32nd International Symposium on Essential Oils

September 9 – 12, 2001

ABSTRACTS

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Published by Department of Chemistry, Agricultural University of Wrocław

ISBN 83-915199-0-2

Druk i oprawa: F.P.H."Elma"

Wrocław Uniwersity
of Technology

Agricultural University
of Wrocław

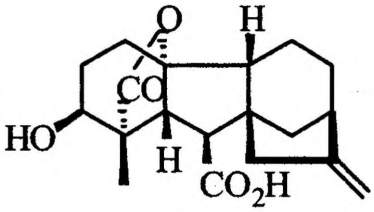
Publication sponsored by
State Committee for Scientific Research (KBN)

PL 1

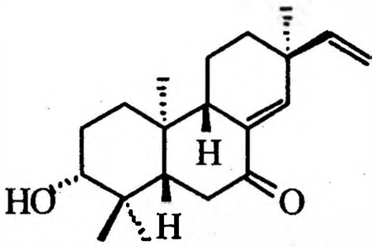
Terpenoids as Semiochemicals and Bioregulators

Kenji Mori
Department of Chemistry, Science University of Tokyo
Kagurazaka 1-3, Shinjuku-ku, Tokyo 162-8601, Japan
Fax: +81-3-3235-2214

Many terpenoids and related compounds are employed by organisms as bioregulators such as hormones and semiochemicals such as pheromones. Roles of terpenoids and relatives as phytohormones [gibberellins, *cf.* GA$_4$ (**1**)], phytoalexins [oryzalexin A (**2**) and phytocassane D (**3**)], insect juvenile hormones [JH I (**4**)] and pheromones [acoradiene (**5**), supellapyrone (**6**), posticlure (**7**) and the secretion (**8**) of *Endoclita excrescens*] will be discussed with special emphasis on their synthetic aspects.



GA$_4$ (**1**)



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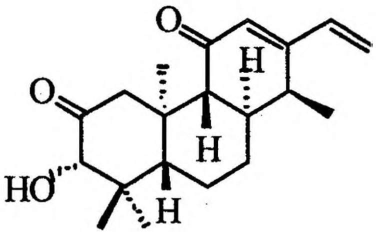
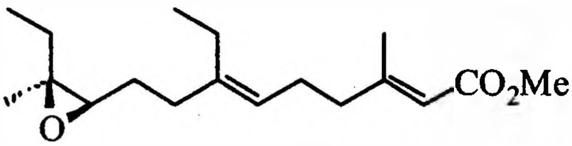
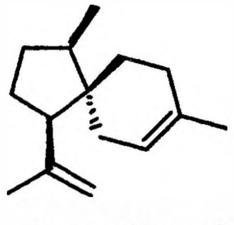


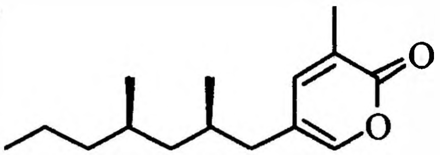
Phytocassane D (**3**)



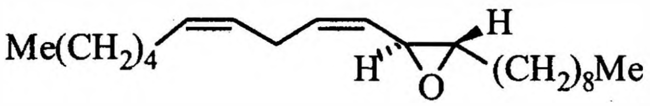
JH I (**4**)



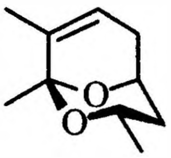
Acoradiene (**5**)



Supellapyrone (**6**)



Posticlure (**7**)



Secretion (**8**) of Endoclita excrescens

PL 2

Quantitative Gas Chromatography-Olfactometry
– Review –

Alain Chaintreau
Firmenich SA, Corporate R&D Division,
P.O. Box 239, 1211 Geneva 8, Switzerland

Since the first paper (T. Acree, 1984) rationalizing the use of the human nose as a detector for gas chromatography, GC-olfactometry (GC-O) has gained an increasing popularity because it allows the analyst to focus the effort of identification on the most potent odorants of the chromatogram.

Three main techniques are known: extract dilution analysis (CHARM and AEDA), odor intensity recording (OSME, FSCM), odor detection frequency (GC-'SNIF'). The first two were initially used to qualitatively determine the most intense odorants from a food aroma. During the last few years, the investigation of quantitative capabilities of GC-O have been undertaken. This review proposes a synthesis of recent studies in this area.

At first, some theoretical considerations are presented to justify the most suitable methods for quantitative purposes, from the viewpoint of psychophysics. The advantage of using a panel is discussed, as olfactograms established by only one or two persons exhibit limitations inherent in human characteristics (specific anosmias, fatigue, etc.). The variability of GC-O peaks has been measured to better support the conclusions drawn from the comparison of olfactograms of smelling products. These results have enabled authors to compare the olfactive profiles of foods or beverages according to their origin, to the process, as well as their evolution during the process. In some cases, a statistical clustering has been performed.

Recent papers report the comparison of GC-O methods applied to different aromas; similarities and differences are discussed.

In conclusion, the application of GC-O to the quantitation of an odorant is exemplified with a flavor compound in a model mixture and a real beverage.

Theoretical Methods in Aroma and Fragrance Research

Peter Wolschann
Institute of Theoretical Chemistry and Molecular Biology, University of Vienna
Währinger Strasse 17, A-1090 Vienna, Austria

Biological active substances interact, in most cases, with biomolecules, triggering specific molecular mechanisms like activation of an enzyme cascade or opening an ion channel, which finally leads to a certain biological response. Structure activity relationships (SAR) and quantitative structure activity relationships (QSAR) correlate this effect with molecular properties of the compounds of interest. These methods are widely accepted for modern drug design strategies, but have been applied successfully in aroma and fragrance research.

A general survey over the various methods of molecular modelling and molecular calculations will be given before some procedures will be discussed. Starting from classical 2D-QSAR methods, where the free energy ΔG of the association reaction between an active molecule and a receptor protein is correlated with different molecular descriptors, depending on steric and electronic interactions as well as on transportation properties, various 3D-QSAR methods will be introduced. In particular, COMFA and related methods will be described more extensively. The application of Artificial Neural Networks for the evaluation of prediction models of non-linear dependencies will be demonstrated.

Since a broad variety of molecular modelling methods have been used for many systems in pharmaceutical chemistry, also some investigations on fragrance and aroma compounds have been performed recently with the help of these methods. Additionally, in the last years more information about the structure of the proteins of the receptor site of odor and aroma compounds has been obtained, which allows more insight into the physico-chemical mechanism of the odor perception. The determination of the sequence of some receptor proteins and their 3D-structures, obtained from X-ray crystallography leads to some knowledge about the geometry of the association complexes between aroma molecules and their receptor targets.

As examples of molecular modelling studies on fragrance and aroma compounds, two systems will be selected: In the case of Sandalwood odorants only less quantitative information about the odor threshold values is known, and SAR investigations are connected with ligand-based similarity studies. For pyrazine-derived aroma compounds, which can be found in foods, threshold values are known to some extent, which allows the application of various QSAR techniques. Moreover, a classification of different aroma impressions can be performed by various procedures.

PL 4

Chemical Composition Variability of Essential Oils in Ontogenesis of Some Plants

J. Góra, A. Lis, J. Kula, M. Staniszewska, A. Wołoszyn
Institute of General Food Chemistry, Technical University of Łódź, Poland

Plant ontogenesis is an individual's development, which includes the period from zygote to the individual's death. All plants biosynthesize secondary metabolites including essential oils, the characteristic feature of which is the variability of yield and chemical composition during ontogenesis. This refers particularly to the oleiferous annual plants, and to nearer extent, to perennial plants (trees, bushes).

There is a distinct dependence between the ontogenesis stage and the contents as well as the chemical composition of essential oils.

The knowledge of this dependence has a significant importance for appointing the most favourable time limit for picking the plant raw material.

In this work we discuss the ontogenetic variability of essential oils of the following plants: *Erigeron canadensis*, *Daucus carota* sap. *carota*, *Achillea millefolium*, *Mentha piperita*, *Coriandrium sativum* and *Anethum graveolens*.

PL 5

Volatile Components of Some Oriental Medicinal Plants and Southern Hemispheric Liverworts

Y. Asakawa
Faculty of Pharmaceutical Sciences, Tokushima Bunri University,
Yamashiro-cho, 180, Tokushima 770-8514, Japan
Fax: +81-88-655-3051
asakawa@ph.bunri-u.ac.jp

1) A number of oriental medicinal plants contain characteristic essential oils, which have been used for food additives, incense sticks, aromatherapy and drugs etc. The volatile components of selected oriental medicinal plants (*Acorus*, *Actinidia* sp., *Artemisia princeps*, *Cinnamomum loureirii*, *Cinnamomum japonica*, *Citrus junos*, *Chamaecyparis obtusa*, *Cryptomeria japonica*, *Cryptoporus volvatus*, *Curcuma* sp. *Houttuynia cordata*, *Illicium japonicum*, *Lindera strychnifolia*, *Lindera umbellata*, *Momordicae grosvemori*, *Perilla frutescens*, *Polygonum hydropiper*, *Solidago* sp., *Taxus cuspidata*, *Wasabia japonica*, *Zanthoxylum piperitum* and *Zingiber mioga etc.*) will be reported.

2) Liverworts are rich sources of mono-, sesqui- and diterpenoids and phenolic compounds many of which show interesting pharmacological activity [1–3]. The isolation, structure determination of volatile components from the Fijian, New Caledonian, New Zealand and Argentinean liverworts will be discussed. The New Zealand fern and liverwort, *Blechnum fluviatile* and *Hymenophyton flabellatum* produce the characteristic pungent components, (–)-polygodial, a sesquiterpene dialdehyde, and 1-(2,4,6-trimethoxyphenyl)-2-but-1-one, respectively. The former compound has been isolated from the Japanese liverwort, *Porella vernicosa* complex and the latter one from the Japanese fern, *Arachinoides standishii.* Occurrence of both compounds in pteridophytes and bryophytes is very important in determining the evolutionary ladder of both terrestrial spore-forming green plants.

[1] Asakawa Y., *Progress in the Chemistry of Organic Natural Products*, ***65***, 1–618, 1995, Springer, Wien.
[2] Asakawa Y., *Recent Advance in Phytochemistry*, ***33***, 319–342, 1999, Kluwer Academic/Plenum Publishers, New York.
[3] Asakawa Y., *Phytochemistry*, ***56***, 297–312, 2001.

L 1

From Natural Terpenes Towards Chiral Auxiliary: New Ideas and New Approach

Alexey V. Tkachev
Novosibisrk Institute of Organic Chemistry, Novosibirsk State University
Academician Lavrentjev Ave. 9, Novosibirsk 630090, Russia

Natural terpenoids are regarded as one of the major primary source of chirality for preparing all the other chiral chemicals for research and industry. Several natural oxygen-containing derivatives such as menthol, pulegone, carvone and camphor as well as boron derivatives of pinene have been studied intensively. At the same time, in spite of extremely high enantiomeric purity and accessibility of some terpene hydrocarbons, the lack of functional group makes them less attractive as chiral auxiliary. Is there a possibility to transform wide spread terpenes to effective chiral agents? What kind of chemical modification has to be performed to convert unfunctionalized terpenes to the chiral agents?

The use of terpenes as starting compounds in the syntheses of chiral auxiliary requires introduction a heteroatomic function to the carbon-carbon double bonds at the first stages of the reaction sequences. Different oxidative processes are well studied in the chemistry of terpenes and are traditionally used to transform unsaturated terpenic hydrocarbons to alcohols and their esters, unsaturated ketones, epoxides, carboxylic acids, etc. From the viewpoint of preparation of chiral auxiliary, it is more preferable to introduce a nitrogen-containing function at the C–C double bond rather than oxygen-containing one. Introduction of a nitrogen containing function usually results in the new possibilities for the molecule to be transformed to a remarkable diversity of derivatives. Addition of simple inorganic reagents with electrophilic nitrogen (like NOCl, NOBr, N_2O_3, etc.) to unsaturated hydrocarbons is the most important transformation leading to a variety of useful nitrogen-containing derivatives. To the moment, we have studied the addition of NOCl to a series of natural terpenes (mono-, sesqui- and di- terpenes), as well as structure, reactivity and chemical properties of the intermediates, and designed new approach to a variety of nitrogen-containing chiral building blocks and auxiliary (chiral amines, oximes, amino oximes, amidoximes, N-hydroxyamino oximes, enaminones, different nitrogen-containing heterocycls, macroheterocycles, mono-, di- and tri- nuclear complexes of transition metal ions).

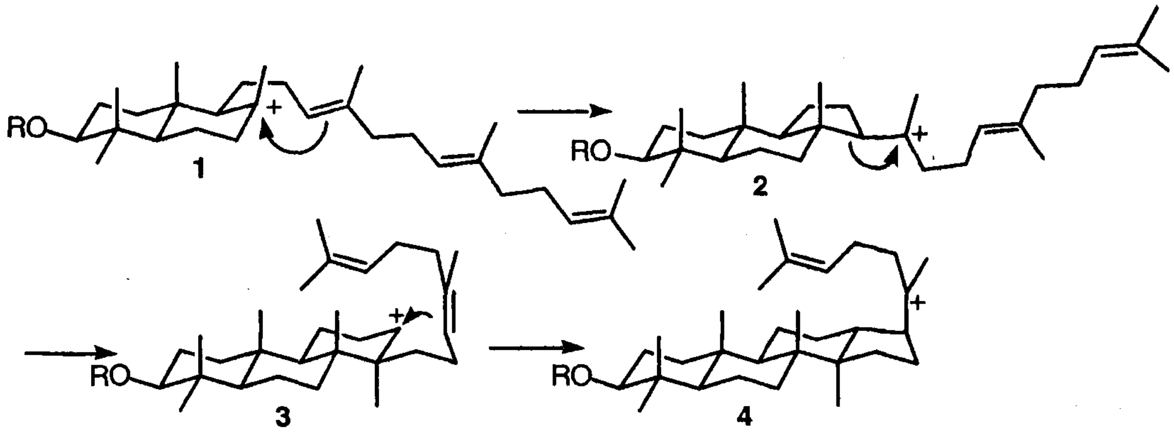
L 2

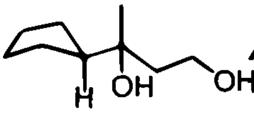
$TiCl_4$-Induced Rearrangement into the Anti-Markovnikov Cation that Observed in the Sterol Biosynthesis

Mugio Nishizawa, Yoshihiro Iwamoto, Hiroko Takao, Hiroshi Imagawa
and Takumichi Sugihara
Faculty of Pharmaceutical Sciences, Tokushima Bunri University
Tokushima 770-8514, Japan
mugi@ph.bunri-u.ac.jp

Based upon the idea of the stepwise mechanism of biomimetic olefin cyclization and enzymatic cyclization of oxidosqualene via conformationally flexible cationic intermediates, significant attention has been focused on each step of sterol biosynthesis. Today, the biosyntheses of phytosterols such as dammaranoid, lupanoid, oleananoid, and tirucallanoid are explained by the cyclization of oxidosqualene via a bicyclic cation **1**, tricyclic 6/6/5-cation (pre-C ring cation) **2**, secondary 6/6/6-cation **3**, and 6/6/6/5 cation **4**. In the animal kingdom, steroids are also constructed through the corresponding boat-form B-ring intermediates.

Transformation of **2** to **3** involves ring expansion of a tertiary cationic substrate into a secondary cation, namely, an anti-Markovnikov cation. We chose a diol **5** as the model compound and investigated the chemical behavior of the cation **6**. Cation **6** generated by the reaction of diol **5** and BF_3Et_2O, $SnCl_4$, $Sc(OTf)_3$, $FeCl_3$, TiF_4 or CF_3SO_3H leads to a hydride shift to cation **7** leading cis ether **10** that corresponds to the initiation of backbone rearrangement. On the other hand, $TiCl_4$ selectively induces rearrangement to secondary cation **8** by the ring expansion that corresponds to the C-ring formation of sterol biosynthesis, and afforded ether **11**, chloride **12** and **13**. $AlCl_3$ and $ZrCl_4$ induce further rearrangement into six-membered ring *tert*-cation **9** and afforded only isomeric ether **14**. Thus, the transformation of **2** to **3** will be able to achieve without enzyme.





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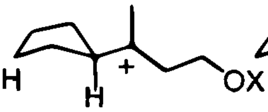

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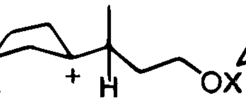

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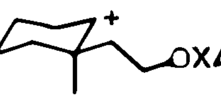

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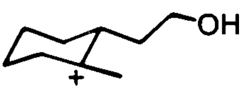

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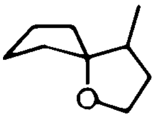

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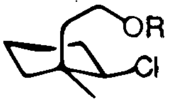

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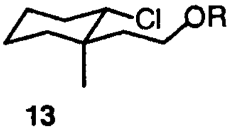

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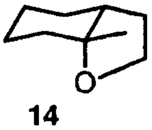


14

L 3

Conventional Inner Diameter Short Capillary Columns: an Approach to Speeding Up GC Analysis of Medium Complexity Samples

Carlo Bicchi[1], Claudio Brunelli[1], Patrizia Rubiolo[1], Mario Galli[2], Albino Sironi[3]
[1]Dipartimento di Scienza e Tecnologia del Farmaco,
via P. Giuria 9, I-10125 Torino, Italy
bicchi@pharm.unito.it
[2]MEGA, via Plinio 29, I-20025 Legnano, Milan, Italy
[3]ThermoQuest Italia, via Rivoltana, I-20090 Rodano, Milan, Italy

There is a continual demand for faster GC separation. Although investigated since the early 60's, only over the last decade, have some approaches been proposed and applied to routine analysis. The various approaches for fast GC have recently been reviewed and discussed in theoretical terms by Cramers et al. [1]. The easiest way to speed up a GC separation is to decrease column length while keeping enough resolving power for the given separation problem. Short columns with narrow inner diameters (i.d.) (0.1 mm or less) offer efficiency and peak capacity comparable to those of columns with conventional length and i.d. (0.25–0.32 mm i.d.) and can be used when high resolution is needed to analyse a complex mixture correctly.

GC separation depends on column efficiency and on stationary phase selectivity. The separation power of capillary columns is mainly based on efficiency, which is so high that selectivity may often be 'neglected' because most separations are achieved without the selectivity of the stationary phase playing a crucial role. This means that the minimal number of theoretical plates required to analyse a given complexity mixture (n_{req}) can also be achieved with the concurrence of a suitable stationary phase. These considerations provide the basis for an approach, different from narrow bore columns, to speeding up GC analysis for medium complexity samples where the efficiency of shorter columns with conventional i.d.s (0.25–0.32 mm) is combined with a selectivity suitable to separate the components in the sample under investigation, so that the resolution power of the chromatographic system is maintained. In this way, the loss of efficiency due to reduction in column length (i.e. 20–50000 theoretical plates instead of the usual 150000 of a conventional 25 m capillary column) is compensated by better selectivity.

The quali-quantitative effectiveness of this approach is demonstrated through the analysis of essential oils with different compositions and plant extracts or distillates containing medium-volatility or thermolabile components. In all examples, GC analysis was five to ten times faster than with conventional columns.

[1] Cramers C. A., Janssen H.-G., van Deursen M. M., Leclercq P. A., *J. Chromatogr. A*, *315*, 856, 1999 and references cited therein.

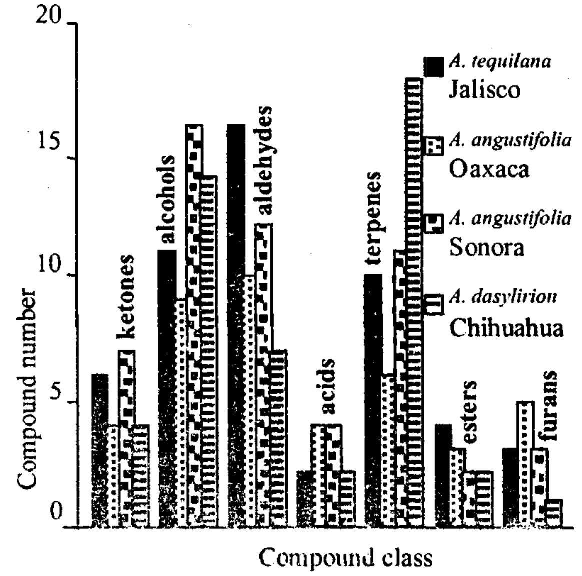
L 4

Volatile Polymorphism in Agave Plants by SPME-GC-MS

Mercedes G. López, Lourdes Macías-Rodríguez
Unidad de Biotecnología e Ingeniería Genética de Plantas,
Centro de Investigación y de Estudios Avanzados del IPN,
Apartado Postal 629, 36500 Irapuato, Gto., México
mlopez@ira.cinvestav.mx

Mexico is considered one of the most important megadiverse countries in the world. The *Agavaceae* family is well-distributed around the country and it has been part of the Mexican culture for 30 centuries. Among the Agave plants, *Agave tequilana*, *A. angustifolia* and *A. dasylirion* are the most popular species, basically because they are used as the starting material for the elaboration of Mexican alcoholic beverages such as tequila, mezcal and bacanora, and sotol, which are very popular today [1]. Solid phase microextraction (SPME) is a rapid, low cost, and solvent-free extraction technique and its coupling to a highly selective analytical tool such as GC-MS opens a new horizon into the aroma study. Therefore, the aim of this work was to study the volatile polymorphism in Agave plants grown in different geographic regions using SPME-GC-MS. Six years old Agave plants were harvested from four Mexican states: *Agave tequilana* (Jalisco), *A. angustifolia* (Oaxaca and Sonora) and *A. dasylirion* (Chihuahua). One gram of the pine (stem) of each plant was incubated at 50 °C for 1h in 4 ml vials. After this time, a SPME fiber (DVB/Carboxen/PDMS) was introduced in the vial and exposed to its head-space for another hour, followed by one min desorption in the injector port of a GC-MS with a HP-FFAP capillary column.

Compound classes and compounds number of Agave plants can be seen in the Figure. Alcohols (ethanol and 1-octen-3-ol), aldehydes (pentanal, and hexanal), ketone (5-hepten-2-one-6-methyl) and an acid (hexanoic acid) were common to all species. Hexanal was the most abundant volatile in *A. tequilana* (30 %) and *A. angustifolia* (32 %), but they can be distinguished by cineole and phenyl ethyl alcohol, respectively. Also *A. angustifolia* grown in Oaxaca presented volatiles such as heptanoic acid methyl ester and 1-octanol that were not observed in the same specie grown in Sonora. On the other hand *A. dasylirion*, was the only Agave that presented more than 15 terpenes (β-myrcene and aristolene). Finally, volatiles common to tequilana-angustifolia (1-octen-3-one), tequilana-dasylirion (fenchol), angustifolia-dasyiirion (2-pentanone-3-methyl) and angustifolia-angustifolia (2-cyclohexen-1-one) were found.



The molecular polymorphism of Agave plants based on specie and geographic origin was determined by SPME-GC-MS. These results might also be considered on the establishment of the plant material (pine) authenticity used in the elaboration of ethnic beverages that are protected by the Mexican norm (NOM-006-SCF1-1994) and an origin denomination.

[1] López M., Mancilla-Margalli N., *Frontiers of Flavour Science*, 523–526, 2000.

L 5

The Use of Essential Oils Ointment Combined with Minor Surgical Debridement in the Treatment of Infected Wounds and Ulcers

Shamai Giler[1*], Dina Giler, Haim Schloss, Avraham Weinberger[*]
Laser Plastic Surgery Clinic, Rothschild Central Clinics
and [*]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel
[1]26 Dakar St., Petah Tikva, Israel

Purpose: Previous experimental and clinical studies conducted in our laboratory and clinic have shown that natural essential oils ointment containing essential oil blends, unsaturated plantar fatty acids and Vit. E has antibacterial and antioxidant activities, thus enhancing the healing process of wounds. Essential oils ointment (ETHERIN) was used as 'postoperative care' following CO_2 laser surgery and for the treatment of various infected wounds. Surgical procedures, either by conventional surgery (scalpel) or CO_2 laser, are used for debridement of necrotic and infected tissues in patients with wounds and ulcers. CO_2 laser ablation enables the surgeon to remove tissues layer by layer and provides hemostatic and sterilizing effects. The present report, on patients with huge infected and necrotic wounds and ulcers, was conducted to study the effectiveness of the combined treatment using topical application of the above natural essential oils ointment and conventional or CO_2 laser surgery used for debridement of the necrotic tissues.

Methods: Minor conventional (scalpel or CO_2 laser ablative surgical) procedures were performed to remove (debridement) the infected and necrotic tissues in 189 patients (45–85 yrs) with huge wounds and ulcers. In most patients, surgery was performed without anesthesia and in the minority, with Xylocaine spray. Two to four surgical or ablative sessions were done in intervals of 2–4 days. Following surgery, essential oils ointment (ETHERIN) containing the above-mentioned ingredients was applied directly on the wound and was changed twice a day.

Results: In most patients, recovery and complete secondary healing appeared within 1 to 3 months (depending on the grade and measurement of the wound). In 19 patients, treatment failed (8 died during the treatment) and in 16, a clean granulated tissue appeared without epithelialization.

Conclusion: No immediate or late complications were observed following topical application of essential oils ointment. CO_2 laser surgery was faster and hemostatic than conventional surgery. Because there are no exact measurements to study the healing process of wounds, clinically, it appears that improvement of healing was achieved by using the combined treatment of CO_2 laser ablative procedure followed by topical application of essential oils ointment (ETHERIN). We, therefore, advocate this combined treatment in patients with infected wounds and ulcers.

L 6

Photo-Aromatherapy — a Novel Combined Treatment Using Low Intensity Non-Coherent Light Source and Essential Oils Ointment for Prompt Healing of Sporadic Infected Facial Lesions

Shamai Giler[1*], Dina Giler, Haim Schloss
[*]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel
[1]26 Dakar St., Petah Tikva, Israel

Purpose: Low intensity visible light (LIL) delivered from coherent (lasers) or non-coherent light sources has been widely used for promoting wound healing. It was reported that LIL generates significant biomodulative effects on biochemical, physiological and proliferative phenomena on various cellular systems and components. As the wavelength increases into visible light of the spectrum, radiation penetrates more deeply into the skin. Recently, a novel compact LIL source with a wide spectral range of wavelengths (400–800 nm) was designed for use in medicine. Previous studies showed that essential oil blends have antibacterial and antioxidant activities, and with a combination of unsaturated plantar fatty acids and Vit. E, enhance the healing process of infected wounds. The term Photo-aromatherapy, which was originated by us, describes the combined usage of the LIL source and an ointment containing the above mentioned natural ingredients. Patients with sporadic infected facial lesions were treated with Photo-aromatherapy in order to study the duration of the healing process.

Patients and Methods: 300 patients (20–62 years) with sporadic facial infected lesions (folliculitis, rosacea, acne) were treated 3 times a day (for 3 min.) with the LIL source apparatus (Emjoi) radiating on the affected lesions which were covered with a thin layer of essential oils ointment (Aromatolin). The lesions were covered permanently with the ointment. The patients were examined and photographed every 12 hours.

Results: In 192 patients, there was a quick improvement in the healing process up to 24 h, and in 65, up to 48 h. In the others, improvement was achieved more slowly, especially in those with acne. There were no complications or hypersensitive reactions.

Conclusions: It appears that the combined effect of the novel LIL apparatus (Emjoi) and essential oils ointment (Aromatolin) — Photo-aromatherapy — enhances the duration of the healing process and we are now using this treatment in patients with various sporadic infected lesions.

L 7

GC-SPME as a Tool for Rapid Estimation of Dill Seed Aroma Compounds and Their Enantiomers

Renata Zawirska-Wojtasiak, Przemysław Czarnomski, Erwin Wąsowicz
Institute of Food Technology of Plant Origin, Agricultural University
ul. Wojska Polskiego 31, 60-624 Poznań, Poland
renazwa@owl.au.poznan.pl

Dill *(Anethum graveolens L.)* is an interesting herb for the production of essential oil with characteristic caraway-like aroma and potato sprouting inhibitor S-(+)-carvone, which is the main odour active compound of dill seeds oil. Dill seeds oil contains also the high amount of limonene; concentration of both the compounds amounted to more than 90 % of total volatiles, but it varied dependently on dill variety, place and year of crop [1].

