldavia* L., *Nepeta cataria* L. and *Nepeta cataria* var. *citriodora* Balb. in connection with essential oils biosynthesis

L-25 A.A. ABENA, J.M. QUAMBA, A. KEITA
Analgesic and anti-inflammatory activities of essential oil of *Ageratum conyzoides* L.

L-26 K. SCHNAUBELT
Aromatherapy - plant medicine or esoteric hoax. Examining the scientific basis of a popular phenomenon

Posters

P-01 J.G. BARROSO, A.C. FIGUEIREDO, L.G. PEDRO, C.A. RIBEIRO, J.J.C. SCHEFFER
Essential oil composition of *Juniperus brevifolia*, an endemic species of the Azores

P-02 J.A.T. DA SILVA, A.C. FIGUEIREDO, J.G. BARROSO, L.G. PEDRO, M. AVILA GOMES, S.S., FONTINHA, J.J.C. SCHEFFER
Essential oil composition of *Melanoselinum decipiens*, an endemic species of the Madeira and Azores archipelagos

P-03 L.G. PEDRO, M.S. PAIS, J.J.C. SCHEFFER
Accumulation of volatiles in cell suspension cultures of *Geranium robertianum* var. *purpureum*

P-04 D. BARTSCHAT, D. LEHMANN, A. DIETRICH, A. MOSANDL
The scent of orchids - Chirospecific analysis, structure and properties of 4-methyl-5-decanolides

P-05 B. FABER, C. FRANK, A. MOSANDL
Recent developments in the authenticity control of essential oils

P-06 P. KREIS, A. DIETRICH, A. MOSANDL
Chiral compounds of essential oils: Elution order of furanoid linalol oxides on common gas chromatographic phases and modified cyclodetrin phases

P-07 A. BELANGER, S. KHANIZADEH
Classification of sixty strawberry genotypes based on their essential oil composition and degreee of mite susceptibility

P-08 C. BICCHI, A. D'AMATO, V. MANZIN
Some Applications of cyclodetrin derivatives in GC enantioseparations of essential oils

P-09 C. BICCHI, M. FRESIA, P. RUBIOLO, O. PANERO
Preliminary results in the study of the composition of *Artemisia roxburghiana* essential oil

P-10 I. GOEHLER, CH. FRANZ, P. RUBIOLO, C. BICCHI
Variability of the essential oils of *Tagetes lucida* Cav. from Guatemala

P-11 G. BINDER, T. VAN DEN BERG, A. ABOU-MANDOUR, F.-C. CZYGAN
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New investigations on the liverworth *Lophocolea bidentata*

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P-53 S. KISTLER
Essential oil production of *Chamomilla recutita* (L.) Rauschert in Germany

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Essential oils of three *Thymus kotschyanus* varieties from Turkey

P-58 M. MIYAZAWA, A. TAKAHATA, T. URANO
Flavour components of Japanese traditional food, dried and roasted sheet "Nori" (*Porhyla yezoensis* F. Narawaensis)

P-59 M. MIYAZAWA, H. NANKAI, H. KAMEOKA
Microbial transformations of acyclic terpenoids by *Glomerella cingulata*

P-60 O.A. ONAYADE
Trans, 2,trans, 4-decadienoic acid isobutyl ester and other constituents of the root essential oil of *Zanthoxylum zanthoxyloides* Lam. (Waterm.) family Rutaceae)

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Analysis of Essential oil of *Nepeta nuda* ssp. *glandulifera* Davis.

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Chemical composition of essential oil from *Calamintha tauricola*

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The essential oil of *Eupatorium adenophorum* Spreng.

P-66 E. SEZIK, M. ASLAN
Chemical composition of the *Helichrysum plicatum* D.C. ssp. *plicatum* essential oil

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Inter- and infra-population variation of the essential oil yield and composition of *Salvia fruticosa* Mill. in Crete

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Aroma types in essential oils of the Bulgarian representatives of the *Mentha spicata* group

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Composition of the essential oil of *Artemisia maritima* L. from Fanø

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General Information

Location and time

The conference and poster session will be held at the Department of Chemistry, Martin-Luther-King-Platz 6. The symposium will commence on Monday, September 11th at 9.30 and close at 13.00, Wednesday, September 13th.

Registration

The registration desk will be located in the lounge of the lecture halls, Martin-Luther-King-Platz 6. It is open on Sunday, September 10th from 17.00 - 19.00. From Monday to Wednesday it will be open from 8.30 onwards; it closes on Wednesday, September 13th at 12.00; phone: 410 76 39 .

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

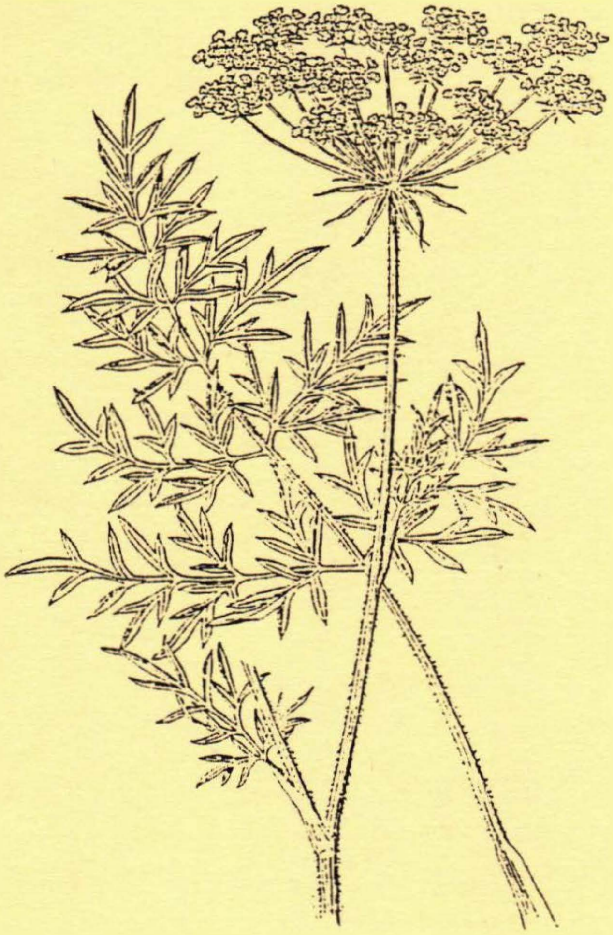
The poster boards are located in the passage way next to the porter's office. They will be labelled corresponding to the numbers listed in the program. Please prepare your poster on Monday mornig, September 11th. You are required to be present at your poster during the formal poster session for discussion of your data, on Tuesday, September 12th from 15.45 to 18.00 h.