Solid-phase microextraction (SPME) was examined for its suitability for isolation of volatiles from seeds of dill, estimation and differentiation between the aroma of different varieties in comparison with the traditional steam distillation method. Two mention above compounds were taken into consideration: carvone and limonene. The SPME technique recently has been successfully used for separation of volatiles organic aroma compounds [2, 3].

Two Supelco SPME fibers were use for the extraction: PAc-polyacrylic 85 µm and PDMS-polydimethylsiloxane 100 µm. The time required to saturate PDMS and PAc fibers was 3 minutes, while distillation of volatiles took 3 hours. To make the whole analysis faster the time required for gas chromatography separation was reduced to 5 minutes by use of microcapillary column HP-5 Crosslinked 5 % PH ME Siloxane.

The standards of limonene and carvone in the range of concentration from 2 to 50 mg per gram of seeds' matrix were used to prepare calibration curves. PAc fiber responses were described by quadratic curve, while PDMS responded lenear, but in the case of limonene only for low concentration values (2–10 mg/g).

The distillation and SPME method were applied for evaluation dill samples of different varieties. The analysis of variance indicated that every methods (SPME-PAc, SPME-PDMS, Distillation) gave different absolute values, but the analysis of regression has shown statisticaly high significant ($\alpha = 0.0008$) regression between the data from distillation and SPME-PDMS for carvone. The elaborated parameters of SPME-PDMS-GC method give the possibility to determine carvone content in dill samples within 10 minutes.

It is known that carvone and limonene are both chiral compounds and occure as two optical isomers. The predominated forms in dill seeds oil are R-(+)-limonene (over 99.1 %) and S-(+)-carvone (over 99.4 %) [4]. This characteristic enantiomeric ratio may be use as the criterium of natural aroma authenticity [5]. The SPME methods were also tested for their suitability for chiral resolution of carvone and limonene from seeds of dill. The enantiomeric separation was done with two chiral gas chromatography columns: Rtβ-Dex s.a. for carvone and Rtβ-Dex s.e. for limonene. The data obtained for enantiomeric ratios were just the same as with distillation.

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L 8

Gas Chromatography-Mass Spectrometric Analysis of the Components of Agarwood (*Aquilaria agallocha* Roxb.) Oil from Assam, India

Abhijit Talukdar[1], G. U. Ahmed[1] and S. K. Dutta[2]
[1]Department of Biotechnology, Gauhati University, Guwahati 781014, Assam, India
[2]Forensic Science Laboratory, Guwahati 781019, Assam, India

Agar oil obtained by hydro-distillation of agar laden wood of *Aquilaria agallocha* Roxb. has been studied for its constituent organic compounds. GC-MS analysis of the oil has shown the presence of at least 35 different compounds, out of which 18 were identified. Three furanoids viz. 3-methyl-2-(2-methyl-2-butenyl)-furan; 2-isobuteyl-3-methyl furan and 3-methyl-2-(2-oxopropyl)furan were identified as the main aromatic components of the oil. Besides these some acids, ketones alcohol and aldehydes were also identified and reported here.

L 9

Aroma of Living Flowers and Fruits™ from Earth to Heaven (MicroGravity)

Braja D. Mookherjee, Subha M. Patel, Robert W. Trenkle and Boaping Jin
Research and Development, International Flavors & Fragrances,
1515 Highway 36, Union Beach, New Jersey, USA 07735
Phone: +1-732-335-2506
braja.mookherjee@iff.com

We, who are associated in Flavors & Fragrance business, are very familiar with the aroma of common flowers and fruits grown in soil on earth. But to our knowledge no one has every studied the characteristic of aroma of living flowering plants when the growth media has changed from soil to water (hydroponics) on the earth. Moreover, no one has also studied, what could happen when the flowering plant has been moved from normal Earth gravity to the microgravity in Space.

The present paper will discuss the mystery of aroma constituents of a flowering plants, particularly rose, grown hydroponically and in microgravity in Space.

L 10

The Essential Oils of *Thymus* Species of Turkey: An Update

K. Hüsnü Can Başer[1], Nese Kirimer[1], Gülendam Tümen[2]
[1]Medicinal and Aromatic Plant and Drug Research Centre (TBAM),
Anadolu University, 26470-Eskişehir, Turkey
http://www.tbam.anadolu.edu.tr/
[2]Department of Biology, Faculty of Science and Letters,
Balikesir University, 10100-Balikesir, Turkey

Sixty-one *Thymus* taxa belonging to forty species are recorded in the Flora of Turkey [1–3]. *Thymus vulgaris*, which is not a native species, is sometimes cultivated. Essential oils of fifty-one taxa comprising 85 % of the total number of *Thymus* taxa of Turkey have been subjected to GC/MS analysis at TBAM.

The paper summarizes the work done at TBAM on several collection of the above mentioned *Thymus* taxa and reports new findings.

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L 11

Aromatic Plants From Corsica: Composition and Chemical Variability of Their Essential Oils

Joseph Casanova
Université de Corse, Equipe 'Chimie et Biomasse', UMR CNRS 6134
Route des sanguinaires 20000 Ajaccio, France
casanova@vignola.univ-corse.fr

Corsica, one of the largest islands of the Mediterranean (8700 km^2), has more than 1000 km of coast and high mountains (Monte Cinto, 2710 m). Corsican landscapes are characterised by a very important diversity, so that more than 2000 species of plants grow wild in the Island, 132 species being endemic. Among them, there are many aromatic plants, which give essential oil.

In our laboratory, we investigated chemical compositions of Corsican essential oils. When necessary, we examined the evolution of the composition along the vegetative life of the plant as well as the chemical variability, if it occurs.

Identification of components has been carried out by (i) the GC retention indices (RI); (ii) comparison of the mass spectra with those of reference compounds; (iii) ^{13}C-NMR spectroscopy, following the pioneering work done by Formàcek and Kubeczka [1] and according to an experimental procedure and a computerised method developed in our laboratory [2]. Depending of the aim of our work, analysis was carried out on the bulk sample or after fractionation by liquid chromatography.

We would like to present some of our latest results of essential oils obtained from Corsican plants:

Labiae: *Thymus herba barona*, *Rosmarinus officinalis*, *Calamintha nepeta*, *Lavandula stoechas*, *Stachys glutinosa*,...

Apiaceae: *Smyrnium olusatrum*, *Chrithmum maritimum*, *Ferula communis*, *F. arigonii*,...

Anacardiaceae: *Pistacia lentiscus.*

Myrtaceae: *Myrtus communis.*

Cupressasseae: *Pinus nigra ssp laricio*, *Juniperus phoenicea*, *J. communis ssp alpina.*

We will also report on the chemical variability of essential oils (leaf oil and peel oil) from Citrus trees grown in the same pedoclimatic and cultural conditions in the germplasm collection orchard of the Station of Agronomic Research of INRA-CIRAD, located at San Ghjulianu, Corsica.

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L 12

The Fragrance Components of Flowers of Quisqualis Indica and the Composition of the Essential Oil

Y. Ramachandra Rao and Prashant K. Rout
Forest & Marine Products Division, Regional Research Laboratory
Bhubaneswar 751013, India

Quisquails Indica syn. Q. Densiflora, family Combretaceae, is a large woody, scandent shrub. It is indigenous to tropical Africa and Indo Malaysian region. It is a hardy creeper grown in gardens for its bright pink to red flowers. The plant is in profuse blooming during March-April and flowers appear in constant succession in drooping clusters. The flowers have a characteristic mild floral sweet but astringent fragrance pervading into the surroundings especially during evenings. Distilling the flowers in a Clevenger apparatus gave deep yellow oil in 0.010–0.015 % yields. The oil is analysed on GC and GC-MS to contain cis and trans linalool oxides (10 %), linalool (11 %), 2,2,6-trimethyl-3-keto-6-vinyl-tetrahydro pyran (9.5 %), α-farnesene (9.4 %), and a hydrocarbon tentatively identified as normenthane (29.5 %) besides several minor constituents. This is the first report of the chemical composition of the essential oil of quisqualis flowers.

Solvent less extraction of the fragrance from a few freshly bloomed flowers using Solid Phase Micro Extraction (SPME) on poly dimethyl siloxane fibre and analysis of the adsorbed components brought to light an altogether different composition. The fragrance analysed to contain normenthane (27 %), 2,2,6-trimethyl-3-keo-6-vinyl-tetrahydro pyran (41 %), 2,2,6-trimethyl-3-hydroxy-6-vinyl-tetrahydro pyran (5 %), α-farnesene (11 %) and 1-Cyano-isoquinoline (5.5 %), besides thirteen other minor constituents. Analysis of fragrance from flowers at variable periods from the time of plucking will be also presented.

The study shows that several major components of the essential oil are the artefacts produced during distillation and a few compounds found by SPME have been totally transformed.

L 13

Stability of Essential Oil Composition

Johannes Novak[1], Heinrich Grausgruber[2], Carla Vender[3], Léon van Niekerk[4],
Wolfram Junghanns[5] and Choldwig Franz[1]
[1]Institute for Applied Botany, University of Veterinary Medicine,
Veterinaerplatz 1, A-1210 Vienna
johannes.novak@vu-wien.ac.at
[2]Department of Plant Production and Plant Breeding, University for Agricultural Sciences,
Gregor-Mendel-Straße 33, A-1180 Vienna, Austria
[3]ISAFA, piazza Nicolini, 6, I-3805 Villazzano di Trento, Italy
[4]Darbonne, 6, bd. Joffre — B.P. 8, F-91490 Milly-la-Forêt, France
[5]MAWEA, Majoranweg 21, D-06449 Aschersleben, Germany

Stability is in plant breeding a concept focusing especially in genotype x ervironment interactions.

A genotype is seen as ‘stable’ if he reacts at different environments equal to its expected value. These concepts may also be of interest for the cultivation of essential oil plants where the essential oil composition should react equally (‘stable’) on different environments to guarantee homogeneous qualities from different environments.

Regarding the essential oil composition, two stability concepts (‘static’ vs. ‘dynamic’ concept) are compared at the example of marjoram (*Origanum majorana* L.) genotypes cultivated at three locations in Europe. Additionally, non-parametric stability calculations of sensorial categorial data (smell and taste) evaluated by a sensorial panel are discussed.

L 14

Essential Oil Composition of the Eight Aroma Plant Species from Iran

M. M. Barazandeh
Research Institute of Forests and Rangelands
P.O. Box 13185-116, Tehran, Iran
mbaraz@rifr-ac.org

As a part of whole project, we report the essential oil composition of the eight aroma plant species, which are important from the views of essential oil and therapeutic aspects. Their essential oils were produced by steam distillation or other methods and analyzed by capillary GC and GC/MS:

Mentha aquatica L. has sixteen compounds which among them menthofuran (52.6 %) and β-caryophyllene (11.0 %) have the highest percentages.

The leaves of *Laurus nobilis* L. which were collected from two different locations (Tehran and Noshahr) have 35 compounds which among them 1,8-cineole (48.5, 44.0 %), α-terpinenyl acetate (12.5, 15.6 %) and sabinene (9.5, 10.3 %) are the major constituents, respectively.

Essential oils of two *Lavandula* species from Iran and France have 42 compounds which linalool (30.9, 31.9 %), 1,8-cineole (20.9, 18.8 %) and borneol (8.9, 10.1 %) are the major constituents, respectively.

Essential oils of *Eucalyptus camaldulensis* L. were produced by three different methods (i.e. steam distillation, clevenger and kaiser) and 24 compounds identified which among them 1,8-cineole (35.6, 48.8, 63.7 %) and spathulenol (6.7, 3.7, 3.9 %) are the major constituents, respectively.

In *Origanum vulgare* 34 compounds identified which among them β-caryophyllene (24.5 %) and germacrene D (15.2 %) have the highest percentages. In addition in an other species of this plant (*O. majorana* L.) 24 compounds identified which among them linalyl acetate (26.1 %) and sabinene (12.0 %) are the major constituents.

Fresh and dry leaves of *Myrtus communis* L. have 32 compounds which α-pinene (29.1, 22.4 %), limonene (21.5, 19.2 %), 1,8-cineole (17.9, 23.4 %) and linalool (11.0, 10.4 %) are the major constituents, respectively.

Twenty-eight compounds were identified in the essential oil of *Artemisia fragrans* Willd. among which 1,8-cineole (52.1 %) and α-thujone (34.8 %) are the major constituents.

L 15

Determination of Gas Chromatographic Retention Indices by Computer Programs

M. M. Barazandeh
Research Institute of Forests and Rangelands
P.O. Box 13185-116, Tehran, Iran
mbaraz@rifr-ac.org

In qualitative analysis of organic compounds by gas chromatography method, using retention indices is inevitable because in some cases (such as many terpenes) their mass spectra are essentially identical and not adequate for identification. This can be due to the initial similarity of structures, or due to various fragmentations after ionization [1]. The retention index is based upon a comparison between the position of an analyte peak and the peaks of two or more normal paraffins and by definition, the retention index for a normal paraffin is equal to 100 times the number of carbon atoms in the compound, regardless of the columns or the chromatographic conditions.

In essential oil analyses by capillary gas chromatography method, sometimes the constituents are more than 100 numbers so that measuring the retention indices one by one is very time consuming. In order to measure accurate retention indices and also fast calculation, we wrote two separate computer softwares for two different polarity columns which most commonly are used in essential oil analyses (i.e. Polyethylene glycol the polar column and Polydimethyl siloxane the nonpolar one). These programs calculate the retention indices only by inputing the name of sample and applied for determination of essenthial oil retention indices. In isothermal condition, retention indices (which are also known as Kovats retention indices), had very good reproducibility but in the case of temperature programmed (which is common method in essential oil analyses), they showed large random deviations. The most accurate retention indices are obtained when the n-alkanes chromatographed together with the solution of the investigated solutes but in most cases the samples have many components so it is not possible to mix the n-alkanes into the analysed sample. Although improvement of the reproducibility of retention indices is possible by using normalized sets of retention times of n-alkane series. In order of measuring these normalized sets, the difference or ratio of the retention time of a member of the n-alkane series measured in the sample and measured separately, should be added to or multiplied by retention times of n-alkane series. Experiences showed that by using the ratio of retention times, reproducibility is better [2].

For applying the above method to the qualitative analyses of essential oils, we first tested the method for measuring retention indices of 40 authentic standard compounds which were available in the lab and after confirmation, we used this method in essential oil analyses. Using the two computer programs and also the normalized retention times of n-alkane series, caused firstly fast and accurate calculating of retention indices and second improvement of the reproducibility.

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L 16

Pharmacological Studies on the Mode of Action of Essential Oils and the Corresponding Hydrosols, Teas and Methanolic Extracts of Scented Pelargonium Species and Cultivars

Maria Lis-Balchin[1], Suzanne Groisse[2], SandraVollmaier[2] and Stephen Hart[3]
[1]School of Applied Science, South Bank University,
Borough Road, London, SE1 OAA, UK
lisbalmt@sbu.ac.uk
[2] Institute of Pharmaceutical Chemistry, University of Vienna,
A-1090 Vienna, Althanstrasse 14, Austria
[3] School of Biomedical Sciences, King's College London,
Guy's Campus, London, SE1 9RT, UK

Some scented *Pelargonium* cultivars give rise to the commercial 'geranium oil' which is used mainly in perfumery, the food industry and also aromatherapy. The many medicinal properties conferred on 'geranium oil' have been erroneously misinterpreted from the herbal use of the *Geranium* species. The scented *Pelargonium*, originating from Southern Africa have not been studied extensively and therefore pharmacological studies were originally instigated to investigate the mode of action of the essential oil including the commercial geranium oil and that obtained from various species and cultivars (Lis-Balchin et al., 1996; Lis-Balchin and Hart, 1998). All the essential oils produced a spasmolytic effect on guinea-pig ileum preparations *in vitro* and the mode of action of many rose-like or minty-like *Pelargonium* essential oils appeared to use cyclic AMP as their secondary messenger.

Teas and alcoholic extracts of Pelargoniums have, however, been largely used in South African herbal Folk medicine, mainly as an anti-diarrhoeal agent. As these were largely more water-soluble extracts, the pharmacological action could be different to that of essential oils. Fresh *Pelargonium* leaves were therefore extracted in methanol and boiling water and their pharmacology studied on smooth muscle *in vitro*. The water-soluble hydrosol, remaining after the essential oil was extracted using steam/water distillation was also tested, in order to determine any commercial viability for this waste product.

The results showed that some of the more hydrophobic extracts elicited a contraction, sometimes followed by a relaxation. The mode of action of the spasmolytic action was shown in many cases to use calcium channels at physiological concentrations, unlike their essential oil equivalents.

The different modes of action of these extracts from different *Pelargonium* species and cultivars will be discussed in detail.

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L 17

Sesquiterpene Hydrocarbons of Some *Fusarium* and *Penicillium* Fungi as Indicators of Their Toxigenicity

Henryk H. Jeleń
Institute of Food Technology, Agricultural University of Poznań,
Wojska Polskiego 31, 60-624 Poznań, Poland

Fungi are a source of odoriferous compounds, which contribute to *Basidiomycetes* mushroom flavor, as well as to off odors generated by some molds. Molds that belong to *Fusarium* and *Penicillium* genera are often associated with agricultural crops spoilage, the first are typical field fungi, whereas the latter invade stored products. Some of them are a severe menace to livestock and humans because of their ability to produce toxic metabolites. Among the most important *Fusarium* toxins are trichothecenes, a family of closely related sesquiterpenoids, whereas *Penicillium roqueforti* produces PR-toxin and related compounds also of a sesquiterpenoid character.

The research was aimed at identification of volatile compounds produced by toxigenic *Fusarium sambucinum* and *Penicillium roqueforti* strains to look for volatile markers of undergoing biosynthesis of trichothecenes and PR-toxin.

Selected strains were incubated on autoclaved wheat kernels medium for up to 17 days. Volatile compounds were isolated by purge and trap, or SPME methods, and compounds were resolved and identified by gas chromatography/mass spectrometry (GC/MS). Selected trichothecenes were analyzed by GC/MS, PR-toxin by thin layer chromatography (TLC).

Trichodiene was the most significant compound among volatile sesquiterpene hydrocarbons produced by *Fusarium* strains, whereas aristolochene was the most important one among *Penicillium roqueforti* volatiles. Both are intermediates in the biosynthesis of trichothecenes and PR toxin skeletons respectively.

Toxigenic strains produced much more volatile sesquiterpenes then nontoxigenic ones and of a different character. Among *Fusarium sambucinum* sesquiterpenes the most abundant were β-farnesene, α-farnesene, β-bisabolene, β-chamigrene, β-himachalene. The most characteristic for *Penicillium roqueforti* were β-elemene, β-patchoulene, α-cedrene, α-himachalene, γ-himachalene, β-himachalene and α-chamigrene. Toxigenic strains were easily distinguishable from nontoxigenic ones by profile of their voatile sesquiterpenes. Profile of sesquiterpene hydrocarbons in investigated strains was characteristic feature of a given strain and not influenced by incubation temperature, solid medium type and water contents in a medium. When dynamics of formation of volatile sesquiterpenes and toxins was investigated high correlation of these processes was observed and characteristic volatile sesquiterpenes were detectable in a medium prior to toxins formation.

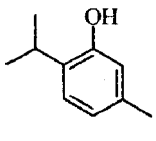
Volatile sesquiterpene hydrocarbons can be a valuable tool in *Fusarium* and *Penicillium* chemosystematics and an indicator of strains toxigenicity.

L 18

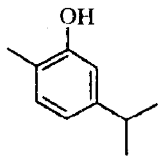
Antifungal Properties of Some Oxygenated Aromatic Essential Oil Compounds against White-Rot and Brown-Rot Fungi

Karmen Voda[1], Margareta Vrtačnik[1], Bojana Boh[1], Franc Pohleven[2]
[1]Department of Chemical Education and Informatics, Faculty of Natural Sciences and Engineering,
University of Ljubljana, Vegova 4, P.O. Box 18/1, SI-1001 Ljubljana, Slovenija
karmen.voda@uni-lj.si
[2]Department of Wood Science and Technology, Biotechnical Faculty,
University of Ljubljana, Rožna dolina, Cesta VIII/34, P.O. Box 2995, SI-1001 Ljubljana, Slovenija

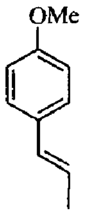
Many scientific studies have confirmed that essential oils and their constituents have antifungal properties [1–3]. Within our study, 22 essential oil phenols, phenol ethers, and aromatic aldehydes have been tested for their antifungal activity against two types of wood decaying fungi: the white-rot *Trametes versicolor* and the brown-rot *Coniophora puteana.* Test organisms were grown on potato-dextrose agar (PDA), minimal inhibitory concentrations (MICs) of the selected compounds were determined by the agar dilution method within the concentration limits of 20 to 0.078 mM, and the results were collected after a seven day incubation period in a growth chamber at 25 °C. The results of the experimental determination of MICs indicate a difference in the tolerance of the fungi towards the tested compounds. However, thymol, carvacrol, *trans*-anethole, methyl chavicol, and cuminaldehyde were the most active, while *o*-cresol, guaiacol, hydroquinone, and coniferaldehyde were the least active compounds in both cases. Our results are consistent with the results of other researchers, who found that thymol and carvacrol are among the most antifungally active essential oil phenols, followed by eugenol, isoeugenol, and phenol ethers such as *trans*-anethole, and methyl chavicol [4,5]. The results of our research also point to a potential relationship between the chemical structure and the experimentally determined antifungal activity of the tested essential oil compounds, which will be further studied by QSAR methods with the purpose of predicting antifungal properties of structurally similar oxygenated aromatic compounds and designing an optimally active wood preservative against white-rot and brown-rot fungi, based on essential oils.



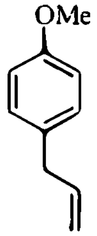
thymol



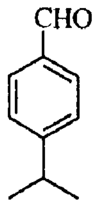
carvacrol



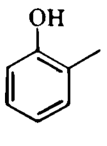
trans-anethole



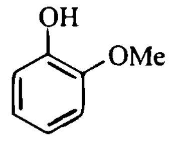
methyl chavicol



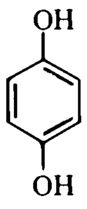
cuminaldehyde



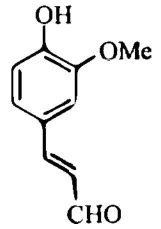
o-cresol



guaiacol



hidroquinone



coniferaldehyde

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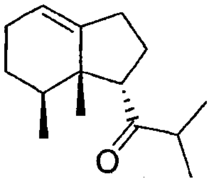
L 19

New Sesquiterpene Derivatives from the Liverworts *Lepidozia fauriana* and *Lepidozia vitrea*

Claudia Paul[1], Wilfried A. König[1], Chia-Li Wu[2]
[1] Unversität Hamburg, Institut für Organische Chemie,
Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany
[2] Tamkang University, Tamsui, Taiwan

From the essential oils of the Taiwanese liverworts *Lepidozia fauriana* and *L. vitrea* several new oxygenated sesquiterpenoids were isolated and spectroscopically characterised. 11,12-Dihydrochiloscyphone **(1)** and 7,10-anhydro-11,12-dihydrochiloscypholone **(2)** were iso-lated with the chiloscyphane backbone, three amorphane derivatives amorpha-4,9-dien-14-al. **(3)**, amorpha-4,9-dien-2-ol **(4)** and 7,14-anhydro-amorpha-4,9-diene **(5)** were identified from *L. fauriana.* From *L. vitrea* two oxygenated elemanes 7-acetoxy-elema-1,3-dien-8-ol **(6)** and elema-1,3-dien-7-ol **(7)** were isolated and identified. For single compounds structure elucidation was carried out by NMR spectroscopy and chemical correlations in conjunction with enantioselective gas chromato-graphy to establish absolute configurations.



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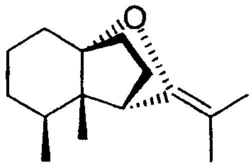

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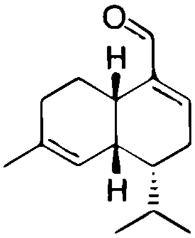

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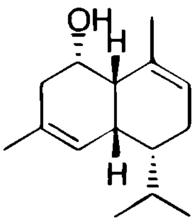

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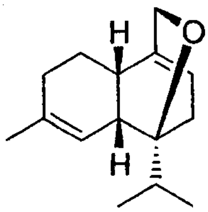

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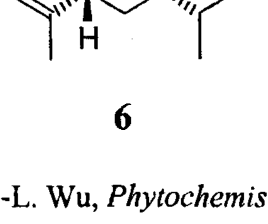

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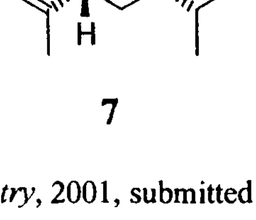

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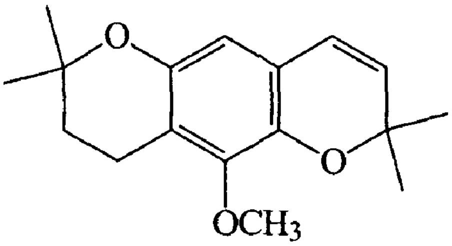
L 20

A Linear Dihydrodichromene Derivative from the Liverwort *Metacalypogeia alternifolia*

Horng-Shing Shy[1], Chia-Li Wu[1], Claudia Paul[2] and Wilfried A. König[2]
[1]Department of Chemistry, Tamkang University, Tamsui, Taiwan 251
clwu@mail.tku.edu.tw
[2]Institute of Organic Chemistry, University of Hamburg,
Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

A novel linear dihydrodichromene derivative, metacalypogin, was identified from the liverwort *Metacalypogeia alternifolia* of which the chemical constituents were first thoroughly investigated. *M. cordifolia* showed nearly identical chemical constituents as those of *M. alternifolia.* This is the first report on the chemical structure of this genus and the natural occurrence of a dihydrodichromene.



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L 21

Constituents of the Essential Oil of *Cymbopogon nervatus* Inflorescences from Sudan

Hatil Hashim Elkamali[1], Hassan ELSubki Khalid[2], Babiker Fadlalla[3],
Mohammed Ahmed Hassan[4], Hizabr Hashim Elkamali[5]
[1] Department of Botany, Omdurman Islamic University,
382, Omdurman, Sudan
[2] Department of Phytochemistry, Medicinal and Aromatic Plants Research Institute,
11496, Khartoum, Sudan
[3] Department of Botany, University of Khartoum,
321, Khartoum, Sudan
[4] Dubi Police Crime Laboratory
jumaa@emirates.net.ae
[5] EL-Wasal Hospital, Pharmacy Department,
Dubi, UAE

Several species of the genus *Cymbopogon* (Poaceae) in Sudan have been the subject of numerous chemical studies [1]. Analysis of the essential oil from *Cymbopogon nervatus* (Hochst.) Chiov. leaves was reported [2]. In this study, we report on the GC-MS analysis of the hydrodistilled essential oil from dried *C. nervatus* inflorescences (1.3 % yield) growing wild in East central Sudan. The oil sample was analyzed in a Hewlet-Parkard GC-MS model 6890 instrument. Identification of compounds were based upon mass-spectra and retention times. Nine compounds were identified, of which cis-p-mentha-1(17),8-dien-2-ol (25.24 %), trans-p-mentha-1(17),8-dien-2-ol (22.93%) and trans-carveol (9.58 %) were the major constituents. A high proportion of oxygenated monoterpenes (94.28 %) were also found in the inflorescence oil. Sesquiterpenoids were not detected. Some differences were found in comparison with *C. nervatus* leaf oil analyzed in earlier studies which may suggest the occurrence of different chemotype.

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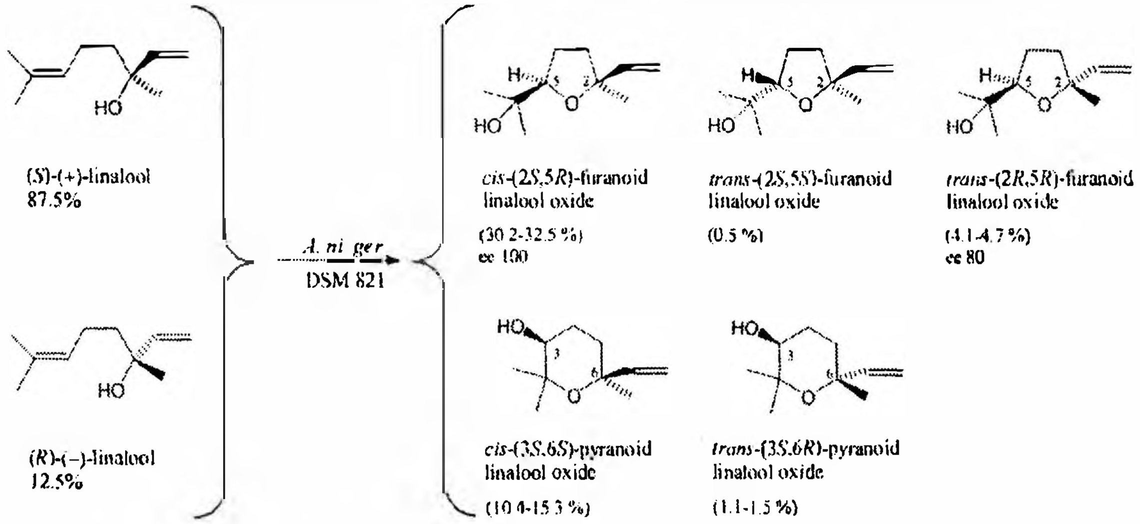
L 22

Biotransformation of (S)-(+)-Linalool and Natural Coriander Oil by *Aspergillus niger*

Jan C. R. Demyttenaere, An Adams, Jan Vanoverschelde, Norbert De Kimpe
Department of Organic Chemistry,
Faculty of Agricultural and Applied Biological Sciences, Ghent University,
Coupure Links 653, B-9000 Ghent, Belgium
jan.demyttenaere@rug.ac.be

In the course of our research devoted to the bioconversion of terpene alcohols by fungi [1, 2], we investigated the biotransformation of (S)-(+)-linalool by different *Aspergillus niger* strains, using submerged shaken liquid cultures. One strain, *A. niger* DSM 821 was able to convert the substrate to *cis*- and *trans*-furanoid linalool oxide (yield 30 % and 5 %, respectively), and *cis*- and *trans*-pyranoid linalool oxide (yield 14 % and 1.5 %, resp.). The bioconversion of natural coriander oil, containing 69 % linalool (ee (S)-(+)-linalool 75 %), was also studied (see Figure). The culture conditions involved such as the composition of the broth, the type and concentration of co-solvent applied and possible adaptation to the substrate during inoculation were investigated. It was found that (S)-(+)-linalool was converted much better than (R)-(–)-linalool, and that no significant chemical conversion of the substrate was noticed in control flasks at pH 3.5. Three co-solvents for improving the solubility of linalool in the culture broths were compared, namely MeOH, EtOH and acetone. The highest bioconversion yields were obtained when the substrate was applied as a diluted solution in acetone.

Figure. Bioconversion of natural coriander oil (ee (S)-(+)-linalool 75 %) by *Aspergillus niger*.



Screening of the fungi for their biotransformation capacity was performed by solid phase microextraction (SPME), a technique which has also been used for the screening for the bioconversion of the monoterpene hydrocarbon limonene by fungi [3].

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**Acknowledgement: The authors wish to thank the Dept. of Org. Chem., Faculty of Sciences, Group of Separation Sciences for their financial support (project no. BOF/GOA 1205898).*

L 23

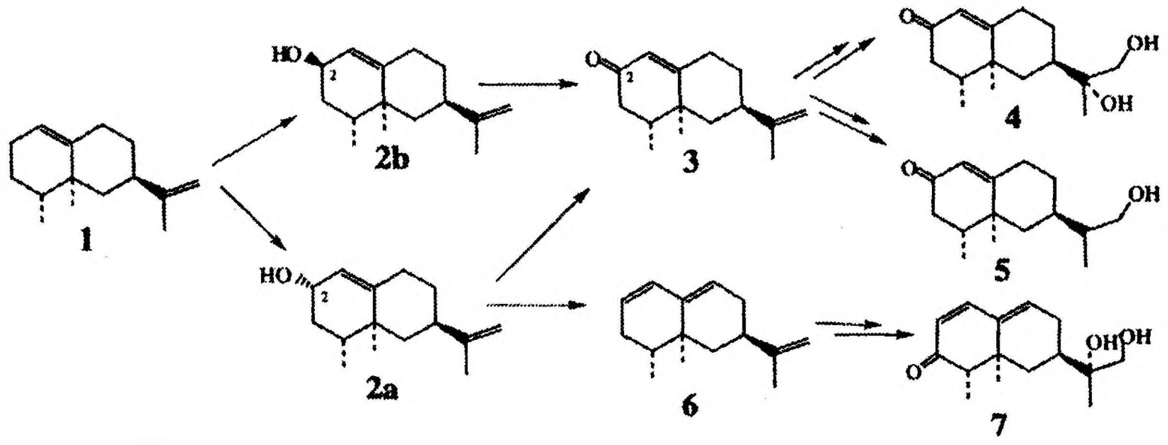
Microbiological Transformation of Valencene, Nootkatol and Nootkatone

Yoshiaki Noma[1*], Toshihiro Hashimoto[2], Chie Murakami[2], Mai Furusawa[1],
Yoshinori Asakawa[2*]
[1]Faculty of Domestic Sciences, Tokushima Bunri University
ynoma@tokushima.bunri-u.ac.jp
[2]Faculty of Pharmaceutical Sciences, Tokushima Bunri University,
Yamashiro-cho, Tokushima 770-8514, Japan
asakawa@ph.bunri-u.ac.jp

We are continuing to study the microbiological transformation of plant secondary metabolites [1, 2] from pharmacological and fragrant point of view.

In this paper, the biotransformation of valencene (1), 2α- (2a) and 2β-nootkatol (2b), and nootkatone (3) by various kinds of microorganisms will be discussed.

Compound 1 was biotransformed mainly to 3 via 2a and 2b by *Chlorella pyrenoidosa* IAM C-28, *Botryosphaeria dothidea* and a soil strain KK. Compounds 2a and 2b were easily transformed to 3 by *Chlorella*, *Euglena*, *Dunaliella*, *Hansenula*, *A. niger*, *A. cellulosae* and *Fusarium*.



Compound **1** was also biotransformed *via* nootkatene (**6**) and 3-oxonootkatene to 3-oxonootkatene-11,12-diol (**7**) and *via* **3** to nootkatone-11,12-diol (**4**) by *A. niger*. Compound **3** was biotransformed to **4** *via* nootkatone-11,12-epoxide together with a small amount of nootkatone-12-ol (**5**) by *A. niger*. When **2a** was biotransformed by *A. niger*, **3**, **6** and **7** were obtained. On the other hand, **3** and **4** were obtained from **2b**. There are different biotransformation pathways in the present substrates by *A. niger*. The production of **3** and **2b** from **1**, a valencia orange constituent, is very significant since each compound is grapefruit fragrance and calcium antagonist [3], respectively.

[1] Noma Y., Asakawa Y., *Biotechnology in Agriculture and Forestry*, Springer-Verlag Berlin Heidelberg, *33*, 62–96, 1995.
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L 24

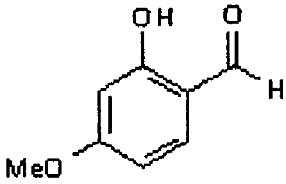
Supercritical Fluid Extraction of *Decalepis hamiltonii* W. & A. and Novel Insecticidal and Antimicrobial Activity of 2-Hydroxy-4-Methoxybenzaldehyde from the Supercritical Extract

J. George[1,2], K. Udaya Sankar[1], J. Pereira[1], N. Keshava[1], S. Divakar[1], M. D. Laing[2], S. E. Drewes[2]
and G. A. Ravishankar[1]
[1]Central Food Technological Research Institute,
Mysore 570 013, India
[2]University of Natal Pietermaritzburg,
Scottsville 3209, South Africa
georgej@nu.ac.za

Decalepis hamiltonii W. & A. (Asclepiadaceae),commonly called 'swallowroot' is a monotypic genus restricted to hilly and forest areas of the Eastern and Western Ghats of peninsular India. The root possesses a sarsaparilla-like taste and is widely used in pickles and Ayurvedic tonics.

Supercritical carbon-dioxide extraction of *D. hamiltonii* roots resulted in 4.42 % of an amorphous, waxy extract containing over 69 % of the flavour compound, 2-hydroxy-4-methoxybenzaldehyde (a). In addition, eight constituents were isolated from the extract, three reported for the first time from this plant. Supercritical extraction afforded a higher yield of around 4.77 times compared to steam distillation [1].

Insect-based bioassays of 2-hydroxy-4-methoxybenzaldehyde from the supercritical extract (SCE) exhibited residual contact toxicity of the compound to red flour beetle *Tribolium castaneum* Hbst., rice weevil *Sitophilus oryzae* L. and lesser grain borer *Rhyzopertha dominica* F. The effective deposit on glass surface for 50 % mortality (ED_{50}) were *T. castaneum* 1.5 µg/cm^2, *R. dominica* 6.36 µg/cm^2 and *S. oryzae* 8 µg/cm^2.



(a)

Antibacterial activity determined by the agar well diffusion assay showed that at 24 µg/ml, the supercritical extract was highly effective against *Staphylococcus aureus, Vibrio cholerae* and *Bacillus licheniformis*. The volatile fraction from the supercritical extract containing 97 % of 2-hydroxy-4-methoxybenzaldehyde inhibited growth of *Salmonella madrasensis, Staphylococcus aureus, V. cholerae, Bacillus subtilis, B. megaterium* and *Escherichia coli* at 24 µg/ml. The volatile fraction from supercritical extract and 2-hydroxy-4-methoxybenzaldehyde were assayed for antifungal activity employing Poisson Bates technique. Complete growth inhibition was observed at 1000 ppm for *Aspergillus flavus, A. oryzae* and *Trichoderma viride.*

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L 25

Essential Oil Content and Composition of Five *Thymus* Species from Iran and Study of Their Anti-Microbial Effects

F. Sefidkon, S. A. Mirmostafa and F. Askari
Research Institute of Forests and Rangelands,
P.O. Box 13185-116, Tehran, Iran

The genus of *Thymus,* with the common Persian name of Avishan, presents 14 species which are found wild in many regions of Iran [1], four of which are endemic. In this project five *Thymus* species, *T. kotschyanus* Boiss & Hohen, *T. persicus* (Ronniger ex Rech. f.) Jalas, *T. pubescens* Boiss & Kotschy, *T. serpyllum* L. and *T. carnosus* L. have been investigated for their oil content and composition and also anti-microbial effect. (The last two species are cultivated in Iran).

The essential oils were isolated by steam distillation from the aerial parts of *Thymus* species in two stages of plant growth (before and full flowering). The oils were analyzed by capillary GC and GC-MS.

The main constituents of the oils (at full flowering stage) were as follow:

***T. kotschyanus**:* carvacrol (41.4 %), thymol (19.6 %), γ-terpinene (10.3 %), p-cymene (5.3 %).

***T. persicus**:* carvacrol (27.1 %), thymol (11.9 %), p-cymene (10.2 %), nerol (9.4 %) and γ-terpinene (6.5 %).

***T. pubescens**:* carvacrol (48.8 %), thymol (13.9 %) and p-cymene (12.7 %).

***T. serpyllum**:* γ-terpinene (22.7 %), p-cymene (20.7 %), thymol (18.7 %) and germacrene D (5.1 %).

T. carnosu: thymol (36.1 %), p-cymene (21.3 %) and γ-terpinene (19.1 %).

All of the essential oils showed anti-microbial effects. The most significant effect has been observed for *T. pubescens* oil.

The complete results about oil content and composition of these species at two stages of plant growth and details of anti-microbial experiments will be presented.

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L 26

The Chemical Composition of *Senecio oreophyton* Essential Oil

Susana Carolina Nuñez Montoya, Alicia Mariel Agnese, José Luis Cabrera
Farmacognosia, Departamento de Farmacia,
Facultad de Ciencias Químicas, Universidad Nacional de Córdoba,
Instituto Multidisciplinario de Biología Vegetal (IMVIB-CONICET)
Córdoba, Argentina
magnese@dqo.fcq.unc.edu.ar

Senecio oreophyton J. Rémy belongs to the Asteraceae family [1]. It is popularly known as 'chachacoma' or 'Yerba del incordio'. It is a typical little shrub that grows between 3100 and 3600 meters above the sea level in the argentine provinces of Salta, La Rioja, San Juan, and Mendoza [1]. In popular medicine it is reputed as cough suppressant and used against other respiratory diseases [2].

S. oreophyton was collected in 'El Leoncito' reservation, San Juan Province, and it was identified by Prof. Dr. L. Ariza Espinar. A voucher specimen has been deposited at CORD as Lujan 66.

Our research group had studied the chemical composition and biological activity of the essential oil of another species of this genus: *Senecio graveolens*, demonstrating that it has antimicrobial activity on highly pathogenic bacterial and fungal species [3]. *S. graveolens* is also used in popular medicine with the same purpose as *S. oreophyton*. This reason and the lack of chemical and biological studies of the latter stimulated us to initiate them in order to determine the essential oil chemical constituents and investigate if it has similar biological activity. In the present work we report the chemical composition and comparison with the essential oil previously studied.

The aerial parts of *S. oreophyton* were subjected to hydrodistillation in order to obtain its essential oil. In this way, a pleasant smell and intense yellow oil with a yield of 2 % v/w was obtained. The density was determined: 0.8770 g/ml.

The components of the essential oil were separated and identified by GLC-MS using a Perkin Elmer Qmass-910 apparatus, and capillary column SE 30, 30 m in length. The injection volume was 0.02 μl with He as carrier, the flow rate 1 ml/min. and the program was 50 °C (T_i) for 5 min., with an increase in rate of 2 °C/min. until it reached 200 °C (T_f). The individual identifications were made by the comparison of the breakdown patterns with those found in the literature [4] and in the library of the mass spectrometer.

As a result, eleven compounds were identified, mainly hydrocarbons, acyclics: beta-ocimene (6 %), and β-myrcene (2.4 %); monocyclics: α-phellandrene (0.16 %), α-terpinene, (0.6 %), limonene (26 %), γ-terpinene (1.3 %), α-terpinolene (34.4 %), p-cymene (0.6 %); bicyclics: α-pinene (6 %), β-pinene (19.7 %), and also one alcohol, terpinen-4-ol (2.8 %).

Comparing the constituents of both essential oils we can say that they have seven components in common (α-pinene, α-phellandrene, α-terpinene, p-cymene, γ-terpinene, α-terpinolene and terpinen-4-ol), nevertheless their percentage is different.

As we are at starting this research, antimicrobial studies will be undertaken in order to determine if *S. oreophyton* essential oil has antimicrobial properties and consequently we would fulfill our objectives.

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P 1

Comparative Chemical Composition of the Essential Oil of Common Juniper Berries Produced by Liquid CO_2 Extraction and Hydrodistillation

Biljana Damjanovic[1], Dejan Skala[2], Dusanka Petrovic-Djakov[2] and Josip Baras[2]
[1]Faculty of Metallurgy and Technology, University of Montenegro,
ul. Cetinjski put bb., 81000 Podgorica, Yugoslavia
biljanad@cg.ac.yu
[2]Faculty of Technology and Metallurgy, University of Belgrade,
ul. Karnegijeva 4, 11000 Belgrade, Yugoslavia

The genus *Juniperus* contains more than 60 species and most widely used in healing is Juniperus communis L. (common juniper), growing well in Montenegro on the high mountain slopes. The essential oil of common juniper berries is frequently used in the liquor and food industry, in pharmacy, in perfume creation and in aromatherapy [1].

Essential oils are traditionally produced by hydrodistillation although this method has some disadvantages, such as the heat instability of the essential oils and the loss of certain water-soluble components. Liquid CO_2 as extraction solvent become very interesting in last few years because it penetrates botanicals quickly thanks to its low viscosity, and its low latent heat of evaporation enables it to be easily removed. Also, liquid CO_2 extracts have not been subjected to any heating at any stage of their extraction and so are the closest in composition to the headspace aroma of the botanical [2].

The aim of the work was to compare chemical composition of the obtained oil with special attention on the presence of very important bioactive compound terpinen-4-ol (Figure 1.). This unsaturated monoterpene alcohol is reported to increase the glomerular filtration rate of the kidneys. For this reason common juniper is commonly utilized as a diuretic to treat conditions involving the kidney and bladder and for relief from symptoms of gout and kidney stones [3].

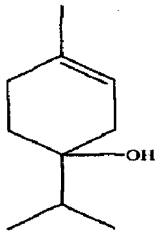

Figure 1. Terpinen-4-ol.

The common juniper berries were collected at Maoce (Pljevlja, Montenegro) at the end of October 1998. The hydrodistillation of the berries was carried out in a Clevenger-type apparatus for 2.5 h. Liquid CO_2 extraction was performed in a laboratory-scale high-pressure plant Autoclave Engineers 'SCE Screening System' with working conditions of 90 bar and 28 °C.

Chemical compositions were determined by GC/MS and results were summarized in the Table. Twenty-nine compounds were identified in the hydrodistilled oil and twenty-eight in the liquid CO_2 oil. The content of terpinen-4-ol was rather same in the both samples. The liquid CO_2 oil contains rather small quantities of monoterpene hydrocarbons, resulting in its higher stability compared to the hydrodistilled oil. Also, liquid CO_2 isolates high percentage of some less-volatile compounds (aromadendrene, methyltranscommunate, globulol), which were not inherent in hydrodistilled oil.

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P2

The Essential Oil Components of some Species of Genus Artemisia Growing in Western Mongolia

S. Shatar[1], TS. Chimiddulam[2], L. Amarjargal, SH. Darjima[3]
[1]Institute of chemistry and chemical Technology of the Mongolian Academy of Sciences, Ulaanbaatar-51, Mongolia
[2]Food Research Production 'Hunstech' of Food and Agricultural Ministry, Mongolia
[3]Pedagogical University of the Mongolia, Ulaanbaatar

The genus Artemisia (Asteraceac) is represented in the Flora of Mongolia by 104 species [1, 2] in Mongolia. A literature search reveahs that the essential oil not been the of previous study. The hydrodistilled essential oils obtained fresh and dried plant material were analysed by GC/MS. A summary of results is as follows.

Artemisia species	Collection place	Main compounds (%)
A. adamsii bess	Mongolian Dahur	β-thujon-67.12 cineol-14.32
A. aksaiensis v. r. ling	Mountains of Mongolian Altai, Bulgan Gol	myrcene-16.08 (E)-beta-ocimene-13.23. germacrene-D-12.40. limonene-8.09. α-zingiberene-5.35
A. capillaris thunb	Khalhiin Gol, Boyr nuur	germacrene-D-29.42, cis-ocimene-11.44, β-pinene-8.70, trans-ocimene-7.52
A. commutata bess	Mountains of Mongolian Altai, Bulgan Gol	germacrene-D-14.28. α-zingiberene-12.76, β-caryophyllene-10.52
A. demissa krasch	Middle Khalkh and Eastern Mongolia	methyl-eugenol-8.51(E)-β-ocimene-8.72. eugenol-7.49. γ-terpinene-6.70. β-pinene 6.58
A. dolosa krasch	Mountains of Mongolian Altai Bulgan Gol	(E)-beta-ocimene-16.04. germacrene-D-14.47. α-zingiberene-8.46 myrcene-6.42. β-caryophyllene-6.97
A. depauperata krasch	Mongolian Altai. Bulgan gol	spathulenol-24.48. cineol-14.20. p-cymene-7.80
A. gracilescens krasch	Gobi-Altai	cineol-40.80. camphor-36.0. terpin-4-ol.3.70
A. laciniata willa	Mongolian Altai, Mongolian Dahur	trans-ocimene-15.03. myrcene-18.42. α-terpinene-18.30. cis-ocimene-7.31
A. latifolia ledeb	Eastern Mongolia Khalkhiin Gol	cineol-29.0 α-pinene-15.90. art. ketone-15.10 menthon-16.0 limonene-6.70. α-terpineol-6.00
A. monostachya bunge	Mongolian Altai Khovd Bulgan	myrcene-20.99. art. curcumene-19.22. germacrene-D-14.30. α-zingiberene-7.70
A. oxycephala kitag	Mountains of Mongolian Altai, Khovd Bulgan	germacrene-D-26.26. (E)-β-ocimene-15.35. myrcene-10.50. art. curcumene-6.43
A. subdigitata mattf.	Dzhungarin Gobi. Khovd Bulgan	eugenol-11.20. methyleugenol-9.40. camphor-9.00. limonen-8.10. sphatulenol-4.80. linalool-4.50. γ-curcumene-4.40
A. sphaerocephala krasch	Gobi-Altai, Eastern Gobi	cineol-29.50. γ-terpinene-15.70. β-pinene-13.00. p-cymene-7.10. α-pinene-6.80. sabinene-7.50

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P 3

The Essential Oil of *Bergenia crassifolia* (L.) Fritsch in Mongolia

Sanduin Shatar, Shataryn Altantsetseg, Luvsan Odsuren
Institute of Chemistry and Chemical Technology of the Mongolian Academy of Science,
Ulaanbaatar-51, Mongolia
sshatar@yahoo.com

The leaves and rhizome of *Bergenia crassifolia (Saxifragaceae)* is widely used in traditional Tibetan and Mongolian medicine [1–3] for the treatment of gastric, stomach intestinal, rheumatism and nervous diseases. It may be characterizied by the contents of biological active principles, such as tannins, which is contained in the leaves about 20 %, while in rhizome about 25 % [1, 4].

The essential oil of *Bergenia crassifolia* was obtained by steam distillation of the rhizomes and chemical characterization of essential oil components was carried out by means of GC-MS analysis. Total yielding of the essential oil is 0.01–0.02 % and about 70 components were identified.

The components were identified by their retention times and their mass spectrum fragmentation in comparasion with bibliographic data reported for references components [5].

A main component was palmitic acid (14.91 %), myristicine (5.74 %), thymol (5.43 %), n-dodecanal (4.79 %), nonanol (3.86 %), (Z)-dihydroapofurnesol (3.83 %), camphor (2.72 %), 2-penthylfurane (2.00 %), β-pinene (1.52 %), ethylhydroquinone, methyleugenol, 2-octenal, cedrol, isoeugenol acetate, farnesol, epi-alpha-cadinol and limonene (0.83–1.52 %) etc.

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P 4

The Essential Oil Composition of *Ocimum basalicum* L. Depending on Mongolian Climate

Shataryn Altantsetseg, Sanduin Shatar
Institute of Chemistry and Chemical Technology of the Mongolian Academy of Science,
Ulaanbaatar-51, Mongolia

The *Ocimum basalicum* L. (*Lamiaceae*) has been reported that it was as food product and also used in folk traditional Mongolian and Tibetan medicine [1, 2]. The results of the chromatographic and mass spectroscopic analysis of the essential oil from *Ocimum basalicum* L. is discussed in Table 1.

Table 1. Basic components of the essential oil from *Ocimum basalicum* L. in Mongolian climate.

Country	Comparative dates (%)			
	Linalool	Methyl chavicol	Methyl eugenol	Eugenol
Mongolia [2]	23.80	51.98	1.09	0.10
Cuba [4]	10.03	56.58	–	–
Taiwan [3]	2.20	68.50	0.78	–
Italy [3]	63.09	30.27	–	–
Izrael [3]	41.72	2.24	–	–
Poland [3]	54.06	8.68	–	–
Egypty [3]	33.0	11.23	0.48	26.49

Among these compounds, 14 are monoterpene hydrocarbons (3.72 %), 11 are oxygenated monoterpenes (80.82 %), 13 are sesquiterpene hydrocarbons and an oxygenated sesquiterpenes were detected in the amount of 15.46 %. The main percentage of essential oil occurs in the oxygenated compounds such as linalool (23.80 %), methylchavicol (51.98 %) and cadinol (4.42 %) respectively.

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P 5

Essential Oil Composition of *Salvia rhytidea* Benth.

S. Ebrahim Sajjadi[1] and M. Khatamsaz[2]
[1]Department of Pharmacognosy, School of Pharmacy,
Isfahan University of Medical Sciences, Isfahan, Iran
[2]Research Institute of Forests and Rangelands, Tehran, Iran

The genus *Salvia* (Sage) of the family Lamiaceae comprises approximately 900 species spread widely throughout the world. Some of these species are used in the food, drug and perfumery industries. Fifty-eight species of the genus *Salvia* are found in Iran, seventeen of them are endemic [1].

Salvia rhytidea Benth. is an Iranian native species distributed in eastern part of the country. Literature survey has shown that *S. rhytidea* has not previously been investigated for essential oil. As part of a screening program of the aromatic medicinal plants of Iran, we decided to analyze the volatile oil of *S. rhytidea.*

The air-dried aerial parts of *S. rhytidea* were powdered and the volatile fraction was isolated by hydrodistillation for 3 hours according to the method recommended in the British Pharmacopoeia. The constituents of the oil were investigated by GC-MS analysis. Identification of components of the oil was based on GC retention indices relative to *n*-alkanes and computer matching with the WILEY275.L library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [2]. The major components appearing in the oil of *S. rhytidea* were p-cymen-8-ol (11.9 %), spathulenol (7.3 %), pulegone (6.4 %), sabinene (5.8 %), terpinen-4-ol (5.5 %), α-copaene (5.3 %) and β-eudesmol (4.8 %).

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P 6

Comparative Study of the Essential Oils of Five Salvia Species Grown Wild in Iran

Shiva Masoudi[1], Abdolhossein Rustaiyan[2]
[1]Department of Chemistry, Central Tehran Branch, Islamic Azad University
[2]Department of Chemistry, North Tehran Branch, Islamic Azad University
soheila@kavosh.net

The genus Salvia (Labiatae) comprises about 700 herbs and shrubs, growing in the temperature and warmer zones of the world. The composition of the oils from leaves and flowers of five Salvia species, *S. hydrangea* Dc. ex Benth. *S. multicavlis* Vahl., *S. sahandica* Boiss. & Buhse., *S. aethiopis* L., and *S. hypolevca* Benth. Of Iranian origin has been analyzed by means of GC/MS in combination with retention indices.

Eighteen Consistuents representing 83 % of the total components in the oil of *S. hydrangea* were characterized by spathulenol (23.1 %) as the main compound. *S. multicavlis* oil contained α-pinene (26.0 %), 1,8-cineole + Limonene (20.0 %) and camphor (19.0 %) among the 16 constituents characterized comprising 94.6 % of the total components detected. β-pinene (34.8 %) and α-pinene (29.4 %) were the main constituents among the 24 characterized comprising 95.8 % of the total components detected in the oil of *S. sahandica.* β-caryophyllene (24.6 %) and α-copaene (15.5 %) as the main compounds characterized twenty-two constituents represent 92 % of the total components in the oil of *S. aethiopis*. *S. hypolevca* oil contained β-caryophyllene (22.0 %), δ-elemene (15.1 %) and bicyclogermacrene (15.0 %) among the 29 constituents characterized, comprising 85.5 % of the total components detected.

As can be seen the above information, the oils (*S. hydrangea*, *S. aethiopis*, *S. hypolevca*) consisted mainly of sesquiterpenes, while in *S. multicavlis* and *S. sahandica* oils monoterpenes predominated over sesquiterpenes.