Exhibition

An exhibition will be held during the meeting from Monday to Wednesday, September 11-13, in the lounge of the lecture halls. Manufacturers and distributors of analytical equipment will show and explain their products.

Lunch

The registration does not include meals. In the vicinity of the conference building are several restaurants (see enclosed leaflet). Furthermore, lunches will be offered during the conference in the near student's dining hall at Van-Melle-Park and Bundesstrasse 55 (GEOMATIKUM).



26th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

September 10 - 13, 1995
University of Hamburg
Hamburg, Germany

First Circular

Call for Papers

February 1995

Organization and Mailing Address:
Prof.Dr. K.-H. Kubeczka
University of Hamburg
Department of Pharmaceutical Biology
Bundesstrasse 43
D-20146 Hamburg, Germany

Tel.: 49-(0)40-4123-3899
Fax.: 49-(0)40-4123-2893

Invitation

After the many successful meetings in Finland, Germany, Italy, Scotland, Switzerland, The Netherlands and last year in France, the 26th International Symposium on Essential Oils will be held in September 10-13, 1995 at the University of Hamburg in Germany. It is therefore a great honour for the Organizing Committee to cordially invite you to participate in this forthcoming meeting.

Call for Papers

The scientific program traditionally deals with all aspects concerning essential oils and similar natural products: occurrence, botany, analysis, fields of application. Furthermore, the use of computers in essential oil research shall be discussed as a special topic of the symposium.
Participants wishing to present a communication (15 min) or a poster are requested to submit the title and a camera-ready copy of the abstract.

Deadline for announcement of the definite title: May 15, 1995
Deadline for submitting the abstract (one page): June 30, 1995

Location

The conference and poster session will be held at the Chemistry Department of the University of Hamburg, Martin-Luther-King-Platz 6, close to the center of the city.

Language

The official language of the Symposium is English. No simultaneous translation will be provided.

Provisional program:

Sunday, September 10, 1995:	Arrival in Hamburg
5.00-7.00 pm	Registration
7.00-9.00 pm	Get-Together-Party
Monday, September 11, 1995:	
9.00-10.00 am	Opening Session
	Welcome Addresses
10.00 am	Scientific communications
Afternoon	Scientific communications
Evening	Free
Tuesday, September 12, 1995:	Continuation
Full day	Scientific communications
Afternoon	Poster presentation
Evening	Symposium dinner
Wednesday, September 13, 1995:	Continuation
Morning	Scientific communications
Afternoon	Special scientific topics
	alternatively: Excursion

Technical Aids

The following technical equipments are at disposal:

- slide projector (5 x 5 cm)
- Overhead projector
- Pin-up walls for posters

Registration

A pre-registration form is enclosed. Please return the form as soon as possible.

The registration fees are as follows

Full participants	180,- DM
Accompanying persons	60,- DM
Students (with legitimation)	50,- DM

The fee includes one copy of lecture and poster abstracts and attendance at the informal welcome on Sunday, September 10.

Luncheons

In the vicinity of the Chemistry Department you will find several restaurants and a students' dining hall serving lunch at reasonable prices.

Accomodation

Registrants are expected to make their own arrangements for hotel accomodation. Reservation forms will be enclosed to the 2nd circular.

Please note:

Participants of the annual meeting of the GA in Halle, Germany, may reach Hamburg by car or by train without any difficulty.

26th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

September 10 - 13, 1995
University of Hamburg
Hamburg, Germany

Second 

Circular

June 1995

Organization and Mailing Address:
Prof.Dr. K.-H. Kubeczka
University of Hamburg
Department of Pharmaceutical Biology
Bundesstrasse 43
D-20146 Hamburg, Germany

Tel.: 49-(0)40-4123-3899
Fax.: 49-(0)40-4123-3895 (new number!)

26th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS
September 10-13, 1995, HAMBURG, GERMANY

Organizing Committee

W. Francke, Hamburg
W.A. König, Hamburg
Chr. Kubeczka, Hamburg
K.-H. Kubeczka, Hamburg
W. Schultze, Hamburg
E. Stahl-Biskup, Hamburg

General Information

With the second circular we are pleased to give you more information on the 26th International Symposium on Essential Oils, September 1995 in Hamburg to facilitate your decision to attend the symposium. You are cordially invited to fill in the enclosed registration and accommodation forms as soon as possible. Only those participants who have **registered and paid** their fees will be allowed to attend the congress. The official language of the Symposium is English.

Scientific Program - The following plenary lectures are scheduled:

Prof.Dr. Yoshinori ASAKAWA, Tokushima, Japan
Biotransformation of terpenoids and aromatic compounds by microorganisms and mammals - preparation of bioactive compounds

Prof.Dr. Carlo BICCHI, Turin, Italy
Reliability of analytical data in the analysis of essential oils

Prof.Dr. Wilhelm BOLAND, Bonn, Germany
Induction of plant volatiles by herbivores and jasmonates

Prof.Dr. Wilfried KÖNIG, Hamburg, Germany
Occurrence of sesquiterpene enantiomers and their identification

Prof.Dr. Peter WEYERSTAHL, Berlin, Germany
Structure elucidation of novel compounds, exemplified on the essential oil of Lantana camara

In addition, more than 20 oral communications (20 minutes) and more than 70 posters have been announced. The organizers will try to enable all contributors to present their posters. In any case, the final decision of the scientific committee and instructions will be mailed directly to those who have announced a presentation. Final **deadline** for submission of **camera-ready abstracts: 15.07.95**

Provisional Program

Sunday, September 10, 1995:

17.00 - 19.00	Registration at the Symposium Office
19.00 - 21.00	Get-Together-Party

Monday, September 11, 1995:

9.30 - 10.00	Opening Session
10.00 - 12.00	Scientific communications
14.00 - 18.00	Scientific communications and informal poster session

Tuesday, September 12, 1995:

9.00 - 12.00	Scientific communications and informal poster session
14.00 - 16.30	Scientific communications and informal poster session
16.30 - 18.30	Formal poster session
20.00	Symposium dinner

Wednesday, September 13, 1995:

9.00 - 13.00	Scientific communications
15.00 - 19.00	Half-day excursions within Hamburg

General Information

Location and time

The conference, the poster session and the get-together-party will be held in the Chemistry Department of the University of Hamburg (Martin-Luther-King-Platz 6, Hamburg). A map of the university area is enclosed.
The conference will commence at 9.30, Monday, September 11th and close on Wednesday, September 13th, 1995.