P 7

Chemical Composition of Essential Oils from the Umbelliferae Family: *Ferulago angulata* Boiss., *Ducrosia assadi* Alva. and *Lomatopodium khorassanicum* Mozaffarian Species Growing in Iran

Soheila Sedaghat, Abdolhossein Rustaiyan & Morteza Khosravi
Department of Chemistry, North Tehran Branch, Islamic Azad University
soheila_67@yahoo.com

The composition of the essential oils from leaves and flowers of *Ferulago angulata* (Schlecht.) Boiss., *Ducrosia assadi* Alva. and *Lomatopodium khorassanicum* mozaffarian has been analyzed by means of GC/MS in combination with retention indices. The genus Ferulago comprises of some thirty-five species of which seven are found in Iran. In the oil of *Ferulago angulata*, twenty-five components were characterized representing 88.7 % of the total components detected with β-Phellandrene (32.0 %) and α-Phellandrene (13.8 %) as the major constituents.

Three species of the genus Ducrosia are found in Iran. *Ducrosia assadi* oil contained Citronellol (38.2 %) and Cis-Chrysanthenyl acetate (11.0 %) among the twenty-nine constituents characterized comprising 94.3 % of the total components detected. Myrcene (28.2 %) and E-β-Ocimene (15.4 %) were the main constituents among the thirty-three characterized comprising 94.5 % of the total components detected in the oil of *Lomatopodium khorassanicum.*

As can seen the above information, all of three oils consisted mainly of monoterpenes.

P 8

Study of Qualitative Properties of Some Sorts of the *Echinacea* Moench Genus

Stefani Vaverkova[1], Magdalena Holla[1], Jozef Tekel[1], Valeria Ostrovska[2], Miroslav Haban[3]
[1]Faculty of Pharmacy, Comenius University, 832 32 Bratislava, Slovakia
vaverkova@fpharm.uniba.sk
[2]Research Institute of Drugs, 900 01 Modra, Slovakia
[3]Faculty of Agronomy, Slovak Agricultural University, 949 76 Nitra, Slovakia

After stormy development of new synthetic, therapeutically usable drugs, it may be seen the world-wide trend of a come back of natural drugs, which are the most acceptable by a human organism. Among the medicinal plants, which became as significant sources for the production of drugs with immuno stimulation activity in the last years, belong some sorts of the *Echinace*a Moench. genus coming from North America. According to the ecological claims they can be divided into several groups. Thus, the *Echinacea* genus contains mainly the mezophytes, which require moderate and/or high supplies of water. Longer soil drying can be withstood only by the sorts, Which possess high adaptability. The area of their original occurrence lies at the near woods – mountain localities on the one side, or at higher positioned sandy ones. The sorts occupying the second týpe of places do not require large water supply, since cylinder-shaped long roots enable them to survive at worse conditions as well.

The informations about constituent compounds from the individual sorts allong with their therapeutical effect were published by several teams of autors (Bauer et al., Bukovsky et al., Vaverkova et al., Horakova et al.)

Influence of technological conditions on the onthogenetic stage of plants and content of oil in three various sorts of *Echinacea* Moench genus were studiet. Comparated were: *Echinacea purpurea* L., *Echinacea atrorubens* NUTT., and *Echinacea pallida* NUTT. The were examined both under-ground and above-ground parts of the plant samples, i. e. roots, stems, leaves, flowers, and their individual parts. They were collected from three localities of their cultivation differing in quality of soil and ecological and climatic conditions. A detailed agro-ecological characteristics of individual localities is archived at the research workplace. Further, the differences in the content of oil in various parts of plants and abundance of individual oil constituents in oils from sorts under study at optimum stage of ripe for harvest were evaluated as well.

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P 9

The Influence of Prometrex 50 WP on the Content and Quality of the Essence of Garden Sage *Salvia officinalis* L.

Stefani Vaverkova, Magdalenka Holla, Jozef Tekel
Faculty of Pharmacy, Comenius University, 832 32 Bratislava, Slovakia
vaverkova@fpharm.uniba.sk

Medicinal plants from an organic compartment of the natural wealth of each state. Aimed cultivation of medicinal and spicy plants is of great importance in the climatic conditions of middle Europe. These are used as sources for the natural drugs production and for isolation of natural dyes or for alcoholic beverages as well [1-3].

Nowadays, the use of modern technologies, wide-scale cultivation is required to ensure sufficient amounts and quality of such medicinal plants, which can be grown in our climatic and edaphic conditions. A chemical protection of the essence plant croppings is a long-term and economically interesting problem at the production of *Salvia officinalis* L. The results gained by the team of authors point at the influence of a herbicide Prometrex 50 WP (based on prometryn) upon the content and quality of main components of oil from *Salvia officinalis* L.

The main criterion of the essence plant quality is the content of essential oil in flowering stalks and leaves and relative abundance of main oil components. Repeated experiments led at two localities during several vegetation periods have had to disclose whether there exist the differences in the content and quality of oil from treated and untreated plants. It has been shown, that application of the herbicide did not affect the plant metabolism to such extent, which might decrease the quality of raw material. The oil content was increasing during the vegetation period with sequence of fenophases up to the one of butonisation, further it remains at steady levels. The GC analyses have shown, that application of Prometrex 50 WP did not cause significant changes in the relative abundance of both main and side components of the oil.

Stalks and leaves of *Salvia officinalis* L. harvested after post emergent treatment with Prometrex 50 WP in the first year of the plant life did not differ from those of control plants with regard to the content and main components of the oil.

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P 10

Composition of the Essential Oil from the Liverwort *Asterella africana* (Mont.) Evans Grown on Madeira

Ana Cristina Figueiredo[1], José G. Barroso[1], Luis G. Pedro[1], Susana S. Fontinha[2],
Manuela Sim-Sim[3], Johannes J. C. Scheffer[4]
acsf@fc.ul.pt
[1]Centro de Biotecnologia Vegetal, Dep. de Biologia Vegetal,
FCL, C2, Campo Grande, 1749-016 Lisbon, Portugal
[2]Jardim Botânico da Madeira, Caminho do Meio,
Bom Sucesso, 9050-244 Funchal, Madeira, Portugal
[3]Centro de Ecologia e Biologia Vegetal, Dep. de Biologia Vegetal,
FCL, C 2, Campo Grande, 1749-016 Lisbon, Portugal
[4]Div. of Pharmacognosy, Leiden University, Gorlaeus Labs,
P.O. Box 9502, 2300 RA Leiden, The Netherlands

Asterella is one of the largest genera of the Marchantiales, that is estimated to consist of ca 80 species worldwide [1]. *Asterella africana* (Mont.) Evans [= *Fimbriaria africana* Mont.] is a Macaronesian and Mediterranean thalloid liverwort, that grows in rather compact small patches [1–6]. The dorsal face shows a bright green colour, while numerous uncoloured, large rhizoids develop along the ventral surface of the keel middle. Large oil bodies are present on the scales that occur on each side of the keel. The ecological behaviour and the reduced distribution of this species in Portugal and Spain led to its inclusion in the Red Data Book of European Bryophytes as a vulnerable species for Europe but it is not threatened on Madeira [7]. The species was recently found in a restricted area of the Portuguese mainland [8].

Asterella species are known to emit intense, more or less pleasant, characteristic scents [9]. In addition, steroids, triterpenoids and dihydrophenanthrene derivatives were detected in members of the genus [10]. Nevertheless, no previous study was reported on the essential oil of this species.

The essential oil from *A. africana* was isolated by distillation - extraction and analysed by GC and GC-MS. Thirty components could be identified, amounting to 89 % of the total oil. The monoterpene fraction was the most representative one (83 %); the sesquiterpenes and a fraction of non-terpenoids amounted to 1 % and 5 %, respectively.

The oil of *A. africana* was dominated by myrtenyl acetate (38 %), α-pinene (13 %), myrcene (13 %), myrtenol (7 %) and limonene (5 %). β-Caryophyllene (0.7 %) and β-caryophyllene epoxide (0.1 %) were the only sesquiterpenes detected, and 2-undecanone (5 %) was the dominant compound of the non-terpenoidal fraction.

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P 11

Chemical Polymorphism of the Essential Oils from *Thymus caespititius* Brot. Grown on the Islands São Miguel and Terceira (Azores) and on Madeira

P. A. G. Santos[1], J. G. Barroso[1], A. C. Figueiredo[1], L. G. Pedro[1], L. R. Salgueiro[2], S. S. Fontinha[3], S. G. Deans[4], J. J. C. Scheffer[5]
[1]Centro de Biotecnologia Vegetal, Dep. de Biologia Vegetal, FCL, C2, Campo Grande, 1749-016 Lisbon, Portugal
[2]Laboratório de Farmacognosia, Faculdade de Farmácia,
Universidade de Coimbra, Rua do Norte, 3000 Coimbra, Portugal
[3]Lardim Botânico da Madeira, Caminho do Meio, Bom Sucesso, 9050-244 Funchal, Madeira, Portugal
[4]Aromatic & Medicinal Plant Group, Food Science & Technology Department,
SAC, Auchincruive, Ayr KA6 5HW, Scotland, UK
[5]Div. of Pharmacognosy, Leiden University, Gorlaeus Labs, P.O. Box 9502, 2300 RA Leiden, The Netherlands

The genus *Thymus* consists of a taxonomically complex group of aromatic plants, that are widely distributed in the Iberian Peninsula. *Thymus caespititius* is an endemic species of the NW Iberian Peninsula that grows wild also in the Madeiran and Azorean archipelagos.

The aerial parts of the plant were collected during its flowering period from fourteen populations growing on the Azorean islands S. Miguel and Terceira and on Madeira. The oils obtained by hydrodistillation were used to estimate the oil yields; those obtained by distillation-extraction were analysed by GC and GC-MS. The sites of collection of the plant material, the corresponding altitudes, the oil yields, the total percentage of the oil components identified, and the main components (>5 % in at least one sample) of each oil sample are given in Table 1.

Table 1. Main components identified in the oils of *Thymus caespititius* grown on the islands S. Miguel, Terceira and Madeira.

Island		**São Miguel**						**Terceira**					**Madeira**		
Population		**SM1**	**SM2**	**SM3**	**SM4**	**SM5**	**SM6**	**TE1**	**TE2**	**TE3**	**TE4**	**TE5**	**MA1**	**MA2**	**MA3**
Altitude (m)		600	700	650	450	900	800	530	550	550	300	650	1185	1170	1800
Components (>5 %)	Sabinene	t	0.1	0.1	0.1	0.1	0.1	t	0.4	0.2	0.3	0.1	15.0	16.3	7.7
	β-Myrcene	t	0.1	t	t	t	t	t	0.1	0.3	t	t	9.0	10.0	7.3
	p-Cymene	5.3	4.0	6.3	4.4	5.6	5.1	10.2	13.5	10.8	14.3	12.8	1.5	2.1	4.1
	γ-Terpinene	2.8	0.9	2.9	1.1	3.2	2.7	2.2	5.2	2.1	3.7	2.7	4.2	4.8	5.5
	Terpinen-4-ol	0.6	0.7	0.8	0.7	0.6	0.8	0.4	0.9	0.7	1.1	0.7	5.2	5.0	3.3
	α-Terpineol	0.1	0.1	0.9	1.1	0.2	2.5	1.0	7.6	0.8	5.5	1.0	33.3	32.8	36.7
	Thymol	0.1	0.1	0.1	0.2	0.1	0.1	50.3	38.4	41.8	34.9	51.1			
	Carvacrol	58.3	64.0	64.8	61.6	55.0	42.6	3.4	2.7	3.3	2.6	3.5	0.4	0.1	t
	Thymyl acetate							14.3	10.9	18.9	10.9	10.1			
	Carvacryl acetate	12.9	13.5	5.9	9.8	18.5	23.8	0.7	0.5	0.8	0.6	0.5			
Total % identified		94.5	95.6	96.1	94.5	95.9	92.5	98.1	96.7	96.8	95.6	99.0	96.2	97.2	95.1
Oil yield (%)		0.4	0.7	0.6	0.6	0.8	0.4	0.8	0.4	0.7	0.6	0.6	0.9	1.2	0.5

t = traces < 0.05 %

The essential oils from the fourteen populations were dominated by their monoterpene fraction (79–89 %) that consisted mainly of oxygen-containing compounds (42–79 %). Oxygen-containing sesquiterpenes (6–13 %) were the major components of the sesquiterpene fraction that attained only 7–16 % of the total oils. The oils showed, however, remarkable differences concerning their main components; the populations grown on the two Azorean islands yielded phenol-rich oils (on S. Miguel and Terceira possessing carvacrol and thymol, respectively), whereas the Madeiran populations gave α-terpineol-rich oils (Table 1.). Although there is a clear chemical polymorphism among the populations growing on the different islands, within the same island the populations are quite homogeneous with regard to their oil compositions.

Acknowledgments: Funded by the Fundação para a Ciência e Tecnologia (FCT) — PRAXIS/P/BIA/11054/1998.

P 12

Composition of the Essential Oils from Nine Populations of *Crithmum maritimum* L. Grown on Four Azorean Islands

Luis G. Pedro[1], Carla S. Duarte[2], A. Cristina Figueiredo[1], José G. Barroso[1], Johannes J. C. Scheffer[3]
[1]Centro de Biotecnologia Vegetal, Dept. de Biologia Vegetal,
FCL, C2, Campo Grande, 1749-016 Lisbon, Portugal
luis.pedro@fc.ul.pt
[2]Unidade de Ciências e Tecnologias Agrárias, Universidade do Algarve,
Campus de Gambelas, 8000-117 Faro, Portugal
[3]Division of Pharmacognosy, Leiden University, Gorlaeus Laboratories,
P.O. Box 9502, 2300 RA Leiden, The Netherlands

Crithmum maritimum L. (Apiaceae), the only species of the genus, can be found along the coast of Europe, Africa, Asia and Australia. This fleshy, glabrous, perennial herb frequently grows in maritime rock-crevices, coarse gravel, breakwaters or sand deposits along the shoreline of the Portuguese mainland and in the Madeiran and Azorean archipelagos. According to Guenther [1], early studies on *C. maritimum* oil showed that all parts of the plant contain volatiles, but the yield and the chemical composition of the oils varied considerably depending on the site of growth and soil conditions. In addition, a study of the seasonal variation in the composition of the essential oil of *C. maritimum* showed major fluctuations in the relative amounts of several components, mainly sabinene (7–42 %) and γ-terpinene (26–55 %) [2]. Later reports on essential oils from *C. maritimum*, collected in Italy, Portugal and Turkey, showed also different compositions [3–5]. These variations, and our previous results on the essential oil of this species collected on other Azorean islands [6], prompted us to continue the studies on this plant.

The aerial parts (leaves and stems) from plants of nine populations of *C. maritimum* were collected, late in the vegetative phase, on four Azorean islands (S. Miguel, Terceira, Flores and Corvo). The oil yields ranged from 0.1 % to 0.2 %. The monoterpene hydrocarbon fraction was dominant in the oils from all the populations, attaining 91–98 % of the total oils. The main components were γ-terpinene (71–88 %) and *p*-cymene (6–19 %). The oxygen-containing monoterpenes attained 2 % and were dominated by carvacrol (0.3–1.2 %). The sesquiterpene fraction contained only hydrocarbons. With the exception of *trans*-α-bergamotene, that attained 0.6 % in the oil of one population, no other component of this fraction was found in a relative amount higher than 0.3 %. The phenylpropanoid fraction, containing only dillapiole, ranged from traces to 0.3 %.

A study performed by Pateira *et al.* [4] on the essential oils from several populations of *C. maritimum* grown on the Portuguese mainland, reported two chemotypes: chemotype 1 (15–47 % dillapiole; 17–35 % γ-terpinene; 10–18 % methylthymol; 7–22 % sabinene) and chemotype 2 (25–44 % γ-terpinene; 17–34 % sabinene; 10–18 % methylthymol; 0–6 % dillapiole). The occurrence of a much higher γ-terpinene content and the lower sabinene, dillapiole and methylthymol contents, recorded in the present study, together with similar results on the essential oils isolated from populations grown on other Azorean islands [6], suggest the existence of a distinct chemotype for plants growing in the Azorean archipelago.

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P 13

Authenticity Assessment of Clary Sage (*Salvia sclarea* L.) Oil

Denys J. Charles
Frontier Natural Products Co-op,
3021 78th Street, P.O. Box 299, Norway, IA-52318, U.S.A.
denys.charles@frontiercoop.com

Clary sage oil (*Salvia sclarea* L.), belonging to the *Lamiaceae* family, was extracted from plants grown organically at Frontier Organic Research Farm, Norway, U.S.A. Plants were grown for two years and monitored throughout the growing season for agronomic data. Each year plants were harvested at the optimum time as commercially practiced. Some plants were dried and then distilled, while others were distilled fresh. Both the fresh and dried plants were steam distilled and hydro-distilled to obtain essential oils. The essential oils were analyzed for specific gravity, optical rotation, refractive index, and chemical constituents separated and identified by gas chromatography-mass spectroscopy (GC-MS). Twenty commercial clary sage essential oils were also analyzed by the above methods to ascertain their purity. To detect the addition of synthetic linalool and linalyl acetate, the oils were analyzed by chiral gas chromatography.

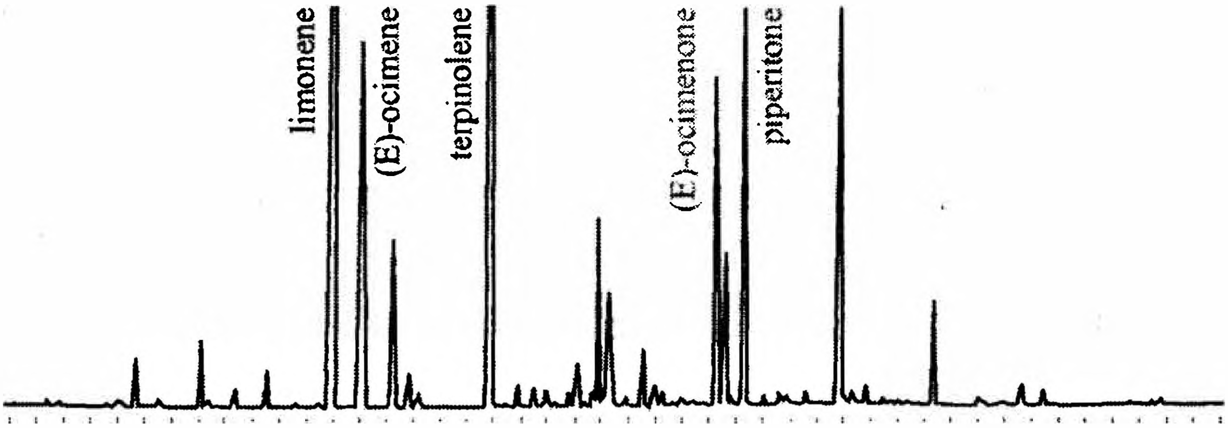
P 14

Characterization of Essential Oils from Different Types of Marigold (*Tagetes spp.*)

Mauro Marotti, Roberta Piccaglia, Bruno Biavati
Dipartimento di Scienze e Tecnologie Agroambientali
Università degli Studi di Bologna, via Filippo Re 6-8, 40126 Bologna, Italy
biavati@agrsci.unibo.it

Marigold (*Tagetes spp.)* is an important source of essential oils rich of biologically active compounds [1]. Due to the interest in the exploitation of these natural substances, in our work, seven species of marigold were grown in an experimental field and harvested at full flowering stage to evaluate the composition of essential oils obtained from flowers and leaves. The oils, extracted by steam distillation, were characterized by GC and GC/MS and thirty-three compounds were identified. The oils obtained from flowers and leaves of the same species differred only in the quantitative composition, whereas important differences both in quali- quantitative compositions were observed among the oils from different species. In particular *T. filifolia* and *T. lucida* were characterized for the high contents of methyl chavicol responsible of the pleasant aromatic flavor of these plants, whereas in the other species the main compounds were dihydrotagetone, tagetone, ocimenone and piperitone. Each species showed a peculiar oil composition confirming that the oil characterization can be a valuable contribution to chemotassonomy. The differences observed in the oil compositions make *Tagetes* a genus with a broad field of application.

Chromatographic profile of essential oil from *Tagetes patula*



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P 15

Essential Oil from Herb and Rhizome of *Peucedanum ostruthium* (L. Koch.) ex DC

Wojciech Cisowski[1, 4], Urszula Sawicka[1], Marek Mardarowicz[2], Monika Asztemborska[3],
Maria Łuczkiewicz[4]
[1]Department of Pharmacognosy, Wrocław University of Medicine,
pl. Nankiera 1, 50-140 Wrocław, Poland
[2]Department of Chemical Physics, Faculty of Chemistry,
Maria Curie-Skłodowska University, sq. M. Curie-Skłodowskiej 3, 20-031 Lublin, Poland
[3]Department of Chemical Physics, Polish Academy of Sciences,
Kasprzaka str. 44/52, 01-224 Warszawa, Poland
[4]Department of Pharmacognosy, Medical University of Gdańsk,
Al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland

Essential oil from herb and rhizome of *Peucedanum ostruthium* (L. Koch.) ex DC underwent qualitative and quantitative analyses. Earlier studies of the essential oil of this old medicinal plant report the presence of 1.4 % essential oil in the rhizome. The oil was reported to contained only d-limonene, dipentene, α-pinene and d-phellandrene [1, 2].

In our investigations the content of the oil obtained by hydrodistillation was 0.95 % in the herb and 1.25 % in the rhizome. Gas chromatography (GC) with MS detection and flame ionisation detection showed that the oil from the rhizome contains 39 compounds, of which 29 were identified. Gas chromatography with flame ionisation detection in chiral columns against standard compounds showed presence of enentiomers of some of the components of the oil [3]. Compounds present in largest quantities are: sabinene (35.2 %, of which (+)-sabinene accounts for 96.54 %) and 4-terpineol (26.6 %, of which (+)-4-terpineol accounts for 65.8 %). 44 components were found in the herb essential oil, of which 39 compounds were identified. It was shown that compounds present in largest quantities were β-caryophyllene (16.1 %) and α-humulene (15.8 %). The content of sabinene in the herb oil was 4.7 %. The following compounds were present in the herb oil only as enentiomers: (+)-sabinene (4.7 %), (–)-limonene (4.4 %), (–)-xpinene (0.4 %). A coumarin (osthole) was detected in both essential oils (5.5% in herb oil and 5.1% in rhizome oil).

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P 16

Comparative Analysis of Volatile Oil in *Achillea grandifolia* Friv. and *Achillea tanacetifolia* All.

Wojciech Cisowski[1], Adam Kowalczyk[1], Marek Mardarowicz[2], Janina Dąbrowska[3]
[1]Dept. of Pharmacognosy, Wrocław Medical Univeristy,
Nankiera 1, 50-140 Wrocław, Poland
[2]Dept. of Chemical Physics and Physico-Chemical Methods of Separation,
Maria Curie-Skłodowska University, M. Curie-Skłodowska sq. 3, 20-031 Lublin, Poland
[3]Institute of Botany, Wrocław Univeristy, Maxa Borna 9, 50-206 Wrocław, Poland

The genus *Achillea* L. numbers over 100 species and is a polyploid complex of di-, tetra-, hexa- and octaploids. When performing chemical analysis, it is advisable to know which is the number in the analysed taxa, since it can not be excluded that the cytotopes may differ qualitatively or quantitatively with respect to the chemical composition of their oil [1].

The objective of this study is a comparative analysis of the composition of volatile oil in the two species: *Achillea grandifolia* Friv. and *Achillea tanacetifolia* All. The examined material was collected during blooming by J. Dąbrowska. Each material, previously air-dried, was distilled with water vapour in Deryng apparatus, according to the method of obtaining and measuring volatile oil in Polish Pharmacopea [2].

Chromatographic analysis was carried out using gas chromatograph coupled with mass spectrometer. Resulting spectra were compared with the data from data library. The differences and similarities of the volatile oil in the studied species are valuable data which may be applied when establishing relationships between taxa of the genus *Achillea* L.

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P 17

Essential Oil of *Phellodendron amurense* Rupr.

Anna Lis[1], Józef Góra[1], Krystyna Świąder[2], Eliza Lamer-Zarawska[2]
[1]Institute of General Food Chemistry, Technical University of Łódź,
Stefanowskiego 4/10, 90-924 Łódź, Poland
[2]Institute of Biology and Botany University of Medicine,
Kochanowskiego 10, 51-601 Wrocław, Poland

Phellodendron amurense Rupr. (Rutaceae) is a tree occurring in the forests in northeast Asia (Russia, China). All plant organs (bark, root, leaf, fruit) have therapeutic properties and are used in Chinese folk medicine.

Essential oil from ripe fruits of *P. amurense* was investigated. Plant material was collected from trees growing in Medicinal Plants Garden University of Medicine in Wrocław (Poland). Essential oil was obtained in 1.8–2.0 % yield by hydrodistillation method from air-dried, crushed fruits. Chemical composition of the oil was analyzed by GC/MS. More than 50 components were identified. Myrcene (70.3 %), β-caryophyllene (6.8 %) and germacrene D (3.7 %) were the main constituents.

P 18

Essential Oil of *Erigeron canadensis* L.

Anna Lis, Józef Góra
Institute of General Food Chemistry, Technical University of Łódź,
Stefanowskiego 4/10, 90-924, Łódź, Poland

Erigeron canadensis L. syn. *Conyza canadensis* Cronq. (Compositae) a weed native to North America is widespread in Europe. Its herb has medicinal properties (antiphlogistic, antihemostatic, antirheumatic, diuretic).

The aim of the present study was the evaluation of the variability of yield and chemical composition of the essential oil depending on the kind of plant organ, ontogenesis phase and plant origin.

Plant material was collected from natural state in central Poland (Łódź). Essential oil was obtained from air-dried plants in yield 0.35–0.83 % from herb, 0.88–0.94 % from leaves and 0.78–1.03 % from flowers. The content of the oil varied during the period of vegetation, the highest was observed at the beginning of flowering phase.

R-(+)-limonene and trans-α-bergamotene were the main constituents of the oil from herb (57.9–81.1 % and 6.7–8.5 %), leaves (73.5–89.0 % and 3.5–6.7 %) and flowers (62.8–75.3 % and 6.3–14.1 %) at all ontogenesis phases. The content of limonene in herb oil increased from 57.9 % at the beginning of vegetation (June) to 81.1 % at the budding phase (July) then decreased to 64.2 % at the end of vegetation (September).

The best time for harvesting of *E. canadensis* herb are the early flowering phases (1–15 July in Poland), when yield of the oil is high (0.71–0.83 %) and chemical composition of the oil is constant (limonene 80.1–81.1 %, trans-α-bergamotene 5.6–8.1 %).

In the second part of the study oils from flowering herb of *E. canadensis* growing wild in other European countries were investigated. Limonene was the main component of French (Alps, 83.5 %), Italian (Rome, 70.3 %), Spanish (Seville, 51.4 %), Belgian (68.0 %), Bulgarian (Plovdiv 87.9 %), Lithuanian (Vilnius 77.7 %) and Israeli (54.9 %) oils. European oils were similar to American, differed from Japanese and Indian that were described in literature.

P 19

The Essential Oil of the Species *Micromeria thymifolia* (Scop.) Fritsch (*Lamiaceae*) from Yugoslavia

M. Couladis[1], O. Tzakou[1], V. Slavkovska[2], B. Lakusic[2], R. Jancic[2], D. Stojanovic[2]
[1]Division of Pharmacognosy, Faculty of Pharmacy, University of Athens,
Panepistimiopolis Zografou, 15771 Athens, Greece
[2]Institute of Botany, Faculty of Pharmacy, University of Belgrade,
Vojvode Stepe 450, 11000 Belgrade, Yugoslavia
vikisl@eunet.yu

Micromeria thymifolia (Scop.) is an endemic species of the west part of the Balkan peninsula, whose eastern areal border is on the mountain of Kopaonik in Serbia [1]. This species inhabits crevices mainly on karst, although, dense populations can also be found on serpentine [2], both on mountains and in deep gorges.