Congress office

Sunday, September 10th, 1995, 17.00 - 19.00 and from Monday 8.30 to Wednesday 13.00 in the Chemistry Department of the University of Hamburg, Martin-Luther-King-Platz 6.

Registration

Registration forms are enclosed. The registration fee is 180,- DM for participants, 60,- DM for accompanying persons and 50,- for students with legitimation. All charges due to bank transfers have to be paid by the participants. The fee includes one copy of lecture and poster abstracts and attendance at the get-together-party.

Accommodation

Registrants are expected to make their own arrangements for hotel accommodation. Reservation forms are enclosed. The reservation fee is DM 6,- per room. All participants are requested to follow strictly the rules for any change or cancellation indicated on the confirmation letter you will get from the "Tourismus-Zentrale Hamburg". **The organizers are in no respect responsible for any accommodation problem.**

Abstracts

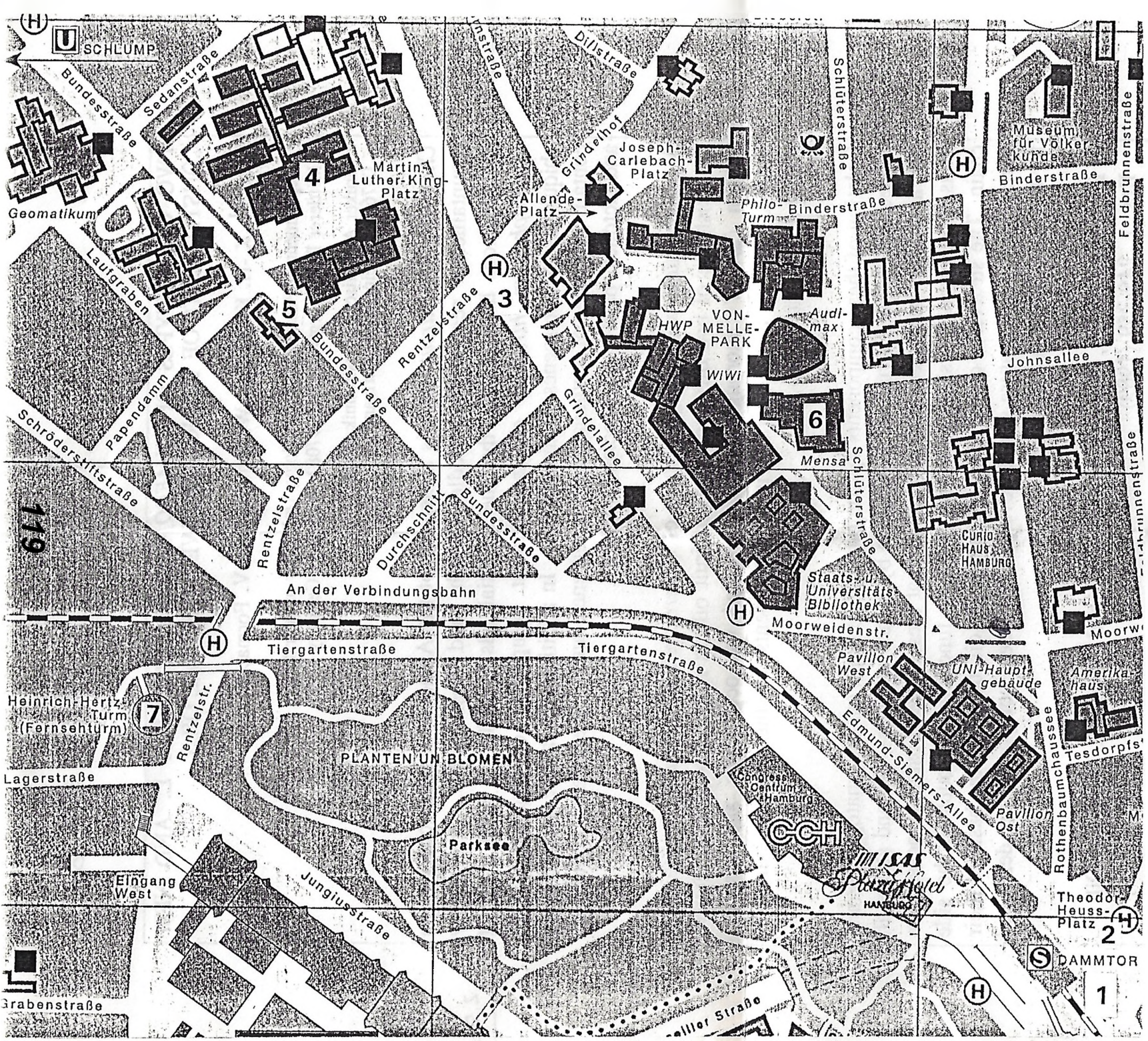
Participants wishing to present a communication or a poster are requested to submit a camera-ready abstract in English of about 200 words (cf. sample copy) **not later than July 15, 1995.** Underline the speaker if more than one author.

Poster presentation

The poster boards will be approximately 1.20 to 1.20 m. Prepare in advance for the top of your poster space a large label indicating the title of your contribution, author(s) and affiliation.

Exhibition

A few selected manufacturers of analytical equipment will show their products during the congress.



1 Railway and "S-Bahn" Station Dammtor

2 Bus stop "Dammtor" (Bus 102)

3 Busstop "Grindelhof"

4 Chemistry Department Martin-Luther-King-Platz 6

5 Department of Pharmaceutical Biology Bundesstrasse 43

6 Students' dining hall

7 Television tower

3 cm

Sample copy

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

I. Bohn[1], K.-H. Kubeczka[1], W. Schultze[1] and V. Formacek[2]

[1] Department of Pharmaceutical Biology, University of Würzburg, Mittlerer Dallenbergweg 64, D-97082 Würzburg, Germany

[2] Bruker Analytische Messtechnik GmbH, D-76287 Rheinstetten, Germany

3 cm

2,5 c.

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 ^{1}H- and ^{13}C-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.,**1**, 249-259 (1989)

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

3 cm

26th International Symposium on Essential Oils
26th ISEO, Hamburg 1995
List of participants

Dr. A.A. Abena
Faculty of Medicine
University Marien Ngouabi
B.P. 69
Brazzaville
Congo
Tel.: 00242-82-18-02
Fax.: 00242-81-18-02

Stanley Allured
Perfumer & Flavourist
South Schmale Road
Carol Stream, IL 60188
USA
Tel.: 001-708-653-2155
Fax.: 001-708-653-2192

Prof.Dr. Y. Asakawa
Faculty of Pharm. Sciences
Tokushima Bunri University
Yamashiro-cho
Tokushima 770
Japan
Tel.: 0081-886-22-9611 (5522)
Fax.: 0081-886-55-3051

Prof.Dr. A. Baerheim Svendsen
University of Oslo
Inst. of Pharmacy
P.O.Box 1068
0316 Oslo
Norway
Tel.: 0047-2285-7499
Fax.: 0047-2285-7494

A.D. Balinova-Tzvetkova
Inst. for Roses, Aromatic and
Medicinal Plants
6100 Kazanlik
Bulgaria
Tel.:
Fax.:

Prof.Dr. Barroso
Dep. Biologica Vegetal
Fac. Ciencias Lisboa
Bloco C2, Piso 1, Campo Grande
1780 Lisboa
Portugal
Tel.: 0035-11-7500069
Fax.: 0035-11-7500048

Dietmar Bartschat
Inst. f. Lebensmittelchemie
Joh.-Wolfg.-Goethe-Universität
Martie-Curie-Straße 9
60439 Frankfurt a. M.
Tel.: 0049-60-789-29208
Fax.: 0049-60-789-29207

Prof.Dr. K.H.C. Baser
Med. Plant Research Centre
Anadolu University
TR-26470 Eskisehir
Turkey
Tel.: 0090-22-335-2952
Fax.: 0090-22-335-0127

Dr. Christian Becker
British-American-Tobacco GmbH
Bahrenfelder Chaussee 139
D-22761 Hamburg
Germany
Tel.: 0049-40-4151-4105
Fax.: 0049-40-4151-4400

André Bélangér
Centre de R & D en hortic.
430, Boulevard Gouin
Saint-Jean-sur-Richelieu
Quebec Canada J3B 3EJ
Tel.: 001-514-346-7740
Fax.: 001-514-346-4494

Thomas van den Berg
Pharmazeutische Biologie
Universität Würzburg
Mittlerer Dallenbergweg 64
D-97082 Würzburg
Germany
Tel.: 0049-931-888-6172
Fax.: 0049-931-888-6158

Prof.Dr. J. Bernath
Univ. Horticulture and
Food Industry
Villanyi Str. 29
H-1114 Budapest
Hungary
Tel.: 0036-1-166-4998
Fax.: 0036-1-166-4998

Prof.Dr. C. Bicchi
Dip. Scienza e Tecnologia
Del Farmaco
Via P. Giuria 9
I-10125 Torino
Italy
Tel.: 0039-11-6707-662
Fax.: 0039-11-6707-687

G. Binder
Pharm. Biologie
Universität Würzburg
Mittlerer Dallenbergweg 64
D-97082 Würzburg
Germany
Tel.: 0049-931-888-6158
Fax.: 0049-931-888-6158

Christina Bischof
Dept. Pharmaceutical Biology
University of Hamburg
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-3892
Fax.: 0049-40-4123-3895

Dr. Dieter Bloß
G. Pohl-Boskamp GmbH & Co.
Kieler Straße 11
D-25551 Hohenlockstedt
Germany
Tel.: 0049-4826-59102
Fax.: 0049-4826-59109

Carsten Blum
University of Hamburg
Pharmaceutical Biology
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-4313
Fax.: 0049-40-4123-3895

Prof.Dr. W. Boland
Institut f. Organ. Chemie
und Biochemie
Gerhard-Domagk-Straße 1
D-53121 Bonn
Germany
Tel.: 0049-228-735 388
Fax.: 0049-228-735 300

R. Bos
Dept. Pharmaceutical Biology
Univ. Centre for Pharmacy
Ant. Deusinglaan 2
9713 AW Groningen
The Netherlands
Tel.: 0031-50-633 354
Fax.: 0031-50-636 908

Harro J. Bouwmeester
Research Inst. for Agrobiology
Soil Fertility
P.O.Box 14
6700 AA Wageningen
The Netherlands
Tel.: 0031-8370-75882
Fax.: 0031-8370-23110

Dr. B. Bradu
Regional Research Laboratory
Canal Road
Jammu Tawi-180 001
India
Tel.: 0091-544-382
Fax.: 0091-191-48006

Herbert Brandauer
Erich Ziegler GmbH
Am Weiher 133
D-91347 Aufsess
Germany
Tel.: 0049-9198-9294-0
Fax.: 0049-9198-239

Prof.Dr. K. Bruns
Inst. f. Organische Chemie
Universität Düsseldorf
Universitätsstraße 1
D-40225 Düsseldorf
Germany
Tel.: 0049-2151-563 477
Fax.: 0049-2151-564 542

Jürgen Brandt
University of Hamburg
Pharmaceutical Biology
Bundesstrasse 43
D-20146 Hamburg
Tel.: 0049-40-4123-3894
Fax.: 0049-40-4123-3895

Dr. Ph.D. Ivan Buben
Statni ustav pro kontrolu leciv
Srobarova 48
10041 Praha
Czech Republic
Tel.: 00412-67310578-2854
Fax.: 00412-744 944

Dr. F. Bucar
Inst. f. Pharmakognosie
Universität Graz
Universitätsplatz 4/I
A-8010 Graz
Austria
Tel.: 0043-316-380-5533
Fax.: 0043-316-388 719

Prof.Dr. G. Buchbauer
Inst. f. Pharm. Chemie
Pharmaziezentrum
Althanstraße 14
A-1090 Wien
Austria
Tel.: 0043-1-31336-8610
Fax.: 0043-1-31336-771

Nils Bülow
University of Hamburg
Inst. of Organic Chemistry
Martin-Luther-King-Platz 6
D-20146 Hamburg
Tel.: 0049-40-4123-2831
Fax.: 0049-40-4123-2893

Prof.Dr. M.C. Cantoria
University of the Philippines
College of Pharmacy
P.O.Box 135
Diliman 1101
Quezon City, Philippines
Tel.: 0063-2-921-6564
Fax.: 0063-2-837-3170

Prof.Dr. Zhong-Liang Chen
Haus Müller
Deutschhausstrasse 29
D-35037 Marburg/Lahn
Germany
Tel.: 0049-6421-61102
Fax.:

Dr. Christian Christiansen
Firmenich S.A.
CP 239
1211 Genf
Switzerland
Tel.: 0041-22-780-3471
Fax.: 0041-22-780-3334

Prof.Dr. C.-C. Chyau
Dept. of Food Nutrition
Huang-Kuang Inst. of Med. and Technology
Taichung, Taiwan
Republic of China
Tel.: 00886-4-631-5017
Fax.: 00886-4-631-5017

Dr. S.G. Claude
Inst. de Chimie
Université de Neuchatel
Av. de Bellevaux 51
2000 Neuchatel
Switzerland
Tel.: 0041-38-23 24 00
Fax.: 0041-38-23 25 11