The object of our investigation was the chemical analysis of two populations of *M. thymifolia* from Western Montenegro (Moraca canyon and mount Orjen, 1600 m a.s.l.) and two populations from Western Serbia (Derventa canyon and Beli Rzav gorge).

The samples were gathered in the flowering period. The essential oil was obtained from dried aboveground parts of the plants by hydrodistillation. The analyses of the oils were carried out using GC/MS. The identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and mass spectra with those obtained from authentic samples and/or the NIST/NBS Wiley libraries [3].

The qualitative composition of the essential oils was similar. Oxygenated terpenes of the menthane type dominated all the oils (>67 %). However, the contribution of certain major components was varying.

The population from Moraca canyon had a high content of piperitone + piperitone oxide (25.81 %), piperitenone (28.67 %) and piperitenone oxide (16.0 %), while the concentration of pulegone amounted to 6.20 %.

The mount Orjen population contained essential oil rich in pulegone (48.01 %) and piperitenone (13,88 %). Piperitone + piperitone oxide and piperitenone oxide were ascertained in smaller quantities (2.83 %).

In the essential oil from the Derventa canyon population there was a considerable presence of piperitone oxide (63.77 %). The quantity of piperitenone oxide was 9.18 %. Piperitenone was present in a very small quantity (0.36 %), while pulegone was found in traces.

Pulegone (37.20 %) and piperitone + piperitone oxide (21.72 %) were the main compounds in the essential oil of *M. thymifolia* from Beli Rzav gorge. The quantities of piperitenone and piperitenone oxide were determined to be 6.27 % and 9.79 % respectively.

The variation in the quantity of some essential oil components can be explained as the result of the influence from different environmental conditions.

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P 20

Composition of the Essential Oil of *Cyclotrichium leucotrichum* (Stapf. and Rech. F.)

Zohreh Habibi, Ahmad Laleh, Tayebeh Biniyaz
Department of Chemistry, Shahid Beheshti University, Evin, Tehran, Iran
zohre1340@hotmail.com

The genus Cyclotrichium from Labiatae family with 4 species is widespread in Iran. In addition *C. leucotrichum* is grown in Iraq and South-eastern of Anatoli.

The aerial parts of plant was collected at flowering period from Eelam. The oil was obtained by hydrodistillation in yield of 0.15 % (w/w). The oil composition was analyzed by GC and GC/MS. 13 compounds representing 99 % were identified in the oil. The major component was p-Cymene with 68.15 %. Four sesquiterpenes (6.52 %) were identified in the oil that Spathulenol (3.7 %) was the main component between them.

P 21

Volatile Oil of *Salvia eremophila* Boiss. from Iran

Tayebeh Biniyaz, Zohreh Habibi, Ahmad Laleh
Department of Chemistry, Shahid Beheshti University, Evin, Tehran, Iran
zohre1340@hotmail.com

The genus Salvia (Labiatae) comprises some species in Iran from which *S. eremophila* Boiss. is endemic to Iran.

The essential oil from aerial parts of *S. eremophila* Boiss. was obtained by hydrodistillation. The oil content was 0.8 % on a dry weight basis.

The oil composition was analyzed by GC and GC/MS. 29 compounds were identified, accounting for 96.58 % of the oil, α-Pinene (24.28 %), Bornyl acetate (18.92 %), Camphene (16.02 %) and Borneol (14.26 %) were the main components.

P 22

The Essential Oil of *Xanthium brasilicum* Vellezo

Ahmad Laleh, Zohreh Habibi, Tayebeh Biniyaz
Department of Chemistry, Shahid Beheshti University, Evin, Tehran, Iran
zohre1340@hotmail.com

The genus Xanthium (Compositae) with 4 species is widespread in Iran. In addition *X. brasilicum* Vellezo. is grown in Afghanistan, Central and Eastern Asia, Japan and South-western Asia.

The aerial parts of *X. brasilicum* was collected during the flowering period from Tehran in April 2000.

Water distilled essential oil of *X. brasilicum*, in 0.3 % yield, was analyzed by GC and GC/MS.

The identification of the compounds was carried out by comparison of their Mass Spectra with those of known compounds together with the relative retention indices. 15 components representing 78.5 % of the oil were characterized. Borneol (26.87 %), Fenchone (20.08 %) and iso-Bornyl acetate (8.97 %) were the main compounds.

P 23

Enantiomeric Distribution of Linalool in Essential Oils

Temel Özek, Nurhayat Tabanca, Fatih Demirci and K. Hüsnü Can Başer
Medicinal and Aromatic Plant and Drug Research Centre (TBAM),
Anadolu University, 26470 Eskişehir, Turkey
http://www.tbam.anadolu.edu.tr/

The enantiomeric compositions of linalool were determined in many essential oils on chiral columns. The essential oils were analyzed by multidimentional gas chromatography mass spectrometry (MD-GC/MS) using a normal phase polar and chiral FSC columns with modified γ-cyclodextrine (Lipodex E) as the chiral stationary phase without previous isolation of the compound from the mixture. The essential oils of *Achillea*, *Ballota*, *Calamintha*, *Micromeria*, *Hedychium*, *Tanacetum*, *Coriandrum*, *Xanthoxylum*, *Ocimum*, *Thymus*, *Lavander*, *Eletteria*, *Cinnamomum*, *Salvia*, *Origanum*, *Satureja*, *Nepeta*, *Stachys* were used for the enantiomeric separation of linalool. Both enantiomers of linalool exist in nature but (–)-linalool was found to be more common than the (+)-enantiomer. Pure (–) and (+)-linalool were also evaluated for their antimicrobial activity.

P 24

The Composition of Essential Oils and Distillation Waters of *Mentha spicata* L. and *Mentha piperita* L.

Nese Kirimer, K. H. C. Baser, M. Kürkçüoglu, Öznur Arslandere
Department of Pharmacognosy, Faculty of Pharmacy and Medicinal
and Aromatic Plant and Drug Research Centre (TBAM),
Anadolu University, 26470-Eskisehir, Turkey
http://www.tbam.anadolu.edu.tr/

The essential oils and distillation waters of *Mentha spicata* L. (spearmint) and *Mentha piperita* L. (peppermint) (Labiatae) obtained by hydrodistillation were analyzed by GC/MS.

Liquid-liquid extraction (with hexane and chloroform), immersion SPME, heated-immersion SPME, Headspace-SPME and heated-Headspace-SPME tecniques were used for the extraction of volatile compounds from mint waters.

P 25

Essential Oil Constituents of *Satureja aintabensis* P. H. Davis from Turkey

Mine Kurkcuoglu[1], Fatih Satil[2], Gülendam Tumen[2], K. Hüsnü Can Baser[1]
[1]Medicinal and Aromatic Plant and Drug Research Centre (TBAM),
Anadolu University, 26470, Eskisehir, Turkey
http://www.tbam.anadolu.edu.tr/
[2]Department of Biology, Faculty of Science and Letters,
Balikesir University, 10100-Balikesir, Turkey

The Genus Satureja is represented in Turkey by 15 species, five of which are endemic. *Satureja aintabensis* P. H. Davis is known as 'Kekik'.

Water distilled essential oil from the aerial parts of *S. aintabensis* was analyzed by GC/MS. Thirtytwo components were chracterizied representing 99.3 % of the oil. p-Cymene (31.5 %) carvacrol (27.9 %) and gamma-terpinene (20.7 %) were characterizied as the main components.

P 26

Composition of the Essential Oil of *Myrrhoides nodosa* (L.) Canon from Turkey

Gülendam Tumen[1], Mine Kurkcuoglu[2], Betul Demirci[2], K. Hüsnü Can Baser[2]
[1]Department of Biology, Faculty of Science and Letters,
Balikesir University, 10100-Balikesir, Turkey
[2]Medicinal and Aromatic Plant and Drug Research Centre (TBAM),
Anadolu University, 26470, Eskisehir, Turkey
http://www.tbam.anadolu.edu.tr/

Water distilled essential oil from herbal parts of *Myrrhoides nodosa* (L.) Canon was analyzed by GC and GC/MS using polar and non-polar columns. Methyl chavicol was characterizied as the main component of this oil which had a district anise-like smell.

P 27

The Essential Oil of *Origanum syriacum* L. var. *sinaicum*

K. Hüsnü Can Baser, Betul Demirci, Mine Kurkcuoglu, Temel Özek
Medicinal and Aromatic Plant and Drug Research Centre (TBAM),
Anadolu University, 26470, Eskisehir, Turkey
http://www.tbam.anadolu.edu.tr/

Water distilled essential oil from herbal parts of *Origanum syriacum* L. var. *sinaicum* cultivated in El Arish, Egypt was analyzed by GC/MS using polar and non-polar columns. Thymol, cis-sabinene hydrate, gamma-terpinene, and terpinene-4-ol were characterizied as main costituents.

P 28

The Essential Oil of *Anthemis cretica* L. ssp. *Leucanthemoides* (Boiss.) Grierson

K. Hüsnü Can Başer, Temel Özek, Fatih Demirci, Ilhan Boydag
Medicinal and Aromatic Plant and Drug Research Centre (TBAM),
Anadolu University, 26470, Eskisehir, Turkey
http://www.tbam.anadolu.edu.tr/

Water distilled essential oil of *Anthemis cretica* L. ssp. *leucanthemoides* (Boiss.) Grierson was analyzed by GC/MS. Main components of the oil were determined as camphor (19.4 %), 1,8-cineole (7.2 %), terpinen-4-ol (5.7 %), borneol (4.5 %) and spathulenol (4.3 %). Enantiomeric distribution of camphor in the oil was determined by MD-GC/MS. Antimicrobial activity of the oil was also investigated.

P 29

Composition of the Essential Oils of Six Endemic *Salvia* Species from Turkey

Betül Demirci[1], K. Hüsnü Can Baser[1], Bayram Yıldız[2], Zeliha Bahcecioglu[2]
[1]Medicinal and Aromatic Plant and Drug Research Centre (TBAM),
Anadolu University, 26470-Eskisehir, Turkey
http://www.tbam.anadolu.edu.tr/
[2]Faculty of Education, Department of Biology,
Balıkesir University, 10100-Balıkesir, Turkey

The water-distilled essential oils of *Salvia blepharochlaena* Hedge & Hub.-Mor., *S. caespitosa* Montbret & Aucher ex Bentham, *S. divaricata* Montbret et Aucher ex Bentham, *S. hypargeia* Fisch. et Mey., *S. longipedicellata* Hedge, *S. pilifera* Montbret et Aucher ex Bentham were analyzed by GC/MS. Percentage yields and main components of the oils are as follows:

***Salvia* Species**	**Oil Yield (%)**	**Main Components (%)**
S. blepharochlaena	0.1	α-Pinene (10.1), α-Copaene (8.6), Camphor (8.5), Spathulenol (7.3)
S. caespitosa	0.2	β-Pinene (22.7), 1,8-Cineol (10.3), α-Pinene (6.8)
S. divaricata	0.8	1,8-Cineole (40.0), α-Pinene (16.6)
S. hypargeia	0.02	Thymol (11.5), Germacrene-D (11.1), Caryophyllene oxide (10.7)
S. longipedicellata	0.04	Caryophyllene oxide (23.3), β -Caryophyllene (16.1)
S. pilifera	0.8	α-Thujone (39.8), α-Pinene (11.2)

P 30

The Application of the Topological Indexes for Evaluation of the TLC Separation of the Selected Essential Oils

Alina Pyka, Katarzyna Bober, Danuta Gurak
Department of General and Analytical Chemistry, Faculty of Pharmacy,
Silesian Academy of Medicine, ul. Jagiellońska 4, 41-200 Sosnowiec, Poland
chemogan@infomed.slam.katowice.pl

One of the current tendencies in chemical investigations is the prediction of physicochemical and biological properties of chemical compounds from their structural parameters. The fundamental establishment of these investigations is fact that the structure of a molecule determines its properties. This fact can be expressed by mathematical dependence [1]:

$$\mathbf{P} = f(\mathbf{S}) \qquad (1)$$

where: **P** is any physical, chemical, pharmacological or toxicological property of a molecule, and **S** is any descriptor connecting with the structural aspects of a molecule.

For this purpose we undertook the attempt of the estimation of usefulness of topological indexes to estimation of chromatographic separation of selected compounds entering in composition of essential oils.

The topological indexes based on connectivity: *Gutman* (M) [2–4], and *Randić* (${}^{m}\chi$, ${}^{m}\chi^{v}$) [2–5], on distance matrix: *Rouvray* (R) [2–4], *Wiener* (W) [2–4, 6], *Pyka* (${}^{0}B$, ${}^{1}B$, A) [4, 7], were calculated for the compounds investigated. The *Rouvray*, *Wiener,* and *Pyka* indexes were calculated by building the distance matrix and determining its elements by means of values given by *Barysz* et al. [8]. The electrotopological states of particular vertexes of graphs of essential oils investigated were calculated [9].

Selected essential oils were separated by TLC technique with using benzene and acetone as well as chloroform and acetone in the different voluminal ratios as mobile phases.

We showed the meaning of some topological indexes in Quantitative Structure Retention Relationships (QSRR) investigation of essential oils.

The further researches of the above problem are continued.

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P 31

New Visualizing Agents for Selected Essential Oils in TLC

Alina Pyka, Danuta Gurak, Katarzyna Bober
Department of General and Analytical Chemistry, Faculty of Pharmacy,
Silesian Academy of Medicine, ul. Jagiellońska 4, 41-200 Sosnowiec, Poland
chemogan@infomed.slam.katowice.pl

The essential oils have been applied as aromatic vegetable compounds used for cosmetic, disinfecting, and curative purposes for a long time. In composition of one essential oil can enter tens different compounds. For this reason, the separation and the detection of the essential oils is an important problem. Gas chromatography is leading technique servant to investigations of essential oils. However thin-layer chromatography is technique much cheaper than gas chromatography. That's way thin-layer chromatography can be used to preliminary qualitative analysis of essential oils investigated.

The aim of the work is use of new visualizing agents in TLC to detection of selected compounds entering in composition of essential oils. Thymol, guaiacol, eugenol, menthol, geraniol, coumarin, cinnamic aldehyde, cineole, trans anethole, (+)-borneol, linalool, (R)-(–)-carvone, farnesol, camphor, (1R)-(–)-fenchon, and vanillin were separated by adsorption thin-layer chromatography. Saturated aqueous solution of variamine blue hydrochloride, 1 % ditizone in methanol, 1 % aqueous $Ni(NO_3)_2$, 2 % aqueous $CuSO_4$, 0.1 % HCl, and 0.1 % aqueous Na_2CO_3 were applied as new visualizing agents for detection of above-mentioned compounds.

The visualizing effects obtained were compared with detection of these compounds by using of iodine vapor and 5 % solution $K_2Cr_2O_7$ in 40 % H_2SO_4.

We showed, that essential oils investigated can be distinguished on chromatogram on the ground of the R_F values and on the ground of the colors of chromatographic spots with definite visualizing systems.

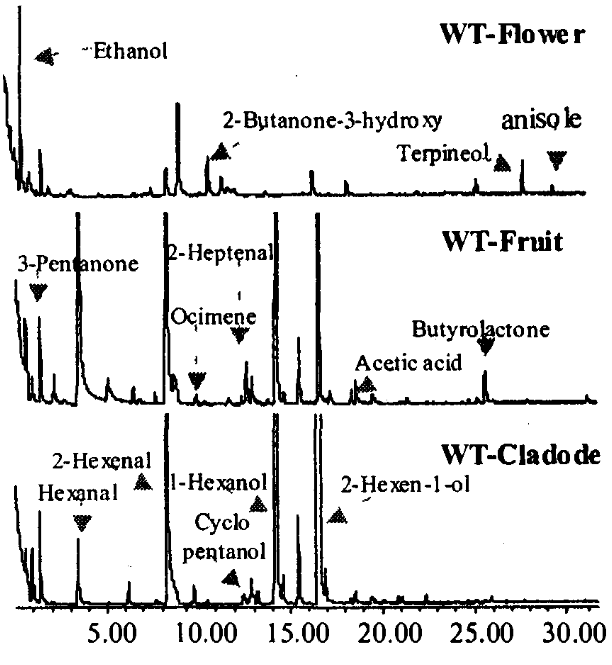
P 32

Nutraceutical-Volatiles of Nopal (*Opuntia* spp.) by SPME-GC-MS

Mercedes G. López, Lourdes Macías-Rodríguez
Unidad de Biotecnología e Ingeniería Genética de Plantas,
Centro de Investigación y de Estudios Avanzados del IPN,
Apartado Postal 629, 36500 Irapuato, Gto., México
mlopez@ira.cinvestav.mx

Nopal (*Opuntia* spp.) is a member of the cacti family. Indigenous people in Mexico has consume it for centuries in their daily diet for its nutritional value. Since then, their flowers, fruits (tuna and xoconostle) and fleshy stems (cladodes) are used in folklore medicine to treat kidney problems and measles, fever and diarrhoea, to lower serum cholesterol levels, blood glucose, regulate blood pressure and control gastric acidity. However, its popularity has increased tremendously since the nutraceutical era begun. Worldwide nopal is mainly used to treat diabetes [1] and it is sold and consumed as pills or capsules. The nutraceutical properties of nopal are well-known, however there are no reports on its volatile composition and role. Therefore, the main goal was to study the volatile profiles of different parts of *Opuntia* spp. plants using SPME-GC-MS. One gram of the flower, tuna and cladode of *Opuntia* sp. (wildtype, WT), tuna and cladode of *O. ficus indica* (cultivated) and xoconostle (*O. joconostle*) were incubated at room temperature for 1h in 4 ml vials. After this time, a SPME fiber (DVB/Carboxen/PDMS) was introduced in the vial and exposed to its head-space for one more hour, followed by one min desorption in the injector port of a GC-MS with a HP-FFAP capillary column.

Different volatile classes such as alcohols (35 %), aldehydes (25 %), esters (15 %) and terpenes (10 %) were identified in flower, fruit and cladode of *Opuntia* spp. Among all materials, fruits presented the largest number of volatiles (35) followed by cladodes (20) and flowers (15). The most abundant volatiles in WT-flower were ethanol (28.0 %) and ocimene (22.1 %), besides some phenolic compounds (anisole) were observed. Unique volatiles were found for WT-fruit-ocimene (0.2 %), cultivated-2,6-nonadien-1-ol (8.3 %) and xoconostle-2-buten-1-ol-3-methyl-acetate (14.3 %). More volatiles were detected in WT-cladodes (24) than cultivated ones (14), but linalool and hexanoic acid; and 1-octen-3-ol and 1-octanol might be markers for WT and cultivated types. Many C5, C6 and C7 compounds (known as green notes) were mainly present in the green part of the plant, cladode.



Volatiles play an important role on aroma and flavour, however volatile compounds like terpenes (linalool and terpineol), phenolic compounds (anisole) and acids (acetic acid) have shown benefic health related effects such as cancer-preventive, anticariogenic and expectorant to mention some examples; therefore volatile compounds found in nopal (flower, fruit and cladode) might play a role on some of the many nutraceutical properties that have been reported for *Opuntia* spp.

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P 33

Changes in Volatile Profiles of Microwaved Avocado with and without Avocado Leaves by SPME-GC-MS

Mercedes G. López[1], Lidia Dorantes[2] and Rosa I. Guzmán[2]
[1]Unidad de Biotecnología e Ingeniería Genética de Plantas,
Centro de Investigación y de Estudios Avanzados del IPN, Apartado Postal 629, 36500 Irapuato, Gto., México
mlopez@ira.cinvestav.mx
[2]Depto. de Alimentos, Escuela Nacional de Ciencias Biológicas del IPN,
Prol. de Carpio y Plan de Ayala S/N, Casco de Sto. Tomás, Del. Miguel Hidalgo, C.P. 11340, México, D.F.

Since the archeological findings in Tehuacán, Puebla, México, it has been determined that avocado (*Persea americana*) is native from Mexico. Besides, this country is the main avocado grower in the world. Currently, its industrialization is being developed mainly for export purposes such as foods. Several methods have been employed to process avocado, mainly heat treatments however, they generate a large number off-flavours. Microwave treatment (MW) is an alternative technology, where high temperature in short time is applied and therefore the damage is minimized. SPME (solid phase microextraction) offers a simple and sensitive technique for volatile analyses. The aim of this work was to detect changes in the volatiles of microwaved avocado with (MWL) and without avocado leaves (MW). Avocado puree at pH 5.5 was microwaved and compared with fresh avocado (FA) and fresh avocado leaves (FAL). Volatiles were collected from 5 g of avocado puree or 0.225 g of avocado leaves (AL) for 30 min on a DVB/CB/PDMS [1] fiber in a 10 ml vial after incubation for 24 h at room temperature and analyzed by GC/MS.

KI	COMPOUND	SAMPLES				
		FA	AL	FAL	MW	MWL
949	α-pinene		10.7	0.43		3.52
960	1-penten-3-one				0.81	0.91
1104	Hexanal			9.89	48.6	25.9
1108	β-pinene		19.6	1.23		9.52
1195	Heptanal				5.04	4.89
1203	Eucalyptol		1.60			1.88
1220	1-butanol-3Me	5.26		10.5		
1228	2-hexenal			25.7	1.03	3.01
1291	2-butanone-3-hydroxy	2.00		4.01		
1295	Octanal				20.7	25.9
1309	1-octen-3-one				24.8	26.4
1328	2-heptenal [E]-			0.65	45.3	46.5
1434	2-octenal [E]-				22.3	21.2
1483	Furfural				1.37	1.41

1-butanol-3-methyl, 2-butanone-3-hydroxy and acetic acid were volatiles detected in FA, while in AL mainly terpenoids (α-pinene, β-pinene, limonene and eucalyptol) were present. When leaves were added to FA: α-pinene and β-pinene, hexanal, 2-hexenal, 2-heptenal as well as 1-octen-3-ol were observed. On the other hand, the main components of microwaved samples (MW and MWL) were aldehydes, alcohols, ketones and Maillard compounds, many of these compounds derived from lipid oxidation. Besides, MW had higher level of hexanal than MWL, while MWL presented a larger increment of the 2-hexenal and terpenes. Aldehydes of C_6 and C_7 are odoriferous compounds that usually impart green and fresh flavour notes.

Previous sensory research by Dorantes have shown that the addition of AL improved avocado flavour. Changes in the volatile profiles of MW and MWL were detected, therefore these results suggest that it is highly probable that some of the volatiles reported here are involved on the flavour enhacement. Finally, it can be said that SPME revealed to be a rapid and sensitive method for the extraction and quantitation of volatiles compounds from avocado.

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P 34

Essential Oil of *Ferula communis* Subsp. *communis* Growing Wild in Corsica

Bernard Ferrari, Félix Tomi and Joseph Casanova
Université de Corse, Equipe 'Chimie et Biomasse',
UMR CNRS 6134 Route des sanguinaires 20000 Ajaccio, France
casanova@vignola.univ-corse.fr

The genus *Ferula* (family *Umbelliferae*, tribe *Peucedaneae*) has some 130 species, distributed throughout the Mediterranean area and Central Asia. These plants, are source of gum-resins, obtained by incision of roots, once used as in medicine for a variety of ailments.

Ferula communis subsp. *communis*, the most widespread species of this genus, is a latex-containing plant, known to be toxic to livestock, causing an often lethal hemmoragic disease known as 'ferulosis'. Prenylated coumarins and daucane sesquiterpenes were identified in solvents extracts of the different parts of the plant. To our knowledge, no studies concerning the chemical composition of the essential oil were reported in the literature.

Continuing our research on essential oil bearing plants growing wild in Corsica [1] we report, on the chemical composition of the essential oil of aerial parts of *Ferula communis* subsp. *communis*. The identification of components has been carried out by (i) the GC retention indices (RI) on apolar and polar columns; (ii) comparison of the mass spectra with those of reference compounds; (iii) ^{13}C-NMR spectroscopy, following the pioneering work done by Formàcek and Kubeczka [2] and according to an experimental procedure and a computerised method developed in our laboratory [3]. In this procedure, the components are identified by comparison of the carbon chemical shift values in the mixture spectrum with those of reference spectra compiled in a computerised data bank.

After fractionation by column chromatography, 46 components representing 96.3 % of the amount of the inflorescence oil were identified. The main constituents were myrcene (53.5 %), limonene (6.9 %) and α-pinene (6.6 %). Several sesquiterpernes were present at appreciable contents: aristolene (8.5 %), (E,E)-farnesol (4.3 %), gurjunene (1.4 %). The oil obtained from the spike has a similar qualitative composition which however differed quantitatively: aristolene (70.0 %) and myrcene (9.3 %) being the major components. Root oil was characterised by the occurrence of sesquiterpenes and the lack of monoterpenes.

The composition of the oil differed drastically to that of *Ferula arigonii* (Bocchieri), also present in the south of Corsica and in Sardinia [1].

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P 35

Composition and Chemical Variability of the Essential Oil of *Juniperus communis* L. ssp *alpina* from Corsica

Marcelle Gonny and Joseph Casanova
Université de Corse, Equipe 'Chimie et Biomasse',
UMR CNRS 6134, Route des sanguinaires 20000 Ajaccio, France
casanova@vignola.univ-corse.fr

The mountain juniper, *Juniperus communis* L. ssp *alpina*, is a subspecies of the common juniper (*Juniperus communis* L.) which belongs to the Cupressaceae family. It's a small shrub, 0.5–1.5 m high, growing wild on the high mountains slopes. In Corsica it is present at 1000 m altitude. The essential oil of juniper is commonly used in the liquor industry (juniperberry oil), in perfume and in pharmacy for its diuretics properties. In a recent publication, it is mentioned that the juniper berry oil possessed repellency against mosquitoes and the common house fly.

The chemical composition of mountain juniper essential oils and solvent extracts has been subject of numerous studies. It has been stated that the main component of twigs and berry essential oils is α-pinene. It is accompanied by a wide range of other monoterpene hydrocarbons, sesquiterpene hydrocarbons and some amounts of oxygenated compounds. The enantiomeric composition of certain monoterpenes in juniper twigs and berry oils has been the subject of several studies. It appears that the enantiomeric excess of monoterpenes depends on the origin of the raw material. Otherwise, an alcoholic extract of juniper berries revealed a qualitative composition similar to that of juniper oil. Several diterpene acids were isolated from the ether extract of juniper leaves.

In our present study, we analysed essential oils obtained from different parts of the mountain juniper from Corsica: twigs, berries and roots. The identification of compounds has been carried out by combination of i) retention indices on apolar and polar columns ii) by ^{13}C-NMR spectroscopy according to an original method first reported by Formacek and Kubeczka, developed and computerised in our laboratories. This method allows the direct identification of the main components of essential oils and extracts without previous separation.

Twigs oils are dominated by limonene, α-pinene and β-phellandrene while the major components the berry oil are limonene, α-pinene and myrcene. Conversely, the composition of root oil, which is to our knowledge reported for the first time, was really different. It was characterised by high content of sesquiterpene compounds: cedrol (36.9 %), longifolene (11.7 %) and longiborneol (9 %).

We also studied the chemical variability of twig oil. Our first results, based on the analysis of 20 samples, showed the existence of three groups of essential oils.

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Composition of the Essential Oil of *Inula viscosa* (L.) from Corsica

Marie-Cécile Blanc, Pascale Bradesi and Joseph Casanova
Université de Corse, Equipe 'Chimie et Biomasse', UMR CNRS 6134,
Route des sanguinaires 20000 Ajaccio, France
casanova@vignola.univ-corse.fr

Inula viscosa (L.) Ait. (syn *Dittrichia viscosa* (L.) Greuter) is a herbaceous plant of the Compositae family widespread in Southern Europe. Decoctions of the plant are known in folk medicine for their anti-inflammatory and antipyretic properties. The extracts of the plant have been widely studied. Several works reported on the isolation and structure elucidation of sesquiterpene lactones, sesquiterpene acids, triterpenoids and flavonoids. The chemical comosition of only two samples of essential oil has recently been reported [1, 2]. An oil from Turkey, contained borneol and isobornyl acetate as main components. Conversely, the main constituents of a sample of Spanish oil were two acyclic sesquiterpene alcohols, fokienol and (E)-nerolidol.