N.F. Collins
Grassroots Natural Products
P.O.Box 16
Gouda 6821
South Africa
Tel.: 0027-236-320 506
Fax.: 0027-236-320 129

Prof.Dr. A.P. Cunha
Faculdade de Farmacia Coimbra
R. Do Norte
3000 Coimbra
Portugal
Tel.: 00351-39
Fax.: 00351-39-27126

Dr. Cvetana Daskalova
Inst. of Botany BAS
23 Acad. G. Bouchev Str.
BG-1113 Sofia
Bugaria
Tel.: 00359-2-7132146
Fax.: 00359-2-758140

Dr. S.G. Deans
Aromatic and Medicinal Plant Group
Dept. Biochem. Sciences
Scott. Agriculture College
Auchincruive, Ayr KA6 5HW
Scotland, UK
Tel.: 0044-1292-520-331
Fax.: 0044-1292-520-640

Peter Degenkolb
Inst. f. Organische Chemie
Straße des 17. Juni 124
D-10623 Berlin
Germany
Tel.: 0049-30-314-236 22
Fax.: 0049-30-314-236 19

Dr. J. Demyttenaere
Faculty of Agricultural and Applied Biological Sciences
Dept. Organic Chemistry
Coupure Links 653
B-9000 Gent
Belgium
Tel.: 0032-9-264-5964
Fax.: 0032-9-264-6943

Daniel Dillenseger
ADRIAN GmbH
Moorhof 2b
D-22399 Hamburg
Germany
Tel.: 0049-40-606 60 86-88
Fax.: 0049-40-602 63 45

Helmut Dittmann
University of Hamburg
Inst. of Organic Chemistry
Martin-Luther-King-Platz 6
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-2831
Fax.: 0049-40-4123-2893

Dr. R. Eberhardt
Fa. Akras
Internationale Essenzenfabrik
Postfach 104
A-2340 Mödling
Austria
Tel.: 0043-2236-62550-230
Fax.: 0043-2236-62443

Birgit Faber
Inst. f. Lebensmittelchemie
J.-W.-Goethe-Universität
Marie-Curie-Straße 9
D-60439 Frankfurt a. M.
Germany
Tel.: 0049-69-798-20206
Fax.: 0049-69-789-29207

Dr. C.A. Figueiredo
Dep. Biological Vegetal
Fac. Ciencias Lisboa
Campo Grande
1780 Lisboa
Portugal
Tel.: 00235-1-175 000 69
Fax.: 00235-1-175-000 48

Andrea Fock
University of Hamburg
Pharmaceutical Biology
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-3894
Fax.: 0049-40-4123-3895

Csaba Fodor
Silvestris & Szilas Ltd.
P.O.B. 10
Vasut ut. 42
H-2143 Kerepsetarcsa
Hungary
Tel.: 0036-28-370-389
Fax.: 0036-28-370-099

Prof.Dr. W. Francke
Inst. of Organic Chemistry
University of Hamburg
Martin-Luther-King-Platz 6
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-2822
Fax.: 0049-40-4123-2893

Dr. Bruno Frank
Kneipp-Werke
Steinbachtal 43
D-97082 Würzburg
Germany
Tel.: 0049-9311-8002-271
Fax.: 0049-9311-8002-275

Prof.Dr. Ch. Franz
Inst. f. Botanik u. Lebensmittelk.
Veterinärmedizinische Universität
Linke Bahngasse 11
A-1030 Wien
Austria
Tel.: 0043-1-71155-270
Fax.: 0043-1-714-9105

M. Freiler
Inst. f. Pharmakognosie
Universität Wien
Althanstrasse 14
A-1080 Wien
Austria
Tel.: 0043-1-31336-8275
Fax.: 0043-1-31336-772

Christiane Fricke
Inst. of Organic Chemistry
University of Hamburg
Martin-Luther-King-Platz 6
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-2824
Fax.: 0049-40-4123-2893

Palle Friies
The Royal Veterinary &
Agriculture University
Chemistry Department
40 Thorvaldsenvej
Copenhagen
DK-1871 Frb. C.
Denmark
Tel.: 0045-3528-2430
Fax.: 0045-3528-2089

Ralf God
Inst. f. Organische Chemie
Am Hubland
D-97074 Würzburg
Germany
Tel.: 0049-931-888-4753
Fax.: 0049-931-888-4755

Axel Görig
Bell Flavors & Fragrances
Schimmelstraße
D-04205 Miltitz
Germany
Tel.: 0049-341-945-1285
Fax.: 0049-341-941-1669

Dr. H.-P. Hanssen
IMB
Lurup 4
D-22927 Großhansdorf
Germany
Tel.: 0049-4102-62155
Fax.: 0049-4102-63028

G.G. Hauptmann
Solon Trading GmbH
Heinrichstraße 36b
D-22769 Hamburg
Germany
Tel.: 0049-40-430 72 19
Fax.: 0049-40-430 83 19

Dr. H. Hendriks
Dept. of Pharm. Botany
University Centre for Pharmacy
Antonius Deusinglaan 2
9713 AW Groningen
The Netherlands
Tel.: 0031-50-633 326
Fax.: 0031-50-633 311

B. Hildebrandt
Plantamed Arzneimittel GmbH
Kerschensteinerstr. 11-15
D-92318 Neumarkt/Oberpfalz
Germany
Tel.: 0049-9181-231-250
Fax.: 0049-9181-21 850

Prof.Dr. R. Hiltunen
Dept. of Pharmacy
Pharmacognosy Division
P.O.Box 56
SF-00014 University of Helsinki
Finland
Tel.: 00358-90-708 59-140
Fax.: 00358-90-708 59-138

Dr. Y. Holm
Dept. of Pharmacy
Pharmacognosy Division
P.O.Box 56
SF-00014 University of Helsinki
Finland
Tel.: 00358-90-708 59-166
Fax.: 00358-90-708 59-138

Dr. S. Hose
ASTA Medica AG, Dept. PEN
Weissmüllerstrasse 45
D-60314 Frankfurt a. M.
Germany
Tel.: 0049-69-4001-2756
Fax.: 0049-69-4001-2740

Dr. J. Ilmberger
Klinik f. Physikal. Medizin
Universität München
Marchioninistr. 15
D-81366 München
Germany
Tel.: 0049-89-709-544 62
Fax.: 0049-89-709-88 36

Katrin Jahn
University of Hamburg
Pharmaceutical Biology
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-4313
Fax.: 0049-40-4123-3898/2893

Dr. H.B. Jakobsen
Research Centre Aarslev
Kirstinebjergvej 12
5792 Aarslev
Denmark
Tel.: 0045-6599-1766
Fax.: 0045-6599-1756

D. Joulain
Robertet S.A.
B.P. 100
F-06333 Grasse
France
Tel.: 0033-93-40 33 66
Fax.: 0033-93-70 68 09

Stefan Jung
Inst. of Organic Chemistry
University of Hamburg
Martin-Luther-King-Platz 6
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-2831
Fax.: 0049-40-4123-2893

Prof.Dr. J. Jurenitsch
Inst. f. Pharmakognosie
Universität Wien
Pharmaziezentrum
Althanstrasse 14
A-1090 Wien
Austria
Tel.: 0043-1-3136-8075
Fax.: 0043-1-3136-772

Dr. D. Kalemba
Inst. of Generell Food Chem.
Technical University
ul. Stefanowskiego 4/10
90-924 Lodz
Poland
Tel.: 0048-42-313 423
Fax.: 0048-42-362 860

Prof.Dr. St. Katsiotis
Dept. of Pharmaceutics
School of Pharmacy
Aristole University
Thessaloniki, 54006
P.O.Box 1589
Greece
Tel.: 0030-31-99 24 76
Fax.: 0030-31-21 45 48

I.K. Kauffmann
Inst. f. Organische Chemie
Straße des 17. Juni 124
D-10623 Berlin
Germany
Tel.: 0040-30-314-236 22
Fax.: 0049-30-314-236 19

Dr. A. Kéry
Semmelweis University of
Medicine
Inst. of Pharmacognosy
Ullöi ut 26
H-1085 Budapest
Hungary
Tel.: 0036-1-1138-609
Fax.: 0036-1-1138-608

S. Kistler
Kistler & Co.GmbH
Lindenstraße 5
D-85254 Sulzemoos/Orthofen
Germany
Tel.: 0049-8134-7055
Fax.: 0049-8134-7757

J. Kloosterman
Trombonestraat 58
NL-3822 CH Amersfoort
The Netherlands
Tel.: 0031-3420-10476
Fax.: 0031-3420-10511

A. Köhne
TU-Berlin, Sekr. TCII
Straße des 17. Juni 124
D-10623 Berlin
Germany
Tel.: 0049-30-314-236 22
Fax.: 0049-30-314-236 19

Prof.Dr. W.A. König
Inst. of Organic Chemistry
University of Hamburg
Martin-Luther-King-Platz 6
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-2824
Fax.: 0049-40-4123-2893

Dr. C. Kohl
Fa. Sanofi Winthrop GmbH
Augustenstraße 10
D-80333 München
Germany
Tel.: 0049-89-52395-560
Fax.: 0049-89-52395-276

Dr. M.R. Kolalyte
VL Komarov Botanical Institute
Dept. Plant Resources
Komarov Botanical Inst.
Prof. Popov Str. 2
197376 St. Petersburg
Russia
Tel.: 007-812-234 845 2
Fax.: 007-812-234 45 12

Dr. B. Konuklugil
Fabriciusstrasse 8
D-40225 Düsseldorf
Germany
Tel.:
Fax.: 0049-21-311-3085

Prof.Dr. K.-H. Kubeczka
Pharmaceutical Biology
University of Hamburg
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-3899
Fax.: 0049-40-4123-3895

W. Krant
Uitenwaardenstraat 13
8081 HJ Elbung
The Netherlands
Tel.: 0031-5250-87215
Fax.: 0031-5250-85905

Prof.Dr. D. Kustrak
Faculty of Pharmacy & Biology
University of Zagreb
P.O.Box 156
HB-41000 Zagreb
Croatia
Tel.: 0038-385-01-445-311
Fax.: 0038-385-51-445-117

Dr. R. Länger
Inst. f. Pharmazeutische Chemie
Universität Wien
Althanstrasse 14
A-1090 Wien
Austria
Tel.: 0043-1-31336-8275
Fax.: 0043-1-31336-772

Dr. D. Lamparsky
Sonnhalde 8
CH-8602 Wangen-Zürich
Switzerland
Tel.: 0041-1-833-1473
Fax.:

Dr. E. Lassak
Phytochemical Services
35, Shinfield Avenue
St. Ives (Sydney), NSW 2075
Australia
Tel.: 0061-2-983-08 28
Fax.: 0061-2-983-07 98

R. Laurent
Robertet S.A.
F-06333 Grasse
France
Tel.: 0033-93 40 33 66
Fax.: 0033-93 70 68 09

P.E. Lebada
Inst. f. Pharmazeutische Chemie
Althanstrasse 14
A-1090 Wien
Austria
Tel.: 0043-1-31336-8612
Fax.: 0043-1-31336-771

P. Liddle
Martini & Rossi
B.P. 50
F-93401 Saint-Oven Cedex
France
Tel.: 0033-1-4945-4873
Fax.: 0033-1-4945-4905

A. Lis
Inst. of General Food Chemistry
Technical University
ul. Stefanowskiego 4/10
PL-90-924 Lodz
Poland
Tel.: 0048-42-313 410
Fax.: 0048-42-362 860

Dr. St. Lochynski
Inst. f. Organische Chemie
Technische Universität Wroclaw
W. Wyspianskiego 27
PL-50-370 Wroclaw
Poland
Tel.: 0048-71-20 24 00
Fax.: 0048-71-21 75 57

Prof.Dr. A.J. Marsaioli
IQ-UNICAMP
CP 6154 Campinas
SP Brasil
Brazilia
Tel.: 0055-192-39 75 51
Fax.: 0055-192-39 38 05

Prof.Dr. I. Máthé
Inst. of Pharmacognosy
A. Szent-Györgyi Medical Univ.
H-6720 Szeged
Hungary
Tel.: 0036-62-312-233/19-40
Fax.: 0036-62-32 41 77

Ch. Mechtler
Inst. f. Pharmakognosie
Pharmaziezentrum
Universität Wien
Althanstrasse 14
A-1090 Wien
Austria
Tel.: 0043-1-31336-8275
Fax.: 0043-1-31336-722

Stephanie Melching
Inst. of Organic Chemistry
University of Hamburg
Martin-Luther-King-Platz 6
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-2814
Fax.: 0049-40-4123-2893

Prof.Dr. M. Miyazawa
Kinki University
Kowakae
Higashiosaka
Osaka, 577
Japan
Tel.: 0081-6-721-2332, 415
Fax.: 0081-6-727-4301

Ulrike Mölleken
Pharmaceutical Biology
University of Hamburg
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-4313
Fax.: 0049-40-4123-3895