Continuing our research on aromatic plants growing wild in Corsica, we report, in the present work, the chemical composition of *I. viscosa* leaf oil. The bulk sample was repeatedly chromatographed, and all the fractions were investigated by GC and ^{13}C-NMR spectroscopy, following a method first reported by Formacek and Kubeczka, developed and computerized in our laboratories.

The identification of the individual components of the oil and the fractions was based: i) on comparison of their GC retention indices (RI) on apolar and polar columns, with those of authentic compounds, ii) on comparison of the resonance in the ^{13}C-NMR spectrum of the mixture with those of reference spectra compiled in our spectral library with the help of laboratory produced software.

In total, 25 compounds were identified. The oil was characterised by high percentages of sesquiterpenes, especially oxygenated ones. The main components are isocostic acid, fokienol and (E)-nerolidol. Unusual bisepoxyfarnesatrienes and epoxyfarnesatrieneol, previously reported in *Tanacetum fruticulosum* [3] were identified for the first time in *Inula* oils or extracts.

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P 37

SPME-Samples of Garlic (*Allium sativum*) from Different Origin: Aroma Compound Analysis Using GC-FID, GC-MS and Olfactometry

Leopold Jirovetz[1], Martin Benoit Ngassoum[2] and Gerhard Buchbauer[1]
[1]Institute of Pharmaceutical Chemistry, University of Vienna,
Althanstrasse 14, A-1090 Vienna, Austria
[2]Department of Applied Chemistry, University of Ngaoundere,
BP 455, Ngaoundere, Cameroon

The aroma compounds of garlic (*Allium sativum*) from Austria, China and Spain were analysed by SPME-GC-FID, SPME-GC-MS and olfactoric evaluations. The typical garlic aroma of the headspace (SPME) of all 3 investigated garlic samples is the result of well-known dominating (concentration higher than 1.0 %; calculated as %-peak area of SPME-GC-FID analysis using a non-polar column) disulfides, like diallyl disulfide (57 .4– 87 .0 %), trans-allyl propenyl disulfide (4.4–28.6 %), methyl allyl disulfide (2.3–5.5 %), cis-allyl propenyl disulfide (0.3–2.6 %) and trans-methyl propenyl disulfide (0.3–1.2 %) as well as methyl allyl trisulfide (0.4–2.6 %).

The target compound for the proof of the natural origin of garlic samples analyzed by gas chromatography, namely 3-vinyl-[4H]-1,2-dithiin, was identified in all 3 headspace samples, but in various concentrations (0.1–1.1 %).

Additional more than 20 SPME-headspace constituents were found in these 3 garlic samples in lower concentrations.

Keywords: Garlic, SPME-Samples, Aroma Compounds, GC-FID, GC-MS, Olfactometry.

P 38

Chemical and Biological Study Natural Recovered Fertility Hybrids of Mentha

Maria Pisova[1], Elena Peleah[1], Vasili Chobanu[1]
[1]Laboratory of Plant Biochemistry, State University of Moldova,
Mateevich str. 60, Kishinev 2012, Moldova
epeleah@yahoo.com

Vegetative proliferation of the interspecific hybrids of Mentha during 7–10 years showed that individual plants had restored normal fertility (2000–3000 seeds per plant). Morphologically the sterile hybrids and the fertile progeny differ insignificantly, there were no changers in terpenoid composition of the oil.

Investigation of the generative selfpolinated progenies of the fertile forms showed that the absolutely majority of this plants have the mother's terpenoid content and degree of stability depend on the peculiarity of the parents forms.

Characteristic features of the hybrids

Origin		Main components of F_1	Main components of F_2			
Mother's form	father's form		mentol	keto-oxides	pulegon	carvon
M.S, mentol	M.i, keto-oxides	MS-41-M_2 mentol	91.1	4.4	1.5	–
M.S, mentol	M.R, keto-oxides M.p, mentol, menton	MS-46-M_1 mentol	87.7	–	12.3	–
M.S, mentol	M.R, keto-oxides	MS401, mentol	85.8	14.2	–	–
M.p, mentol, menton	M.sp, carvon	LS108, mentol, mentol	83.4	13.3	–	3.3
M.p, mentol, menton	M.ar, mentol	Moscvicika, mentol	76.5	16.2	1.5	1.8

Note: M.S — *M. sachalinensis (Briq) Kudo*; M.i — *M. incana Wild*; M.R — *M. Royleana L.*; M.sp — *M. spicata L.*; M.ar — *M. arvensis L.*; M.p — *M. piperita L.*

So, chemical divergence in the generative progenies do not always give us the conception of the species' origin.

All these findings show that natural fertility recovery in sterile generative hybrids results in the stabilization of the terpenoid content and other biological characteristics.

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Studies of the *Mentha arvensis* Citral Chemotype

Elena Peleah[1], Maria Pisova[1], Vasili Chobanu[1], Anastasia Zueva[1]
[1]Laboratory of Plant Biochemistry, State University of Moldova,
Mateevich str. 60, Kishinev 2012, Moldova
epeleah@yahoo.com

A vary interesting chemorase of *Mentha arvensis* from the Far East was introduced in the mint collection of the State University and studied. Thought the morphological features of this form of mint correspond to *M. arvensis*, in its smell there was citral and geraniol notes.

The essential oil was studied by a combination of common technics such as absorption column chromatography, TLC, UV-spectroscopy, GLC. Oil components were isolated and identified by a comparison with pure terpenoids.

As the result of the investigation was revealed that the essential oil composition is unusual for the Mentha genus and contain 27 % of alcohols (geraniol, nerol, mentol, citronellol), 30 % of aldehydes and cetones (such as citral A and B and menton), 37 % of complicated etheres (geranil-acetat, neril-acetat, mentil-acetat) and up to 3 % of monoterpenes (α-pinen, β-pinen, limonen).

Thought, the yield of the oil is non very high (only 0.6–0.8 %), this mint may be useful for perfumery, cosmetics and pharmaceutical purposes thanks the combination of mentol, citral and geranilacetat. Also this ecotype is off interest for hybridization and selection.

P 40

Fast Analysis of Herb Components by Headspace — Solid Phase Microextraction for Quality Control and Variety Recognition

Claudia Holste[1], Jörg Weber[1], Christine Bartzsch[1], Karl-Heinz Feller[1], Brigitta Sander[2]
[1]Department of Medical Engineering, University of Applied Science,
Carl-Zeiss-Promende 2, 07745 Jena, Germany
[2]Bell Flavors & Fragrances Duft und Aroma GmbH Miltitz,
Schimmel-Straße 1, 04205 Miltitz, Germany

The SPME-technique represents an alternative method to the transfer of a aliquot part of the gaseous headspace to the injector of a gas chromatograph. The conventional method for separation of volatile components from its matrix is the distillation or the liquid–liquid extraction. These methods are time and material consuming.

In quality control fast results of analysis are required. Therefore our main intention was to develop a fast, easy and representative method for analysing volatile compounds in natural sources. The use of HS-SPME coupled to gas chromatography offers a lot of advantages in analysis herbs and other essential oil raw materials.

Influences on SPME-extraction process e.g. sample volume and extraction time were studied on thyme and chamomile samples to develop a appropriate method. For these investigations a 85 µm PA-SPME fibre was used. Samples were homogenised by grinding, directly transferred in a 1.8 ml-vial and the volatile compounds extracted from headspace by SPME subsequently. Beyond maceration extracts of thyme and chamomile were analysed by SPME-GC also and the results were compared.

Quantitative determination of volatile compounds were carried out. Results and the efficiency of the developed method will be discussed. In addition several species of thyme were analysed based on the method for variety classification. Some important aspects of these results will be presented to.

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P 41

Essential Oil of Brazilian *Croton urticifolius*

Daíse Lopes[1], Humberto R. Bizzo[1], José C. Albuquerque[2], Vanda C. R. Maia[3],
Luci S. Valle[3]
[1]EMBRAPA Food Technology, Av. das Américas,
29501 Rio de janeiro 23020-470, Brazil
dlopes@ctaa.embrapa.br
[2]Far-Manguinhos, Fiocruz Rua Sizenando Nabuco,
100 Rio de Janeiro 21041-250
[3]Museu Nacional, Universidade Federal do Rio de Janeiro,
Quinta da Boa Vista, Rio de Janeiro

The Genus *Croton* L. belongs to Euphorbiaceae, subfamily Crotonoideae, and comprises approximately 800 species growing in the tropical and subtropical regions [1]. *Croton* species are wide spread in Brazil and up to now, only a few species collected in the Amazonian and Northeastern areas have already been studied due to their biological activities and the occurrence of essential oils. Recently, the chemical composition of the essential oil from leaves of *Croton cajucara* was published. The concentration of linalool varied in different plants collected in several localities of Amazonian region, from 30 to 45 % [2]. Herein we report the results of a representative sample of velame (*Croton urticifolius*) essential oil. This shrub is commonly found in Rio de Janeiro seashore. The oil was obtained by hydrodistillation (4 hours) of aerial parts (leaves and slender stems) using a Clevenger apparatus, yield: 0.1 % (fresh weight). Analyses were performed by GC/MS and identification of the oil components was obtained by comparison of retention indices and, whenever possible, by co-injection with an authentic sample. Comparison and interpretation of fragmentation patterns in mass spectra with those stored in the NIST computer database and published in reference books [3] were also applied for the identification of the compounds. The sesquiterpene fraction predominated in this oil (98.3 %). The major constituents were δ-elemene, α-cubebene, α-copaene, β-bourbonene, β-cubebene, β-elemene, β-caryophyllene, aromadendrene, α-humulene, seychellene, germacrene-D, bicyclogermacrene, germacrene A, *trans*-calamenene, cadina-1,4-diene, *trans*-nerolidol and spathulenol.

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Comparative Studies on Olibanum Resins with SPME

S. Basar[1], A. Koch[2], W. A. König[1]
[1]Institut für Organische Chemie, Universität Hamburg,
Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany
[2]Frohme-Apotheke, Frohmestr. 14, D-22457 Hamburg, Germany

Olibanum belongs to the group of oleogum resins that exudes from incisions in the bark of trees of the *Boswellia* genus (Burseraceae). The trees yielding the resin are native to Ethiopia, Somalia, the Arabian peninsula and India.

The use of olibanum in folk medicine as anti-inflammatory and antiseptic agent may be attributed to the occurance of higher terpenoid constituents. Its non-medical uses as ingredient for perfumery products and as insence in churches are related to the presence of mono- and sesquiterpenes that dominate the essential oil [1].

Our previous studies, which were carried out with samples of various olibanum resins of *B. carterii* and *B. serrata* and *B. frereana*, exhibited characteristic differences in their essential oils. The inspection of the GC-MS data with the comparison of mass spectra and retention indices with our database showed that both species contain many commonly found mono- and sesquiterpenes in which octylacetate and α-pinene are the major components for *B. carterii* and *B. serrata*, respectively. The presence of the diterpene derivatives incensole, incensole acetate as well as cembrene (cembra-2,4,7,11-tetraene), cembrene A (cembra-3,7,11,15-tetraene), cembrene C (cembra-1,3,7,11-tetraene) and verticilla-4(20),7,11-triene in *B. carterii*, cembrenol (cembra-3,7,11-triene-1-ol) in *B. serrata* and several isomeric dimers of α-phellandrene in *B. frereana* serve as diagnostic marks for each specie [2].

In this study we would like to present results of SPME experiments performed for these species under different conditions and the comparison of the results with those obtained by hydrodistillation.

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Phytochemical and Genetic Analysis of *Juniperus communis* L.

Natalia Filipowicz[1], Monika Asztemborska[2], J. Renata Ochocka[1]
[1]Department of Biology and Pharmaceutical Biology, Medical University of Gdańsk,
al. Hallera 107, 80-416 Gdańsk, Poland
nata@biology.pl
[2]Department of Physical Chemistry, Polish Academy of Sciences,
ul. Kasprzaka 44/52, 01-224 Warszawa, Poland

Juniperus communis L. (juniper) is an aromatic plant, which is widely used in pharmaceutical, cosmetic and food industry. Juniper so-called 'berries' are the source of essential oil, but oil is also present in leaves and wood. Although many authors have reported juniper oil content, many problems concerning its composition have to be solved. These are especially ones connected with admissible content of individual components and its chiral recognition. Although these are rather phytochemical problems, there are powerful tools of molecular biology, which can support time consuming and difficult chromatographic analysis. It is known that chemical profile obtained from GC (Gas Chromatography) analysis shows correlation with the genetic one [1].

In this study *Juniperus communis* L. samples collected in Northern Poland and *Juniperus nana* L. (considered as a subspecies of *Juniperus communis* L.) samples from Polish mountains were investigated. Oil was obtained using steam distillation method and analysed using GC in achiral and chiral conditions. Genomic DNA was isolated and genetic profile was investigated using RAPD-PCR (Random Amplified Polymorphic DNA — Polymerase Chain Reaction).

The aim of the study was to find the correlation between results obtained from these two methods and to characterise diversity of Northern Poland juniper population.

The results obtained so far seem to confirm this assumption.

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Dependence of Etherial Oil Composition on the Terpenoid-Type and Taxonomical Status of Asterinae Subtribe Several Species

Elena Korolyuk
Researcher of the Terpenoids Laboratory, Novosibirsk Institute of Organic Chemistry,
Acad. Lavrentjev Ave. 9, Novosibirsk 630090, Russia
koroljuk@nioch.nsc.ru

Dependence of secretory structures type (form) on composition of essential oils of 10 species belonging to subtribe Asterianae (*Heteropappus altaicus, Galatella angustissima, G. hauptii, G. divaricata, G. biflora, G. villosa, Tripolium vulgare, Brachyactis ciliata, Erigeron acris, Asterothamnus central-asiaticus, Conyza canadensis)* was analyzed. The factors taken into account were as follows:

1) taxonomical status,
2) geographical areal,
3) the ecological-phytocoenotic niche,
4) the phytocoenotic role of investigated species,
5) the type and form of terpenoid-containing structures,
6) the qualitative and quantitative content of essential oils,
7) the method of preparation and assay of etherial oil.

Two types of secretorial structures are characteristic for the plant group investigated. The type and form of terpenoid strucrure are connected foremost with evolution age of native species' groups and their adaptation to ecological niches. The glandular trichomes of steppe, montane-steppe, desert and semidesert species are developed in the higher degree. These glands are ones with subcuticular cavity, of large capitate-type, placed on small mono- or bicellular pedicles. Shizogenous cavities are characteristic to them scarcely (species of genus *Galatella, Heteropappus*). The well-developed secretorial cavities trichomes (glandular trichomes — sparse, non-capitate; gen. *Erigeron, Tripolium, Btachyactis*) are inherent to boreal and halophytic species studied.

The species probably connected with ancestral *Aster alpinus* species (the steneendemic genus *Asterothamnus, Rinactinidia)* — also probably possess non-specialized parenchymal cells as essential oils storages.

As exemplified by *Asterienae* subtribe, it could be said, that among the plants of this group the maximal quantity of essential oil is produced by plants with well-developed glandular structure and the minimal one — is produced by species with schizogenous storages. Nevertheless, exceptions are present. The *Conyza canadensis* species — is a recordholder on the quantity of oil produced; at the same time this species has well-developed storages but the glandular trichomes are occasional for it. Above all, according to the sum of parameters investigated (non-asian, an adventitious flora element, weedy, annual plant, fallow- and burned-over-lands-dominant, less than 10 components are found in essential oil) it is individual species. The component composition of essential oil is possible more in connection with the evolutionary age of mutually native groups of species, whereas the content of terpens — with the ecologycal-phytocoenotic niche and their community role.

It should be noted the all the investigated species accumulate in essential oils several components which are characteristic for subtribe as a whole. The evolutionary youngest and broad-areal-species (*Erigeron, Heteropappus)* accumulate the greater quantity of components in their composition. Among these components the sesquiterpenoids' fraction grows up. Additionally, the quantitative oil yield and amount of terpenoid structures, formed per organ area unit of the noted species' group is in close connection with the role of the species in the plant community. The last factor gives rise to the competition capacity of a species in the natural coenosis.

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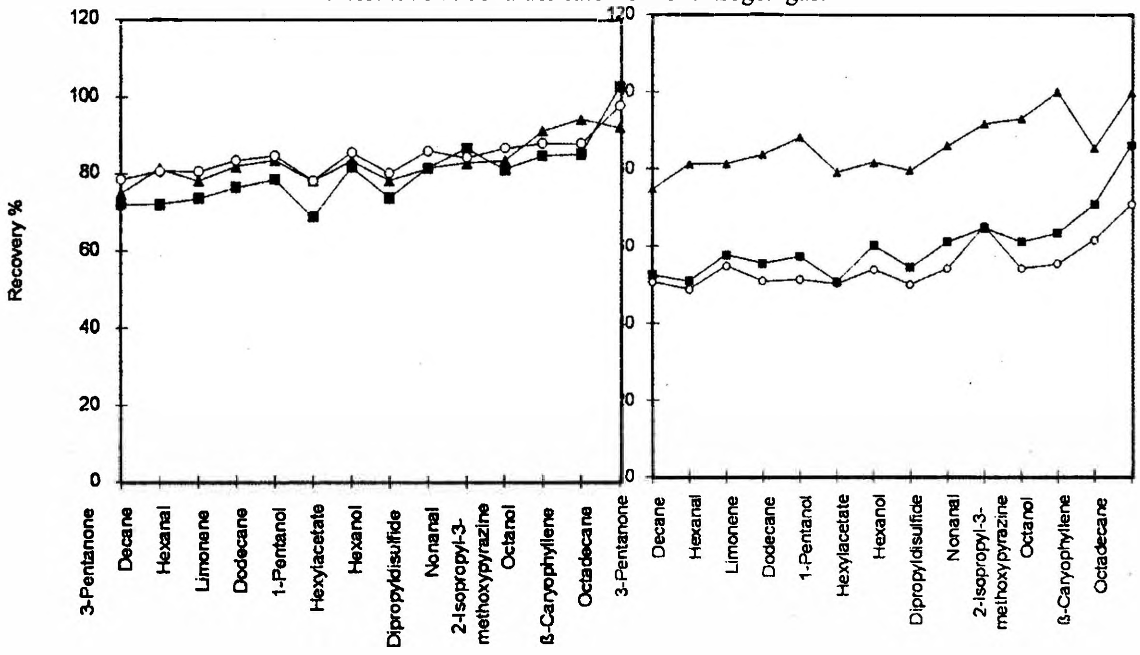
A Comparison of Methods Used for Pre-Concentration of Organic Volatile Solutions

Henrik Byrial Jakobsen, Mette Rindom Nørrelykke, Lars Porskjær Christensen,
Merete Edelenbos
Department of Horticulture, Danish Institute of Agricultural Sciences,
Kirstinebjergvej 10, DK-5792, Aarslev, Denmark
henrik.byrial@get2net.dk

Eight pre-concentration methods were compared for their capacity to retain volatile and semi-volatile solutes during evaporation of solvent. The 2 ml test-samples contained 0.2 ppm or 2 ppm (v/v) of 3-pentanone, decane, hexanal, limonene, dodecane, 1-pentanol, hexyl acetate, hexanol, nonanal, 2-isopropyl-3-methoxypyrazine, dipropyldisulfide, octanol, ß-caryophyllene. And octadecane. Dichloromethane was used as solvent. The samples were concentrated to 1 ml, 0.2 ml and 0.05 ml.

Leaving the 0.2 ppm or 0.02 ppm solutions in a test tube in the fume hood at 22 °C produced the best recoveries at (Figure 1.). The pre-concentration time from 2 ml to 0.05 ml was 19-20 h for this method. The evaporation may be speeded up by engulfing the test tube in a 47 °C water bath. This decreases the evaporation time to 1–2 h and the recoveries are only slightly lower than those obtained at 22 °C. Both these methods are superior to evaporation of solvent under a stream of nitrogen gas (Figure 1.). This method is among the most commonly reported used for the pre-concentration of small volume dilute samples from e.g. extraction of volatiles from polymers after dynamic headspace trapping. The efficiency of the traditional Vigreux and Snyder columns were only satisfactory at pre-concentration to 1 ml. These methods could not be used for pre-concentration to 0.05 ml because all of the solution had to be evaporated before the total reflux became as low as 0.05 ml. The Spinning band technique gave very high recoveries at the 1 ml and 0.2 ml level, but again this method could not be used for further concentration of the sample. The experiments clearly demonstrated that more solute is lost during the pre-concentration of very dilute samples (0.02 ppm) compared to more concentrated solution (0.2 ppm).

Figure 1. The percentage of recovered solute after pre-concentraction of 2 ml samples to 1 ml (▲), 0.2 ml (○) and 0.05 ml (■) using two different methods. Left: concentration in test tube at 22 °C. Right: concentration in test tube under a delicate flow of nitrogen gas.



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Composition of the Essential Oil of *Nepeta persica* Boiss from Iran

Katayuon Javidnia[1], Ramin Miri[1], Fujan Safavi[1], Ali Azarpira[1] and Abbas Shafiee[2]
[1]Department of Medicinal Chemistry, Faculty of Pharmacy,
The Medical Sciences University of Shiraz, Shiraz, Iran
kjavidnia@alberta.com
[2]Department of Medicinal Chemistry, Faculty of Pharmacy,
The Medical Sciences University of Tehran, Teheran, Iran

The feline attractant properties of several *Nepeta* species have been known for a longtime. The extract of many *Nepeta* species are used in domestic medicine. *Nepeta cataria L.*, commonly known as catnip is the most intensively studied species. It is used as a fortifier, a disinfectant and cure against colds [1]. The extract of some species is used also because of their diuretic properties and slight bacteriostatic activity and in ointments to heal skin disorders of the eczema type [1]. Alcoholic preparations of *N. hindostana* decrease the level of serum lipids and lipoproteins, and might thus supplement remedies against atherosclerosis [2].

In this study the composition of the essential oil of *Nepeta persica* Boiss (Lamiaceae) was investigated by GLC and GC-MS [3–5].

The yield of the oil from *N. persica* was 0.08 %. Fourthy components representing 84.5 % of the oil were characterized. The major components were 4aα,7α,7aα-nepetalactone (26.5 %), 1,4-hexadiene-2,3,4,5-tetramethyl (10.9 %), 3,4β-dihydronepetalactone (3.6 %), 2-Methyl-2,3-dihydroindole (0.9 %), α-copaene (0.8 %), β-bourbonene (2.4 %), cis-β-farnesene (4.4 %) and caryophyllene oxide (0.9 %).

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Constituents of the Essential Oil of *Chimonanthus fragrance* Lindle Flowers

Ramin Miri[1], Katayoun Javidnia[1], Maryam Chericki[1] and Abbas Shafiee[2]
[1]Department of Medicinal Chemistry, Faculty of Pharmacy,
the Medical Sciences University of Shiraz, Shiraz, Iran
mirir@sums.ac.ir
[2]Department of Medicinal Chemistry, Faculty of Pharmacy,
the Medical Sciences University of Tehran, Tehran, Iran

Chimananthus fragrance Lindle (Calycanthaceae) is distributed in different parts of Shiraz. It is renowned in folk medicine for its medical uses for its treatment of burns [1].

Study of the essential oil of *Chimananthus fragrance* has been considered for its flavor odor. Except one report on the constituents of the essential oil in China [2], no study of the volatile constituents of this plant in Iran could be found in the literature. In this work, we report on the composition of the oil obtained by steam distillation of the flowers.

The constituents of the essential oil of *Chimonanthus fragrance* Lindle (Calycanthaceae) were investigated by GLC and GC-Mass [3-5].

Twenty components representing 97 % of the oil were characterized. The essential oil was obtained from the flowers in 0.12 % yield. The main constituents of the oil were sesquiterpenes such as β-Elemene, β-Caryophyllene, γ-Cadinene, γ-Bisabolene, δ-Cadinene, β-Elemenone and α-Eudesmol. The sesquiterpenes region showed one compound (3.9 %) which couldnot be identified by RI and mass spectra.

The essential oil of *Chimonanthus fragrance* has a flavor odor and can be considered as a flavoring agent or in fragrance industrial in next researches.

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The Essential Oil of *Lantana camara* L. Occurring in Iran

Fatemeh Sefidkon
Research Institute of Forests and Rangelands,
P.O. Box 13185-116, Tehran, Iran

The genus of *Lantana,* with the common Persian name of Shah-pasand, presents some species that are cultivated in South Iran [1]. The most beautiful and full flower species is *Lantana camara* that cultivated in many regions of South Iran as ornamental.

Hydro- and steam-distilled volatile oils from the leaves and flowers of *Lantana camara* L. were analyzed by a combination of GC and GC/MS. Forty-four components in hydro-distilled oil and tweny-five components in steam distilled oil were identified. The main characterized constituents were sabinene (16.5 % and 7.3 %), β-caryophyllene (14.0 % and 22.5 %), 1,8-cineole (10.0 % and 6.0 %), bicyclogermacrene (8.1 % and 18.4 %) and α-humulene (6.0 % and 10.8 %) in hydro- and steam distilled oils respectively.

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Chemical Composition of Two Samples of Essential Oil of *Valeriana sisymbriifolia* Vahl from Iran

Mohd. Bagher Rezaee[1], Kamkar Jaimand[1]
[1]Phytochemistry group, Medicinal plants Department,
Research Institute of Forests and Rangelands,
P.O. Box 13185-116, Tehran, Iran
Fax: +98-21-6026575
mrezaee@mail.rifr-ac.org

The volatile constituents from roots and rhizomes of two samples of essential oils of *Valeriana sisymbriifolia* Vahl from Iran origin were collected during the flowering periods from two difrenet locality, sample-1 from Hamedan on west and sample-2 from Jolfa on west-north of Iran. The essential oils obtained by hydrodistilled for 4 h in a Clevenger-type apparatus to give a yellow oils in 0.08 % and 0.31 % yields, analyzed by GC and GC/MS. The major constituents of sample-1 were α-pinene (14 %), borneol (11.4 %), bornyl acetate (6.5 %) and valeranone (3 %), and sample-2 were α-pinene (16.5 %), borneol (9.7 %), α-bulnesene (7.3 %), and viridifloral (5 %).

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Constituents of Essential Oils from *Tripleurospermum disciform* (C. A. Mey) Schultz-Bip

Kamkar Jaimand[1] and Mohammad Bagher Rezaee[1]
[1]Phytochemistry group, Department of Medicinal Plants & By-products,
Research Institute of Forests & Ranglands,
P.O. Box 13185-116, Tehran, Iran
Fax: +98-21-6026575
kjaimand@hotmail.com

The volatile constituents of *Tripleurospermum disciforme* (C. A. Mey) Schultz-Bip from Iran were extracted from flowers by steam and hydrodistillation methods and analyzed by capillary GC and GC/MS. Plants were collected on 20th July 1998, from Hamedan Botanical Garden, plant speciment determined by Iranian Botanical Garden (IBG) staff. The major constituents for steam and hydrodistillation samples were respactly trans-trans-Matricaria ester (68.9–64.2 %), cis-β-farnesene (7–4.2 %), (E)-Methyl isoeugenol (3.8–4.2 %), and β-sesquiphellandrene (3.6–2.2 %).

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Chemical Composition of the Essential Oil of Sweet Fern (*Comptonia peregrina* L.)