Dr. Morgenstern
British American Tobacco
Bahrenfelder Chaussee 139
D-22761 Hamburg
Germany
Tel.: 0049-40-4151 41 05
Fax.: 0049-40-4151 44 00

Dr. H.-P. Münzing
Pater-Rupert-Mayer-Str. 1
D-82049 Pullach
Tel.: 0049-89-74425-161
Fax.: 0049-89-793 49 66

Dr. R. Naef
Firmenich S.A.
P.O.Box 239
Route des Jeunes 1
CH-1227 Acacias-Geneve
Switzerland
Tel.: 0041-22-
Fax.: 0041-22-780-3532/-3334

Dr. G. Nagy
Dept. of Pharmacognosy
Albert Szent-Györgyi Medical Univ.
Eötvös Str. 6
H-6720 Szeged
Hungary
Tel.: 0036-62-324-177
Fax.: 0036-62-324-177

Dr. E. Németh
Univ. Horticulture and
Food Industry
Villanyi Str. 29
H-1114 Budapest
Hungary
Tel.: 0036-1-166-4998
Fax.: 0036-1-166-4998

K. Nendel
Frey & Lau GmbH
Immenhacken 12
D-24558 Henstedt-Ulzburg
Germany
Tel.: 0049-4193-99 55 17
Fax.: 0049-4193-99 55 80

Prof.Dr. A. Nikiforov
Inst. of Organic Chemistry
Waehringer Str. 38
A-1080 Wien
Austria
Tel.: 0043-1-31367-2210
Fax.: 0043-1-31367-2280

Prof.Dr. Y. Noma
Faculty of Domestic Sciences
Tokushima Bunri University
Yamashiro-cho
Tokushima
Japan
Tel.: 0081-886-22-9611
Fax.: 0081-886-55-3051

Dr. C. Nussbaumer
Givaudan-Roure Aromen AG
Überlandstr. 138
CH-8600 Dübendorf
Switzerland
Tel.: 0041-1-824-2607
Fax.: 0041-1-821-4478

Dr. A.O. Onayade
P.O.Box 918
Ile-Ife
Osun State
Nigeria
Tel.: 00234-36
Fax.: 00234-36-231 733

M. Orth
Universität Würzburg
Inst. of Pharmaceutical Biology
Mittlerer Dallenbergweg 64
D-97082 Würzburg
Germany
Tel.: 0049-931-888-61 79
Fax.: 0049-931-888-61 58

Dr. A. Pauli
Fürther Str. 13
D-90513 Zirndorf
Germany
Tel.: 0049-911-60 62 21
Fax.: 0049-911-600 19 29

Dr. L. Pedro
Dept. Biologica Vegetal
Faculdade de Ciencias
Edificio C-2, Campo Grande
P-1780 Lisboa
Portugal
Tel.: 00351-1-750 00 69
Fax.: 00351-1-750 00 48

Dr. R. Piccaglia
Dipt. Di Agronomia
V. Filippo Re, 6-8
I-40126 Bologna
Italy
Tel.: 0039-51-351510
Fax.: 0039-51-351511

Jens Achim Protzen
Pharmaceutical Biology
University of Hamburg
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-3897
Fax.: 0049-40-4123-3895

K.-D. Protzen
Paul Kaders GmbH
Eschelsweg 27
D-22767 Hamburg
Tel.: 0049-40-380 30 80
Fax.: 0049-40-380 30 827

Lothar Quanz
Pharmaceutical Biology
University of Hamburg
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-3898
Fax.: 0049-40-4123-3895

Angela Rieck
Inst. of Organic Chemistry
University of Hamburg
Martin-Luther-King-Platz 6
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-2814
Fax.: 0049-40-4123-2893

M. Röthlisberger
John Wiley & Sons
Laupenstrasse 5a
CH-3008 Bern
Switzerland
Tel.: 0041-31-991 23 08
Fax.: 0041-31-382 04 09

Dr. O.R. Roque
Faculdade de Farmacia
Rua do Norte
P-3000 Coimbra
Portugal
Tel.: 00351-39-410 99 95
Fax.: 00351-39-271 26

Dr. P. Rubiolo
Dip. Szienza e Tecnologia del Farmaco
Via P. Giuria 9
I-10125 Torino
Italy
Tel.: 0039-11-670 766 2
Fax.: 0039-11-670 768 7

C. Rupp
Univ.-Klinik f. Psychatrie
Innsbruck
Anichstrasse 35
A-6020 Innsbruck
Austria
Tel.: 0043-512-504 42 45 (36 55)
Fax.: 0043-512-504-36 28

Dr. I. Salamon
Research Inst. of Agroecology
Spitalska 1273
SK-071 01 Michalovce
Slovakia
Tel.: 0042-496-25065
Fax.: 0042-496-24429

Dr. L.M.R.P. Salgueiro
Lab. Farmacognosie
Universidade de Coimbra
R. Neate
P-3000 Coimbra
Portugal
Tel.: 00351-39-410 99 95
Fax.: 00351-39-27 126

Prof.Dr. E. Sarer
Faculty of Pharmacy
Ankara University
06100 Tandogan-Ankara
Turkey
Tel.: 0090-312-212 68 05
Fax.: 0090-312-213 10 81

Yücel Saritas
Inst. of Organic Chemistry
University of Hamburg
Martin-Luther-King-Platz 6
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-2824
Fax.: 0049-40-4123-2893

I. Seelmann
IOC Sekr. TCII
Strasse des 17. Juni 135
D-10623 Berlin
Germany
Tel.: 0049-30-314-224 87
Fax.: 0049-30-314-236 19

Prof.Dr. E. Sezik
Gazi Üniversitesi
Eczacilik Fak.
06330 Ankara
Turkey
Tel.: 0090-4-312-215 11 13
Fax.: 0090-4-312-223 50 18

Prof.Dr. E. Shaaya
Dept. of Stored Products
The Volcani Centre
IL-50250 Bet-Dagan
Israel
Tel.: 00972-3-96 83 586
Fax.: 00972-3-96 04 428

M. Skoula
Dept. of Nature Products
Mediterranean Agron. Inst.
P.O.Box 85
73100 Chania
Greece
Tel.: 0030-821-811 53
Fax.: 0030-821-811 54

U. Splittgerber
TU Berlin
Strasse des 17. Juni 124
D-10623 Berlin
Germany
Tel.: 0049-30-314 236 20
Fax.: 0049-30-314 236 19

Dr. H. Surburg
Haarmann & Reimer GmbH
Postfach 1253
D-37601 Holzminden
Germany
Tel.: 0049-5531-901 384
Fax.: 0049-5531-

Dr. K. Svoboda
Scottish Agric. College UK
Auchincruive Ayr KA6 5HW
Scotland, UK
Tel.: 0044-1292-520 331
Fax.: 0044-

A. Schefe
Strasse des 17. Juni 124
D-10623 Berlin
Germany
Tel.: 0049-30-314 236 22
Fax.: 0049-30-314 236 19

Prof.Dr. J.J.C. Scheffer
Div. of Pharmacognosy
Leiden Univ., Gorlaeus Lab.
P.O.Box 9502
NL-2300 RA Leiden
The Netherlands
Tel.: 0031-71-27 44 74
Fax.: 0031-71-27 42 77

S. Schlüter
Inst. f. Lebensmittelchemie
Grindelallee 117
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-4348
Fax.: 0049-40-4123-4348

Dr. G. Schmaus
Dragoco Gerberding & Co.AG
Dragocostrasse
D-37601 Holzminden
Germany
Tel.: 0049-5531-97 15 49
Fax.: 0049-5531-97 11 58

Andrea Schmidt
Pharmaceutical Biology
University of Hamburg
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-3893
Fax.: 0049-40-4123-3895

Dr. M. Schmidt
Schlingbreede 25
D-33332 Gütersloh
Germany
Tel.: 0049-5241-6 80 86
Fax.: 0049-5241-6 80 89

Dr. W. Schmidt
Bell Flavors & Fragrances
Schimmelstrasse 1
D-04205 Miltitz
Germany
Tel.: 0049-341-945 12 85
Fax.: 0049-341-941 16 69

PD Dr. W. Schultze
Pharmaceutical Biology
University of Hamburg
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-4731
Fax.: 0049-40-4123-3895

N. Schuster
Bio Rad Laboratories GmbH
Bischofstrasse 86
D-47809 Krefeld
Germany
Tel.: 0049-2151-5159-0
Fax.. 0049-2151-5159-11

M. Schwarz
Pharmazeutische Biologie
Universität Regensburg
Universitätsstrasse 31
D-93040 Regensburg
Germany
Tel.: 0049-941-943 47 61
Fax.: 0049-941-943 47 62

Prof.Dr. E. Stahl-Biskup
Pharmaceutical Biology
University of Hamburg
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-3896
Fax.: 0049-40-4123-3895

Dr. R. Stevens
Flav. & Fragr. Journal
Low House, Threlkeld
Keswick, Cumbria
CA12 4SQ
U.K.
Tel.: 0044-17687-79 624
Fax.: 0044-

Prof.Dr. R. Tabacchi
Inst. de Chimie
Université de Neuchatel
Av. de Bellvaux 51
CH-2000 Neuchatel
Switzerland
Tel.: 0041-38-23 24 00
Fax.: 0041-38-23 25 11

Y. Tada
Faculty of Pharm. Sciences
Tokushima Bunri University
Yamashiro-cho,
Tokushima 770
Japan
Tel.: 0081-886-22-9611 (5522)
Fax.: 0081-886-55-3051

A. Takahata
Fuso Chemical Industry Co.Ltd.
2-6-6 Nitaka, Yodogawa
Osaka 532
Japan
Tel.: 0081-6-391-3156
Fax.: 0081-6-391-7931

Prof.Dr. F. Tateo
Dipt. di Sci. & Tecnologia
Universita Degli Studi di Milano
2, Via Celoria
I-20133 Milano
Italy
Tel.: 0039-2-70 63 08 56
Fax.: 0039-2-26 63 05 7

J.A. Teixeira Da Silva
Lisbon University
Rua Bartolomeu de Gusmao,
25,3,Dto
S-Joao Do Estoril
P-2765 Estoril
Portugal
Tel.: 00351-1-468 81 61
Fax.: 00351-1-467 33 99

K. Thefeld
IOC Sekr. TCII
Strasse des 17. Juni 124
D-10623 Berlin
Germany
Tel.: 0049-30-314 224 87
Fax.: 0049-30-314 236 19

Dr. K.G. Tkachenko
Komarov Botanical Inst. of RAS
2, prov. Popova Str.
St. Petersburg
Russia
Tel.: 007-812-234 84 50
Fax.: 007-812-234 45 12

Dr. G. Tümen
Balikesir Universitesi
Necatibey Egitim Fak.
Biology Dept.
Mani 10100 Balikesir
Turkey
Tel.: 0090-266-241 27 62
Fax.: 0090-266-241 12 12

M. Vincent
TU Berlin
Strasse des 17. Juni 124
D-10623 Berlin
Germany
Tel.: 0049-30-314 236 22
Fax.: 0049-30-314 236 19

B. Volkert
TU Berlin
Strasse des 17. Juni 124
D-10623 Berlin
Germany
Tel.: 0049-30-314 236 22
Fax.: 0049-30-314 236 19

Carsten Vollmann
Pharmaceutical Biology
University of Hamburg
Bundesstrasse 43
D-20146 Hamburg
Germany
Tel.: 0049-40-4123-3898
Fax.: 0049-40-4123-3895

B. Vonach
MITU Laborbedarf GmbH
Landsberger Strasse 13
D-86926 Greifenberg
Germany
Tel.: 0049-8192-999 085
Fax.: 0049-8192-8868

Dr. D. Warncke
Fa. Merckle GmbH
Graf-Arco-Strasse 3
D-89079 Ulm
Germany
Tel.: 0049-731-402 77 69
Fax.: 0049-731-402 76 51

T. Wegener
Braunholzstrasse 17
D-33378 Rheda-Wiedenbrück
Germany
Tel.: 0049-5241-6 80 86
Fax.: 0049-5241-6 80 89

Prof.Dr. P. Weyerstahl
Inst. f. Organische Chemie
TU Berlin
Strasse des 17. Juni 124
D-10623 Berlin
Germany
Tel.: 0049-30-314-223 55
Fax.: 0049-30-314-236 19

D. Wolf
Inst. f. Organische Chemie
TU Berlin
Strasse des 17. Juni 124
D-10623 Berlin
Germany
Tel.: 0049-30-314-236 20
Fax.: 0049-30-314-236 19

Dr. A. Zänglein
MCM Klosterfrau GmbH &
Co.KG
Postfach 10 21 55
D-50461 Köln
Germany
Tel.: 0049-221-1652-374
Fax.: 0049-221-

B. Zimmer
Frey & Lau GmbH
Immenhacken 12
D-24558 Henstedt-Ulzburg
Germany
Tel.: 0049-4193-99 55 11
Fax.: 0049-4193-99 55 80