André Bélanger[1], Éléonore Tremblay[1], Christina Pink[2], Andrea Biegel[2]
and Gerhard Buchbauer[2]

[1]Centre de recherche et de développement en horticulture (CRDH),
Agriculture et Agroalimentaire Canada, St-Jean-sur-Richelieu, Québec, Canada, J3B 3E6

[2]Institut für Pharmazeutische Chemie, Pharmaziezentrum,
Universität Wien, Althanstrasse 14, A-1090 Wien, Austria

Comptonia peregrina (L.) Coulter is a plant of the Myricaceae family that grows without difficulty on dry pour soil and is easily reproduce by rhizomes wich makes it one of the first plant to sprout on a burned site. It is found especially in from Canada to North Carolina and west to Indiana and Saskatchewan. The foliage have fern like morphology and a spicy aromatic fragrant when bruised. The leaves have been used as infusion to treat various skin problems, and as a bath for poison ivy rash it was also used in 'pot pourrie' to relieve symptoms of dysentery

Essential oils from *Comptonia Peregrina* (sweet fern) have been harvested from Dolbeau in Lac Saint-Jean (Québec) at three different times during the growing season. The essential oils of the plants were obtained by steam distillation and hydrodistillation from fresh plant, plants dried for 24 hours or 48 hours. The essential oils were analysed by capillary gas chromatography (GC) and compounds were identified by their mass-spectra and retention indices (Kovat's) relatives to n-alkanes on polar and apolar capillary column. The main components found were β-caryophyllene (30 %), myrcene (8 %), 1,8-cineole (4 %) α-selinen (3 %), E-nerolidol (4 %), caryophyllene oxide (4 %). Comparing all together the four collections that were done in June, July, August and September, the best harvesting time seams to be in July with a yield of 0.17 % for the fresh plant and 0.20 % for the 24 and 48 hours dried plant.

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Chemical Composition of the Essential Oils from *Thymbra capitata* (L.) Cav. Collected During Vegetative and Flowering Phases

M. Graça Miguel[1], A. Cristina Figueiredo[2], José G. Barroso[2], Luis G. Pedro[2]
[1]Unidade de Ciências e Tecnologias Agrárias, Universidade do Algarve,
Campus de Gambelas, 8000-117 Faro, Portugal
mgmiguel@ualg.pt
[2]Centro de Biotecnologia Vegetal, Dept. de Biologia Vegetal, FCUL, C2,
Campo Grande, 1749-016 Lisbon, Portugal

Thymbra capitata (L.) Cav. is a perennial Labiatae aromatic undershrub, up to 40 cm tall. Blossoming from June to August this plant can be found, growing wild, in the countries of the Mediterranean basin. In Portugal is particularly diffused in Algarve.

This species has been used as herbal home remedy, owing to the antiseptic, tonic, stimulating, gastrosedative and diuretic properties [1]. Some authors [2] also demonstrated a remarkable antifungal activity of the essential oils from *Thymbra capitata* against some soil-borne phytopathogenic fungi.

In this work we report on the chemical composition of the hydrodistilled essential oils isolated from collective samples of *T. capitata*, collected during the vegetative and flowering phases of the plant at Algarve. The plant parts studied, the oil yields, the dates of collection of the plant material and the main components (≥ 2 % in at least one sample) of each oil sample are given in Table 1.

Table 1. Main components of the essential oil of *Thymbra capitata* grown on Algarve.

Plant part / Collection date	**Flowering phase**			**Vegetative phase**	
	All plant	**Flowers**	**Leaf**	**Aerial parts**	
Components	**Jun**	**Jun**	**Jun**	**Feb**	**Apr**
β-Myrcene	2.2	1.4	1.6	2.4	1.5
α-Terpinene	1.6	1.0	1.0	1.6	1.3
p-Cymene	6.2	3.2	8.3	9.4	4.3
γ-Terpinene	7.1	4.1	4.6	5.2	7.9
Carvacrol	68.4	77.8	69.2	67.0	74.7
β-Caryophyllene	2.3	2.4	1.8	0.9	2.7
% Identification	98.9	97.6	95.9	96.1	99.7
Grouped components					
Monoterpene hydrocarbons	22.1	12.6	19.3	22.7	17.9
Oxygen containing monoterpenes	72.6	81.5	73.3	71.5	78.0
Sesquiterpene hydrocarbons	3.5	2.7	2.5	1.4	3.1
Oxygen containing sesquiterpenes	0.5	0.3	0.6	0.3	0.4
Diterpenes	t	0.1	0.1	t	0.1
Phenylpropanoids	t	0.1	t	t	0.1
Others	0.2	0.3	0.1	0.2	0.1
Oil Yield (v/w)	3.2	2.5	2.2	2.0	1.2

Oxygen-containing monoterpenes were dominant in all oil samples studied (72–82 %), carvacrol being always the main component (67–78 %). Monoterpene hydrocarbons were the second most important group of compounds (13–23 %), dominated in all oils by *p*-cymene and γ-terpinene (3–9 % and 4–8 %, respectively). β-Caryophyllene (1–3 %) was the main component of the sesquiterpene fraction that attained a relative amount not higher than 4 % of the total oils.

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Chemical Composition of the Essential Oils from *Thymus carnosus* Boiss. Collected During Vegetative and Flowering Phases

M. Graça Miguel[1], A. Cristina Figueiredo[2], Francisco F. Silva[1], José G. Barroso[2], Luis G. Pedro[2]
[1]Unidade de Ciências e Tecnologias Agrárias, Universidade do Algarve, Campus de Gambelas,
8000-117 Faro, Portugal
mgmiguel@ualg.pt
[2]Centro de Biotecnologia Vegetal, Dept. de Biologia Vegetal, FCUL, C2, Campo Grande,
1749-016 Lisbon, Portugal

Thymus carnosus is a south western Iberian Peninsula endemic Labiatae, growing wild in coastal sands, mainly in the Estremadura, Alto Alentejo, Baixo Alentejo and Algarve (Portugal) as well as in Huelva (Spain).

The chemical composition of the essential oils isolated by hydrodistillation from *Th. carnosus*, collected at Alto Alentejo (Lagoa de Melides) was evaluated in both the vegetative and the flowering phases of the plant. The plant parts studied, the oil yields, the dates of collection of the plant material and the main components (≥ 2 % in at least one sample) of each oil sample are given in Table 1.

Table 1. Main components of the essential oil of *Th. carnosus* grown on Lagoa de Melides.

Plant part / Collection time	**Flowering phase**		**Vegetative phase**	
	Flowers	**Leaf**	**Leaf**	**Leaf**
Components	**July**	**July**	**Feb**	**May**
α-Thujene	3.5	2.7	1.1	3.7
α-Pinene	4.4	3.0	2.1	2.8
Camphene	9.9	8.0	9.0	7.7
Sabinene	1.7	1.5	1.1	1.8
β-Pinene	2.3	1.8	1.5	1.8
α-Terpinene	2.7	4.0	4.1	4.0
p-Cymene	0.5	1.2	2.6	0.9
trans-β-Ocimene	1.6	0.7	0.1	1.3
γ-Terpinene	4.7	7.3	7.8	7.3
trans-Sabinene hydrate	4.9	3.1	1.9	3.5
Terpinolene	1.1	1.7	1.8	1.7
cis-Sabinene hydrate	9.2	7.4	4.8	7.5
Camphor	0.6	0.9	1.7	0.8
Borneol	22.3	20.3	22.8	17.5
Terpinen-4-ol	11.1	16.4	19.3	18.1
Bornyl acetate	4.0	3.8	4.9	4.3
Elemol	1.8	1.4	0.4	1.1
% Identification	95.3	96.1	94.8	95.9
Grouped components				
Monoterpene hydrocarbons	35.2	34.2	32.6	35.7
Oxygen containing monoterpenes	56.4	58.4	60.6	56.4
Sesquiterpene hydrocarbons	1.1	0.9	0.2	1.0
Oxygen containing sesquiterpenes	2.3	2.3	0.9	2.6
Diterpenes	0.3	0.2	0.3	0.1
Phenylpropanoids	t	t	t	t
Others	t	0.1	0.2	0.1
Oil Yield (v/w)	0.8	0.8	0.1	1.0

The essential oils from *Th. carnosus* were dominated by the monoterpenic fraction (92–93 %), the oxygen-containing monoterpenes attaining 56 % to 61 % of the total oils. Borneol (18–23 %) and terpinen-4-ol (11–19 %) were dominant in all the oils studied. Monoterpene hydrocarbons represented also an important group of compounds, ranging from 33 % to 36 % of the oils, its main component being camphene (8–10 %). The sesquiterpene fraction ranged from 1 % to 4 %, the oxygen-containing components attaining 1-3 % of the total oils. Elemol was the main component of this fraction (0.4–2 %).

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GC and GC-MS Investigation of Extractive Components of Birch Buds

V. Isidorov[1], J. Jaroszyńska[1], U. Krajewska[1], E. Dubis[2] and L. Vetchinnikova[3]
isidorov@noc.uwb.edu.pl
[1]Institute of Chemistry, Białystok University, 15-443 Białystok, Poland
[2]Custom Laboratory of Białystok Custom-House, 15-339 Białystok, Poland
[3]Institute of Forest, Karelian Branch of RAN, 185610 Petrozavodsk, Russia

Birch buds are widely used in medicine. However, up to the present the composition of components extracted from this raw material has not been investigated in detail. This paper presents the results of GC and GC-MS study of the content of essential oils and other components in buds of several birch species growing in the north-western part of Russia (Karelia).

Essential oils were extracted (2×50 ml) with hexane from 3–5 g of air-dry buds collected in March 2001. These combined extracts were concentrated on a rotor evaporator to a volume of 4–5 ml. After extraction with hexane and subsequent air-drying, the buds were additionally extracted (2×25 ml) with acetone, and combined extracts were evaporated to complete dryness. Dry residue was subjugated to silanization by BSTFA with the addition of pyridine and catalytic amounts of trimethylchlorosilane. Gas chromatographic analysis was carried out using a HP-4890D instrument with a fused silica capillary column packed with DB-5 methylphenylsilicon and using a Turbo Mass GC-MS instrument (Perkin-Elmer).

More than 50 components of different classes were detected in the hexane extract: sesquiterpene hydrocarbons and their oxygen-containing derivatives as well as a series of diterpenes and C_{17}–C_{25} *n*-alkanes. Considerable difference in the composition of terpene components were found in buds of different birch species. In particular, one of the main components in hexane extracts of *B. pubescens* and *B. dalecarlia* species is the $C_{15}H_{26}$ hydrocarbon cedrane which is completely absent in extracts of *B. karelica* and *B. pendula* species.

In the acetone extract in the form of trimethylsilyl derivatives, components belonging to four classes of organic compounds are identified: phenolcarboxylic acids (gallic and caffeic acids), mono- and disaccharides (glucose, fructose, galactose, mannose and saccharose), saturated C_{14}–C_{17} carboxylic acids and unsaturated acids (oleic and linalic acids). Just as in the case of terpene compounds, considerable interspecific differences were detected in acetone-extracted compounds.

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Comparison of Different Methods for Isolating the Components of Essential Oils of Plants

V. Isidorov[1], N. Brzostowski[2] and U. Krajewska[1]
isidorov@noc.uwb.edu.pl
[1]Institute of Chemistry, Białystok University, 15-443 Białystok, Poland
[2]Białystok Politechnical Institute, 15-328 Białystok, Poland

Essential oils composition recorded by methods of gas chromatography depends to a considerable extent on the procedure of sample preparation for the analysis. This paper reports the results of comparison of four methods of volatile components isolation taking as an example the essential oils of pine (*Pinus sylvestris* and *P. strobus*). Investigations were carried out with the same sample of pine needles collected in March 2001 in vicinity of Białystok. The sample was analyzed using an HP-4890D instrument on a fussed silica capillary column (30 m × 0.25 mm) with the DB-5 stationary phase in the temperature programming regime from 40 to 220 °C.

Essential oils were isolated from an averaged needle sample with a mass of 300 g by distillation with water vapor. Needle samples (25 g) were extracted for 24 h with n-hexane and ethyl acetate. Two other methods of sample preparation included different variants of head-space analysis (HSA): static HSA proper and solid-phase microextraction (SPME). In both cases the needle feed (3.5 g) was palsied in a glasses 12.5 vial and thermostatted at 85 °C. In the static HSA variant, the 0.5 ml sample was collected with a gas syringe. In the SPME variant, a syringe for microextraction (Supelco Inc., USA) with polydimethylsiloxane coated fiber (100 μm) was used. The exposure time was 60 min.

The investigations showed great difference in the composition of volatile components. Thus, fraction of monoterpene hydrocarbons was 87.5 % for HSA, 74.4 % in extraction by hydrodistillation, but only 32.1, 35.0, and 26.7 % in SPME, and extraction by hexane and ethyl acetate, respectively. The greatest contribution of sesquiterpene fraction was recorded in the SPME variant (56.3 %) and in the extraction by both organic solvents (37.5–38 %). Sesquiterepenoids fractions in the sum of compounds extracted by hydrodistillation and SPME procedures were approximately equal (4.5–5.4 %). However, the solvents extract them more effectively (13.5–16.3 %). Furthermore, in the last variant, considerable amounts of diterpenes and diterpenoids were found (8.1–9.1 %). They were completely absent in the chromatograms obtained when the sample was prepared by hydrodistillation and the HSA methods.

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Establishment of the Aromatic Profile of Different Tunisian Floral Waters

Kjell Spoormans[1], Christine Raynaud[1], Naceur Ayed[2], Tigani Karmous[2], Thierry Talou[1]
[1]LCA/CATAR, ENSIACET,
118 Route de Narbonne, 31077 Toulouse, Cedex 4, France
craynaud@ensct.fr
[2]Department of Colours and Natural Additives, NIAST,
676 Centre Urbain Tunis Nord, 1080 Tunis, Tunisia

Essential oils are used to give flavour and fragrance not only to coffee, tea, wine and other distilled liquors, but also to pastry and yoghurt [1]. In particular the production and domestic use of floral waters in Tunisia goes back up to ancientness due to a historical production of as well the flower as its forthcoming floral water. This study has as aim to identify the aroma profile of different flower species; *Geranium rosat*, *Rosa centifolia*, *Rosa canina*, *Citrus bigaradia*, produced by traditional (domestical) and industrial mode and to investigate their sensory properties.

The domestical produced samples were done by three different systems. System one consists of a distillation unit made of terra-cotta containing water and flowers followed by a basic cooling system. By using the second system, there is a separation between water and floral material. The flowers are laid on a sieve, thus this provides a manner so that water can bubble through the vegetal material. The third system is identical to the first one, except for the material of the distillation unit, namely steel instead of terra-cotta. The compounds of the aromas of floral waters were analysed by a dynamic headspace concentration method (DHCM) [2], and the sensory evaluation was carried out using the field of odours [3] as reference.

Concerning the chemical analysis of geranium, the most important peak for the commercial sample was 6-methyl-5-hepten-2-one (59.15 %), an ‘herbaceous’, ‘green’, ‘oily’ and ‘pungent’ compound. Compounds identified in the traditional samples contribute to the range of odours in an ‘ethereal’, ‘herbaceal’, ‘camphoraceal’ and even ‘anisedical’ way. Interesting was the presence of some compounds (e.g. rose oxide) who were only found in the samples of system one and three and not in the sample made by system two. This is probably due to direct contact between water and floral material in system one and three, which is absent in system two. Another point of interest was the change in concentration in some compounds, especially menthone which showed a severe decrease in concentration during storage.

As well for rose, orange and dog rose bush one of the most important compounds for the commercial sample was 6-methyl-5-hepten-2-one, ranging from 13.82 % (dog rose bush) to 77.34 % (orange). The same investigation as for geranium was carried out for the traditional distilled flowers with all the three other species.

Sensory studies could be correlated with aroma profile results. The odours of the floral waters from the three systems were almost the same in term of odour composition. Only the strength of odour was different between systems. While system one and three were strong in odour, the sample from system two was much weaker in strength of odour.

The DHCM used to analyse samples allowed to identify volatiles from the top note of floral waters odours, which were not yet identified. Samples from system one and three were richest in term of volatiles compared with system two and could be differentiated as well by chemical composition and sensory properties.

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P 57

On-Line Monitoring of Essential Oils Hydrodistillation by Using a Simplified Electronic Nose

Hilde Squire[1], Brigitte Dubreuil[1], Krishna Persaud[2], Thierry Talou[1], Christine Raynaud[1]
[1]LCA-CATAR, ENSIACET, 118 Route de Narbonne, 31077 Toulouse, Cedex 4, France
talou@cict.fr
[2]DIAS-UMIST, Sacksville Street M60 1QD Manchester UK
krishna.persaud@umist.ac.uk

Qualification and quantification of aroma volatiles emitted by plant extracts (essential oils, oleoresins) are important factors of the quality control methodology used by the aromatic and food industries. Physico-chemical techniques (GC, GC-MS, HPLC) and sensory analysis (dynamic olfactometry, evaluation by a 'Nose' or by panellists) are two classical methods used for this purpose, but they are time consuming, expensive and do not allow an immediate decision (off-line methods).

Considerable interest has arisen in the use of arrays of gas sensors together with an associated pattern recognition technique to quantify, differentiate and identify complex mixtures of volatile compounds. The principle of deduction in such apparatus, labelled 'electronic noses', is based on the reversible electrical resistance changes of the sensing element (metal oxides or conducting polymers) in the presence of volatiles combined with on-line computerised statistical dataprocessing [1].

The objective of this study is to monitor the hydrodistillation of aromatic plants in order to obtain standardised quality of essential oils by controlling on-line VOC's emission with smart gas sensors. For an optimal response of the sensors the humidity surrounding them was lowered by a hydrophobic interface combined with a peristaltic pump and purified air to ensure a satisfactory dilution of the vapours. By using a selfconstructed electronic device (SX-mos), 20 commercial MOS, singly located at the top of the distillation column and at the end of the condenser, and 3 kinds of hydrophobic membranes (PTFE, silicon and chemically hydrophobed cotton) were tested in different process conditions (cooling and heating temperatures) and on various substrates (boiling water, water additivated with Caraway seeds (*Carum carvi Linné*) essential oil and real hydrodistillation of seeds) in a hydrodistillation pilot unit (10 L). TGS816/TGS831 gas sensors and the silicon membrane were respectively the best couple of gas sensors for controlling the distillation/condensation of volatiles and the most efficient barrier for water vapour.

With this set-up the distillation of Caraway was monitored allowing the detection of defects such as leaks in the system, changes of fall or rise of temperature or waterflow even as differences in initial operating conditions (vapour pressure and cooling flow).

Although promising results were obtained a further optimisation is in progress for accurate monitoring of the distillation.

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P 58

Rapid Characterization of Essential Oils by Micro Mid-Infrared Diamond ATR Spectrometry

Hartwig Schulz, Rolf Quilitzsch, Sven Pfeffer
Federal Centre of Breeding Research on Cultivated Plants, Institute for Quality Analysis,
Neuer Weg 22-23, D-06484 Quedlinburg
h.schulz@bafz.de

Recently, the successful application of ATR/FT-IR in combination with sophisticated chemometrical algorithms has been demonstrated for various citrus and mint oils [1, 2]. In most cases the terpenoid substances occurring in the individual oils show characteristic key bands which can be used for quantitative predictions. This study presents the potential of a newly developed micro mid-infrared diamond ATR spectrometer for the simultaneous determination of most valuable substances in various commercially produced essential oils. Reference values were obtained by usually applied GC methods. The MIR spectra were registered in the range from 700 to 4000 cm^{-1}. The spectra were processed with a PLS (partial least square) algorithm and the accuracy of the individual chemometrical results is characterized by the statistical parameters R^2 (multiple coefficient of determination) and RMSECV (root mean standard error of cross validation). All components occurring in the essential oils with amounts of more than 1 % can be predicted with very high accuracy. To give an example, the MIR correlation statistics are presented here for chamomile oil (Table 1.). Relating to these results MIR diamond ATR has been found to be a rapid and reliable method for determining the composition of various essential oils. Since only very small sample amounts are necessary (approx. 10 µl) it provides an interesting alternative to the commonly used GC measurements which are presently employed in quality control laboratories of the industry. In combination with existing efficient clean-up procedures (e.g. solvent or CO_2 extraction, micro hydro-distillation) the rapid determination of individual essential oil compositions can be also performed in freshly collected essential oil plant species. Therefore, in principle the mobile ATR-IR spectrometer presented here can be also used for the evaluation of wild plants collected from natural habitats.

Table 1. MIR correlation statistics for most valuable substances in chamomile oil (N = 53).

Oil component	Range (%)	R^2	RMSECV (%)
α-Bisabolol	0–55.51	98.2	2.0
Bisabololoxide B	0–14.1	90.7	1.1
Chamazulene	0–16.84	96.3	0.9
cis-Spiroether	0–14.47	98.6	0.5
α-Farnesene	0–10.23	95.3	0.8
β-Farnesene	0–54.15	98.3	2.3
β-Cubebene	0–4.94	95.1	0.3

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Potential of Near-Infrared Spectroscopy (NIRS) for Rapid Quality Evaluation of Essential Oils

Sven Pfeffer, Hartwig Schulz, Hans Krüger
Federal Centre of Breeding Research on Cultivated Plants, Institute for Quality Analysis,
Neuer Weg 22-23, D-06484 Quedlinburg
h.schulz@bafz.de

In the past, near-infrared spectroscopy (NIRS) has been successfully used for various applications in agriculture but up to now only few studies have addressed the potential of this sophisticated technique to determine the composition of minor plant substances such as essential oils. Therefore this study presents newly developed NIR methods for the simultaneous determination of various oil components within less than one minute. Reference values were obtained by usually applied GC methods. The NIR spectra (range: 1100 to 2500 nm) were registered in the transflection mode using quartz cuvettes, equipped with a diffuse gold reflector (path length 0.2 mm). The presented data indicate that very good estimations can be achieved by NIRS technique in conjunction with suitable chemometric algorithms. The statistical results show that in most cases the individual SECV is not significantly higher than the standard error of the GC method. Generally, the multiple coefficients of determination (R^2) reach values above 0.97, representing a very high correlation with the GC reference data. The developed NIRS calibration statistics can be used not only for rapid quality control purposes but they can be also successfully introduced to monitor the enrichment of valuable oil components during distillation or extraction processes. As an example, the NIR correlation statistics are presented here for the essential oil of milfoil (Table 1.). Based on the presently existing experience, it can be assumed that in principle NIR is also applicable for the caracterization of various other essential oils receiving a similar prediction quality. Due to the very short analysis time and the high accuracy of the results there is some reason to substitute presently performed GC oil measurements by NIRS for some special fields of application.

Table 1. NIR correlation statistics for most valuable substances in the essential oil of milfoil (N = 74).

Oil component	**Range (%)**	**R^2**	**SECV (%)**
Anethole	0.25–69.61	0.999	0.43
Artemisia-ketone	0.31–30.47	0.997	0.48
Camphor	0.32–28.54	0.993	0.80
1,8-cineole	0.31–25.30	0.991	0.37
borneol	0.56–13.36	0.973	0.53
β-pinene	0.42–16.63	0.995	0.36
pro-azulene	0.26–18.69	0.994	0.63

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P 60

Composition of Essential Oils from *Eryngium amethystinum*, *E. alpinum* and *E. planum* (Apiaceae)

Nahla Ayoub[1] and Karl-Heinz Kubeczka[2]
[1]Department of Pharmacognosy, Faculty of Pharmacy,
Ain-Shams University, Cairo, Egypt
[2]Department of Pharmaceutical Biology,
University of Hamburg, Bundesstrasse 45, D-20146 Hamburg, Germany
kubeczka@gmx.de

The genus Eryngium with approximately 220 species is represented in Europe by 26 different species, growing mainly in the Mediterranean area. Continuing our investigations of the essential oils from Eryngium species, we have analysed the essential herb oils from *E. amethystinum*, collected in northern Italy and the two species *E. alpinum* and *E. planum*, cultivated in the Botanical Garden of the University of Hamburg.

The essential oils were obtained by hydrodistillation of the fresh herb and analysed by GC, GC-MS and NMR spectroscopy, exhibiting quite different compositions.

Main constituent of the essential oils from *E. amethystinum* and *E. planum* is germacrene D with 44.9 and 60.6 %, respectively. In addition, palmitic acid (13.5 %) and the two benzaldehyde derivatives 2,3,4- and 2,3,6-trimethylbenzaldehyde (10.6 and 3.1 %, respectively) are detected in considerable amounts in the herb oil from *E. amethystinum*. However, the volatile trimethylbenzaldehydes have to be considered as artifacts formed during hydrodistillation from ferulol and isoferulol esters by hydrolysis and proton-catalysed rearrangement as described earlier [1].

The essential oil from *E. alpinum* contains mainly the polyacetylene derivative falcarinol with 67.8 % whereas germacrene D amounted only to 7.1 %. Further constituents were *(Z,E)*-α-farnesene (9.0 %), bicyclogermacrene (3.9 %), and *(E)*-ß-farnesene (2.0). Finally the occurrence of the rarely found sesquiterpene hydrocarbon prezizaene (0.5 %) in this oil shall be mentioned.

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Essential Oil Composition of Some Medicinal Plants Growing in South-East of Tunisia

Akrout Ahmed[1], Chemli Rachid[2], Chreïf Imed[3] and Hammami Mohamed[3]
[1]Institut des Régions Arides, Laboratoire eau-sol-végétaux,
4119 Medenine, Tunisia
[2]Faculté de Pharmacie, Laboratoire de Pharmacognosie,
5000 Monastir, Tunisia
[3]Faculté de Médecine, Département de Biochimie, Laboratoire USCR/SM,
5010 Monastir, Tunisia

Essential oils were isolated by hydrodistillation from *Artemisia herba alba*, *Thymus capitatus*, *Rosmarinus officinalis* and *Juniperus phoenicea*, growing spontaneously in the south-east of Tunisia and usually used in folk medicine. The essential oil yield was respectively 0.65, 2.75, 2.50 and 0.70 %. These oils were investigated by GC-MS. *Artemisia herba alba* oil was mainly composed of α-thujone (44 %), sabinyle acetate (17 %) and β-thujone (10 %). The major components of *Thymus capitatus* oil were thymol (66 %), p-cymene (6 %) and sabinene (4 %). The most abundant components of the oil of *Rosmarinus officinalis* were 1,8-cineole (27 %), camphene (23 %) and β-pinene (8 %). The oil of *Juniperus phoenicea* was mainly composed of α-pinene (68 %) and p-cymene (6 %).

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Daily Variation in the Essential Oil Composition of *Pycnocycla spinosa* Decne. & Boiss

Gholamreza Asghari[1], Gholamali Houshfar[2] and Zahra Mahmoudi[1]
[1]Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I. R. Iran
[2]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I. R. Iran

The genus *Pycnocycla* (Umbelliferae) has eight species in Iran. *Pycnocycla spinosa,* an aromatic plant, is distributed in central parts of Iran [1]. Samples of *P. spinosa*, growing wild in Isfahan University campus, were collected at different times of the day. The hydrodistilled aerial parts oils of *P. spinosa* were analyzed by GC and GC/MS. Thirty four components were identified, of which the major constituents were geranyl isopropionate, caryophyllene oxide, β-eudesmol, citronellol, elemicin, ρ-cymene, citronellyl acetate, and α-cadinol. Several studies carried out on aromatic plant have shown that essential oil composition may vary considerably throughout a 24 h period [2, 3]. Evaluation of phenylpropanoid components showed maximum concentration (percent of essential oil) of methyl eugenol and elimicin at 1:00 and 7:00 p.m. and their minimum concentration at 7:00 a.m. and 1:00 p.m. respectively. Geranyl isopropionate, ρ-cymene, and α-cadinol showed the highest concentration at 7:00 p.m. Plant harvested early in the morning at 7:00 a.m. provided essential oil with a high β-eudesmol, sabinene, and limonene content. Citronellol and citronellyl acetate reached their highest level at 4:00 and 1:00 p.m. respectively, while both showed lowest levels at 7:00 p.m. Production of α-pinene, β-pinene, linalool, and other minor terpenoid compounds (percent in <1 % amount in the essential oil) did not exhibit any definite diurnal trends.

Variations in essential oil components in *P. spinosa* in response to diurnal changes may indicate that essential oil of differ qualities can be obtained according to the harvest time of the plant.

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Six Chemotypes of Essential Oils of *Thymus pulegioides* L. Growing Wild in Vilnius District (Lithuania)

Genovaite Bernotiene and Danute Mockute
Institute of Chemistry, A. Gostauto 9, LT-2600 Vilnius, Lithuania
Fax: +370-2-617018
albmal@takas.lt

The odours of more than 2000 plants and their essential oils were determined in 12 habitats in 1995–2000. The plants with citric (citral-geraniol chemotype) and 'phenolic' (carvacrol-chemotype) odours were found in all investigated localities. The plants with 'sweet' (geraniol chemotype) odour were found in more than half of investigated habitats. Only in one locality plants with 'ester' odour (α-terpenyl acetate chemotype) were found among plants with citric and 'phenolic' ones. The plants with 'hydrocarbon' (γ-terpene — p-cimene chemotype) and 'spicy' (β-caryophyllene chemotype) odours also grew in only habitat among the plants with citric, 'phenolic' and 'sweet' ones.

The samples of the plants weight 0.1–1 kg. The plants were air-dried at the room temperature (20–25 °C). The essential oil was prepared by hydrodistillation of 10–100 g of air-dried plants.

The GC analysis was carried out with a Hewlett-Packard (HP) 5890 II gas chromatograph equipped with a flame ionisation detector and a fused silica capillary column (25 m × 0.2 mm) coated with FFAP (Hewlett Packard) stationary phase. The oven temperature was programed from 70–210 °C at 3 °C min^{-1} using He as carrier gas (0.7 ml/min).

The GC/MS analyses were performed on HP-MS system coupled with gas chromatograph HP 6890 equipped with a HP-5MS (30 m × 0.25 mm) capillary column and operating under the same conditions as discribed above, the carrier gas flow rate — 0.5 ml/min. The percentage of composition of the essential oil was computed from GC peak areas without using correction factors. Qualtitative analysis was based on the comparison of the retention data on both columns and mass spectra with that of the corresponding components of reference oils and the data in the literature.

The major constituents of essential oils were: 1) citral-geraniol chemotype — cis- and trans-citral (neral + geranial, 16.4–30.2 %), geraniol (10.4–23.2 %), β-caryophyllene (7.4–13.1 %) and nerol (5.0–12.1 %); 2) carvacrol chemotype — carvacrol (16.4–33.3 %), β-caryophyllene (9.5–15.9 %) and β-bisabolene (10.5–12.9 %); 3) geraniol chemotype — geraniol (23.1–25.5 %), β-caryophyllene (10.8–13.5 %) and β-bisabolene (9.5–10.2 %); 4) α-terpenyl acetate chemotype — α-terpenyl acetate (49.5–70.4 %) and β-caryophyllene (6.2–11.5 %); 5) γ-terpinene — p-cimene chemotype — γ-terpinene (15.5–17.2 %), p-cimene (7.7–17.0 %), carvacrol (4.6–13.5 %) and β-caryophyllene (9.6–10.5 %); 6) caryophyllene chemotype — β-caryophyllene (18.0–19.7 %), β-bisabolene (14.5–15.9 %), germacrene D (12.5–14.6 %) and trans-nerolidol (8.2–13.8 %).

The α-terpenyl acetate, caryophyllene and γ-terpinene — p-cimene chemotypes were not described earlier in *Thymus pulegioides* L. species.

The diferent compositions of the essential oils with the investigated odours caused bioactivity of plants characteristic for their constituents. The healing power of plants and their essential oils depended on the quantity of the bioactive constituents.

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Comparative Pharmacological Studies of Essential Oils and the Corresponding Hydrosols, Teas and Methanolic Extracts of Lavandula Species and the Controversial Justification for Usage of Lavender Oil in Aromatherapy

Maria Lis-Balchin[1], Suzanne Groisse[2], SandraVollmaier[2], Gerhard Buchbauer[2] and Stephen Hart[3]
[1]School of Applied Science, South Bank University,
Borough Road, London, SE1 OAA, UK
lisbalmt@sbu.ac.uk
[2]Institute of Pharmaceutical Chemistry, University of Vienna,
A1090 Vienna, Althanstrasse 14, Austria
[3]School of Biomedical Sciences, King's College London,
Guy's Campus, London, SE1 9RT, UK

Essential oils prepared from *L. angustifolia* Mill., *L. viridis* and *L. stoechas* L. were spasmolytic on intestinal smooth muscle and this activity is probably due to the linalool and linalyl acetate and/or camphor. The mechanism of action is myogenic involving cAMP at low concentrations with block of calcium channels also being involved at high concentrations. There is also some spasmogenic activity in some commercial samples of *L. angustifolia* and this is probably due to the presence of α- and β-pinene and other monoterpenes and 1,8-cineole [1].

In the whole animal, the oils of lavender cause sedation [2]. Lavender oil also depresses skeletal muscle activity in the rat but this is probably not important in humans.

The fact that some water-soluble extracts of *Lavandula angustifolia* have a strong spasmogenic action on smooth muscle is somewhat disturbing, as so many modern herbal and aromatherapy books state that the teas are sedative and these are often prescribed for upset stomachs. The spasmolytic results shown for the water-soluble extracts of the more camphoraceous *L. stoechas* supports the early usage of spike lavender teas. These results suggest that information has been mistakenly transcribed from early herbals like those of Culpeper [3] where *L. spica,* a more camphoric lavender was used medicinally and not the very floral, *L. angustifolia,* which has always been used, as it is used today, mainly in perfumery and cosmetic products. Many attributes are given to spike lavender tea and also its alcoholic extracts, but also a warning that the latter may be too strong and even abortifacient.

The aromatherapists' reasons for massaging the essential oil of *Lavandula angustifolia* on the grounds of ancient medicinal value are therefore grossly mis-informed. The value of *Lavandula angustifolia* oil seems to lie mainly in its aroma. The use of the wrong species in clinical aromatherapy trials could be a further reason why these have not yielded any significant therapeutic results.

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P 65

Effect of Different Growing Conditions on Essential Oil Content and Its Constituents of *Cymbopogon olivieri*

Reza Omidbaigi[1], Mohammad Tabatabaei[2], Hossein Mirjalili[2]
[1]Department of Horticulture, College of Agriculture, Tarbiat,
Modaress University, P.O. Box 16415-381, Tehran, Iran
omidba-r@net1cs.modares.ac.ir
[2]Department of Horticulture, College of Agriculture,
Tehran University, Tehran, Iran

Lemongras (*Cymbopogon olivieri*) is distributed in south (Jiroft region), south-east (Sarbaz region) and west (Dezful and Masjed Soleiman region) area of Iran. Collection and phytochemical evaluation of this plant from four different growing conditions were done during years of 1999–2000. The results showed that different growing conditions have significant effect on the essential oil contents and their constituents of *Cymobopogon olivieri.* So that Masjed Soliman and Sarbaz samples have the highest (3.33 %) and the lowest (1.41 %) essential oil content respectively. Twelve constituents belong to mono- and sesquiterpenoids identified by GC-MS analysis and in these analysis results showed that piperitone monoterpenoid was the major component.

The diversity and amount of constituents showed significant differences so that Sarbaz and Jiroft samples had more constituents variability than the others. But the plants grown in Masjed Soleiman and Dezful had the highest and the lowest piperiton in the essential oil respectively.

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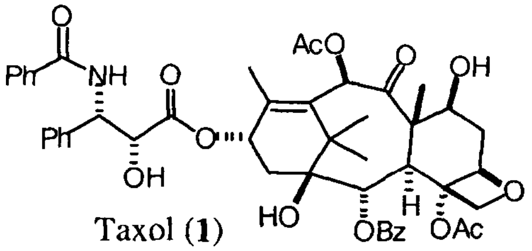
P 66

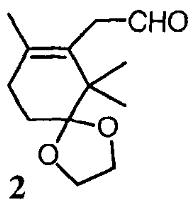
Synthetic Approach into Taxane Skeleton by a Biomimetic Entry

Mariusz Skwarczynski, Kamal K. Kapoor, Hiroshi Imagawa, Takumichi Sugihara
and Mugio Nishizawa
Faculty of Pharmaceutical Sciences, Tokushima Bunri University,
Tokushima 770-8514, Japan
mugi@ph.bunri-u.ac.jp

Taxol (**1**) is a diterpene derivative employed as a significant clinical application against human cancers such as breast, ovarian, and lung cancer. Since the supply of taxol is a serious problem, numerous synthetic studies have been carried out and several groups have accomplished total syntheses. We have developed an efficient olefin cyclization agent, mercuric triflate $Hg(OTf)_2$, and applied it for the syntheses of a variety of carbocyclic terpenoids. We also have been interested in the synthetic application of $Hg(OTf)_2$ for the construction of a taxane skeleton according to a biomimetic entry. In a previous communication from this laboratory, it was demonstrated that the A-ring synthone **2** could be efficiently synthesized by $Hg(OTf)_2$ induced cyclization of homogeranyl acetate as the key step [1]. Conventional introduction of the side chain moiety and McMurry cyclization of the derived keto aldehyde **3** afforded diol with a bicyclo[9.3.1]pentadeca-triene skeleton and the diol was converted to 2α-p-nitrobenzoate **4**. Upon treatment of **4** with $Hg(OTf)_2$, serious rearrangement-cyclization product **5** was characterized by X-ray crystallography [2]. Then, we designed cation **6** as the precursor for the transannular cyclization on the basis of the calculation with CONFLEX-MOPAC program. The cation **6** will be able to generate from tert-alcohol **7**. Therefore, we would like to describe the detail of the synthetic study. During the synthesis, we also found that $Hg(OTf)_2$ is distinguished catalyst for the hydrolysis of terminal alkyne to methyl ketone under neutral and very mild conditions even in the presence of tetramethylurea.



Taxol (**1**)



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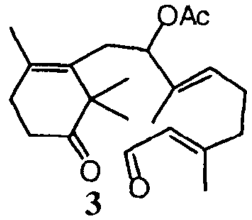

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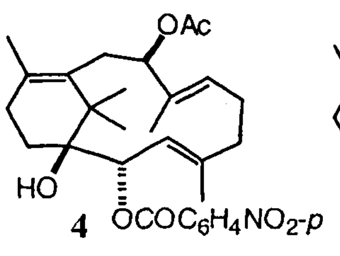

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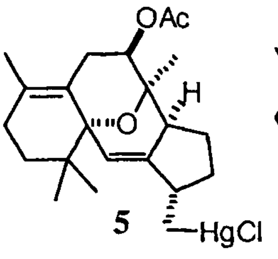

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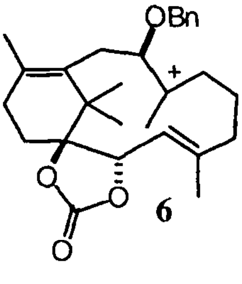

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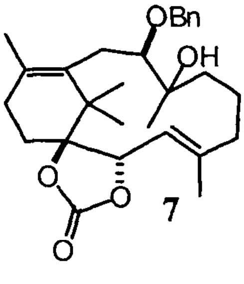

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P 67

Application of Vegetable Roots Enzyme Systems to the Transformation of Aromatic-Aliphatic Components of Essential Oils

Wanda Mączka, Agnieszka Mironowicz
Department of Chemistry, Agricultural University,
ul. Norwida 25, 50-375 Wrocław, Poland
amiron@ozi.ar.wroc.pl

Terpenes constitute an important part in the odoriferous compounds. Nevertheless, in some plants aromatic-aliphatic alcohols or ketones being components of essential oils can be also found. Phenylethyl alcohol occurs in rose oil obtained from *Rose damascena, R. centifolia, R. gallica and R. alba* [1] and in lime-tree leaves (*Tilia parvifolia* Rhrh., *T. grandifolia* Rhrh.) [2], while acetophenone is an element of a fragrance composition of compound in North American lobelia (*Lobelia syphilitica* L.) [3].

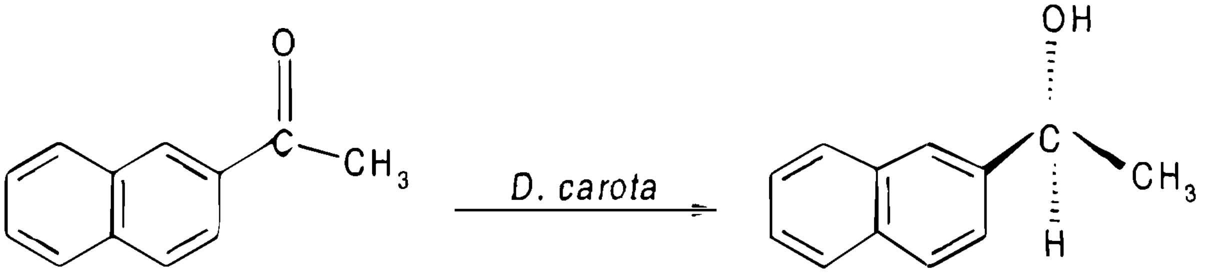
In search of easy and inexpensive methods of achieving enantiopure, biological active compounds, including the essential ones, racemic aromatic-aliphatic esters and prochiral ketones were subjected to biotransformation by means of comminuted plant roots [4].

The following racemic acetates and ketones were used for biotransformations: (±)-1-phenylethyl acetate (**1**), (±)-1-(2-naphthyl)ethyl acetate (**2**), (±)-1-(1-naphthyl)ethyl acetate (**3**), acetophenone (**4**), methyl-(1-naphthyl)ketone (**5**) and methyl-(2-naphthyl)ketone (**6**).

As biocatalysts were used comminuted vegetable roots: celeriac (*Apium graveolens* L. var. *rapaceum*), carrot (*Daucus carota* L.) and horse-radish (*Armoracia lapathifolia* Gilib.).

Each acetate was hydrolyzed to appropriate alcohols (5–100 % hydrolysis; ee = 20–82 %), but only 1-phenylethyl alcohol was subsequently oxygenated to acetophenone by means of celeriac (35 %).

Methyl-(2-naphthyl)ketone (**6**) was reduced (100 %) to pure enantiomer S-(–) (ee = 100 %) by means of carrot.



2-acetylnaphthalene (**6**)

S-(-)-1-(2-naphthyl)ethanol
100%; ee=100%

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P 68

Leaf Essential Oil Composition of Different Accessions of *Vitex agnus-castus*

Johannes Novak and Chlodwig Franz
Institute for Applied Botany, University of Veterinary Medicine
Veterinaerplatz 1, A-1210 Vienna, Austria
johannes.novak@vu-wien.ac.at

Since the fruits of *Vitex agnus-castus* (Verbenaceae) are widely used in folk-medicine and phytotherapy with a growing importance during the last years, much interest was laid recently into the elucidation of the chemical compounds influencing especially the female hormonal equilibrium.

Vitex agnus-castus contains also an essential oil present in the leaves, flowers and fruits. Many previous reports dealt with it's composition and found remarkable differences depending on geographical distributions and environmental factors. In this communication we report the composition of leaf-essential oils of different accessions of *Vitex agnus-castus* cultivated at one location to exclude environmental influence.

15 different accessions of *Vitex agnus-castus* were cultivated in an experimental field in Mallorca, Spain. The leaves of single plants were harvested in the plant's secondary year. The dried leaves were distilled with modified clevenger apparatus and the essential oli analysed using a HP-GC/MS system.

The analyses of the leaves revealed an essential oil rich in α-pinene, sabinene, limonene and 1,8-cineol. The essential oil composition varied strongly between as well as within accessions. The most striking differences were the presence of three chemotypes regarding the contents of α-pinene, limonene and β-caryophyllene.

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Effect of Plant Fertilization with Ionic or Elementary Sulphur on the Contents of Some Fatty Acids in Oil Obtained from Star Spring Rape

Jerzy Gaca[1], Piotr Szulc[2], Grażyna Wejnerowska[1], Piotr Szterk[3]
[1]Technical and Agricultural University,
Department of Chemical Technology and Engineering,
Seminaryjna str. 3, 85-326 Bydgoszcz, Poland
gaca@atr.bydgoszcz.pl
[2]Technical and Agricultural University,
Department of Agriculture,
Bernardyńska str. 6/8, 85-029 Bydgoszcz, Poland
[3]Technical and Agricultural University,
Department of Animal Husbandry,
Mazowiecka str. 28, 85-084 Bydgoszcz, Poland

Our studies aimed at determination of the effect of fertilization with various doses of ionic or elementary sulphur applied into soil or foliar on the yield of crude oil obtained from 'Star' spring rape seeds and on the contents of essential fatty acids (EFA) myristoleic (C14:1), palmitooleic (C16:1), oleic (C18:1), eicosenic (C20:1) and saturated fatty acids (SFA) palmitic (C16:0) and stearic (C18:0) in the Star spring rape seeds.

It was found that sulphur at the dose of 20 kg × ha^{-1}, independently of the form (ionic and elementary) had an insignificant effect on the total amount of the oil obtained. The amount of oil obtained was significantly decreased as the dose was increased to 60 kg × ha^{-1}.

Fertilization with sulphur stimulated increase of EFA total contents and decrease of unsaturated fatty acid contents in educed oil. The highest total increase of EFA and simultaneously the decrease of SFA contents were obtained while applying sulphur in elementary form to the foliar.

The content of individual acids was dependent both on the form (ionic, elementary) of sulphur applied and the method of its dosage (into soil, foliar). It was pointed out that fertilization with elementary sulphur stimulated the increase of oleic, linolic, palmitooleic and stearic acids contents, however it caused the decrease of eicosenic and palmitic acid contents. Fertilization with ionic sulphur stimulated the increase of oleic and palmitooleic acid contents and decrease of eicosenic, palmitic, linolic and stearic acids contents.

P 70

Characterization and Biological Activity of the Essential Oil from a Wild Italian Thyme (*Thymus capitatus* (L.) Hoff. & Lk.)

Bruno Biavati, Roberta Piccaglia, Mauro Marotti
Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi di Bologna,
via Filippo Re 6-8, 40126 Bologna, Italy
biavati@agrsci.unibo.it

A wild thyme, collected, at beginning of summer, in Favignana (Egadi isles, Southern Italy) at full flowering stage, was classified as *Thymus capitatus* (L.) Hoff. & Lk., which is a typical species of the Mediterranean area and Portugal [1]. This thyme is known as a medicinal plant with a well established antimicrobial activity mainly due to the phenol contents [2]. The essential oil, obtained by steam distillation of flowering tops (1.32 % yield on fresh matter) and characterised by GC/MS, showed carvacrol as main component (71 %) and p-cymene, γ-terpinene and β-caryophyllene in relatively abundant amounts (about 5 %).

Our data referred to the antimicrobial activity of the essential oil *Thymus capitatus*, were obtained using M.I.C. (minimal inhibitory concentration). This was defined in the range 200–2000 ppm with intervals of 200 ppm. The results were quite homogeneous between all genus tested, with the exception of phytopathogens: *Xanthomonas pruni* and *Erwinia carotovora* showed the highest inhibition (both 400 ppm) while *Agrobacterium vitis* was the lowest (>2000 ppm). Six spp. of *Lactobacillus,* six spp. of *Bifidobacterium*, six spp. of *Clostridium*, six spp. of *Bacillus*, eight spp. of yeast, eight phytopathogens and thre spp. of *Streptococcus* were analysed.

Considering average data: genus *Bacillus* (400 ppm), yeast (400 ppm), *Clostridium* (500 ppm), are more inhibited than *Streptococcus* (600 ppm), *Bifidobacterium* (700 ppm), *Lactobacillus* (900 ppm). In conclusion our results show that the essential oil of *Thymus capitatus* is very active; this suggests a lot of applications in different areas.

Antioxidant analysis was performed in agar plate added with linoleic acid and ß-carotene. Filters (6 mm) soaked with essential oil (18 µl) were put on the agar plate and after 4 h at 45 °C the zone of colour retention was measured. The antioxidant activity of *Thymus capitatus* is 19 mm.

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P 71

Comparative Studies of Some Strains of Mint

Vasili Chobanu[1], Elena Peleah[1], Maria Pisova[1]
[1]Laboratory of Plant Biochemistry, State University of Moldova,
Mateevich str. 60, Kishinev 2012, Moldova
epeleah@yahoo.com

New hybrids of mint were obtained in Research Laboratory of chemistry and biochemistry of essential oils as a result of distinct hybridisation and complicated selection. The comparison of essential oil of some wild mint species from different localities of Moldova and mint introduced in collection of mint of Moldavian State University have been carried out: *M. piperita L.* from Russia, Brazil and local popultions of Moldova; *M. longifolia L.* from Crimea, Caucase and some Moldavian areas; *M. spicata L.*, *M. silvestris L.*, *M. incana L.*, *M. Royleana Benth.* from Moldova, Bulgaria, the Ukraine, *M. citrata Ehrh.* from Cuba; *M. arvensis L.* from Vietnam and Guinea.

As a result of interspesific hybridisation were advanced forms of mint, which can be used as sources of pharmaceutic leaves, oil, free menthol and other biologically active substances such as carvon, linalool, linalilacetat etc. *M. arvensis L.* from the Far East is of special interest because synthesises menthol and citrale simultaneously. The best results we received using *M. sachalinesis* (Briq.) Kudo as the maternal form and *M. Royleana* L. and *M. incana* Wild as the parental form.

Thanks to attractive characteristics of these varieties, their essential oils could be used in perfumery and cosmetics as well as traditionally in phytotherapy and pharmacy.

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Antifungal Activity of Essential Oils of *Artemisia pontica* L. and *Achillea ageratum* L.

Krystyna Świąder[1], Jolanta Krzyżanowska[1], Danuta Kalemba[2], Anna Kurowska[2]
[1]Department of Pharmaceutical Biology and Botany, Medical Academy,
Al. Kochanowskiego10, 51-601 Wrocław, Poland
[2]Institute of General Food Chemistry, Technical University,
ul. Stefanowskiego 4/10, 90-924 Łódź, Poland

The antifungal activity of essential oils of two plants from Asteraceae family: *Artemisia pontica* L. and *Achillea ageratum* L. against *Candida albicans*, *Rhodotorula rubra* and *Aspergillus fumigatus* was tested using the agar well method. This activity was determined by measuring the diameter of the fungus free areas zone of inhibition after 48 and 72 hours.

The essential oils were obtained by hydrodistillation from flowering plants cultivated in the Medicinal Plant Garden in Wrocław. The chemical constituents were identified (after distillation under vacuum and repeated flash chromatography) by GC-RI, GC-MS and ^{1}HNMR.

From about 60 components identified in each essential oil-1,8-cineole was the main one: *A. pontica* — 57.3 % and *A. ageratum* — 57.5 %.

The tests revealed good inhibitory proprties of *A. pontica* essential oil on the mycelia growth of all three phyla only in the concentration of 1 %. Lower concentrations had weaker effects. The 1 % oil from *A. ageratum* has very strong inhibitory activity on the growth of *Candida albicans* and quite good against *A. fumigatus* and *R. rubrum.* The lower concentrations of this oil had weak antifungal activity.

P 73

Stressing Factors Influences on the Variation of Essential Oil and Coumarins in Marine Fennel — *Crithmum maritimum* L.

K. Wierzchowska-Renke[1], K. Głowniak[2], D. Marek[1], R. J. Ochocka[1], A. M. Zobel[3]
[1]Dept. of Biology and Pharm. Botany, Medical University,
ul. Hallera 107, 80-416 Gdańsk, Poland
[2]Dep. of Pharmacognosy, Medical University,
ul. Peowiaków 12, 20-007 Lublin, Poland
[3]Dept. of Chemistry, Trent Univeristy,
Peterborough, ON, K9JB8 Canada

Crithmum maritimum L. (Umbelliferae) — marine fennel is a fleshy, glabrous, perennial herb, which grows along the Mediterranean and European Atlantic coast. The whole plant is eaten. An infusion of the plant is used medicinally, as a diuretic and stomachic. It is mostly investigated for the content of volatile oils. Furthemore, in phytochemical screening investigations, the presence of flavonoids, coumarins and gums in *C. maritimum* was found [1–3].

The study followed the influence of stressing factors — environmental condition, fertilization, on cumulation and composition of essential oil and coumarins in marine fennel. The presented study is a small part of our research aiming to determine the factors which change the chemical composition of medicinal plants [4, 5]. The studied material was overground parts of *Crithmum maritimum* L. collected from different natural sites and from cultivated plants in the Garden of the Medical University in Gdańsk.

Total content of essential oil in the plant material was determined by steam water distillation and quantity of main essential oil components (α-pinene, β-pinene, p-cymene, limonene, γ-terpinene) was analysed by gas-chromatography. The observations have shown that Ti ions fertilization positively influenced on the weight and development of marine fennel, the freeze resistance of plants and caused changes in their chemical composition. The leaves of *C. maritimum*, fertilized with 0.04 % Tytanit solution (containing titanium ions) and collected in the first year of vegetation were significantly more rich in volatile oils than the controls, the content of five evaluated monoterpens (α-pinene, β-pinene, p-cymene, limonene, γ-terpinene) increased.

Coumarin was analysed after exhaustive extraction with petroleum ether and methanol. The obtained extracts were purified from polar components by liquid-liquid extraction and separated by column chromatography on silica gel 60 (230–400 mesh) stationary phases and then by preparative TLC on silica gel 60 plates, 0.5 mm thickness. Several coumarin fractions have been obtained in such way. As homogenous fractions, scopoline and scoparone have been isolated, they have been confirmed by comparison of their chromatographic retention data with standards in TLC and RP-HPLC analysis; similar composition of coumarins mainly in etheral and methanolic extracts from plant material collected in different natural sites (Crete, Malta, Cyprus, Tenerife, France) and cultivated in Poland were established.

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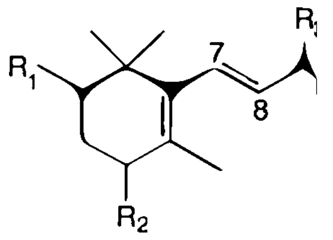
Microbial Transformations of α-Ionone and β-Ionone

Bogna Latacz, Alina Świzdor, Jadwiga Dmochowska-Gładysz
Department of Chemistry, Agricultural University,
ul. Norwida 25, 50-375 Wrocław, Poland

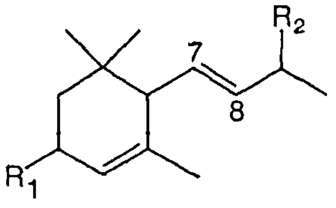
The α-ionone and β-ionone are terpenoid compounds often occurring in the plant kingdom. The ionones and its derivatives are important components of essential oils. They belong to a group of substances creating the scent and fragrance of fruits, vegetables and flowers. Chemical methods of the ionones derivatives syntheses are complicated. Microbial transformations of terpenoids often proceed regioselectively and enantioselectively. They can be useful in obtaining of various derivatives. Fermentation of terpenoid compounds is potentially interesting for practical application especially in the flavour and fragrance industry.

We have developed methods for the microbial transformations of β-ionone and α-ionone. We examined twenty microorganisms and we have selected a few transforming ionones to one or two products with high yield. We have obtained oxygenated and hydroxygenated derivatives of α- and β-ionone. These transformations often proceed enantiospecyfically and regiospecyfically.



β-ionone R_1=H ; R_2=H ; R_3= =O

1. R_1=H ; R_2=H ; R_3=OH
2. R_1=H ; R_2=OH ; R_3= =O
3. R_1=OH ; R_2=H ; R_3= =O
4. R_1=H ; R_2= =O ; R_3= =O
5. R_1=H ; R_2=H ; R_3=OH ; 7,8 -hydrogenated
6. R_1=H ; R_2=OH ; R_3= =O ; 7,8 -hydrogenated
7. R_1=H ; R_2= =O ; R_3= =O ; 7,8 -hydrogenated



α-ionone R_1=H ; R_2= =O

1. R_1=OH ; R_2= =O
2. R_1= =O ; R_2= =O
3. R_1= =O ; R_2= =O ; 7,8 -hydrogenated
4. R_1=H ; R_2=OH

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Secondary Metabolites and Sensory Characteristics of Essential Oils of Walnut and Black Walnut

Karol Bal[1], Beata Ćwikowska[1], Jadwiga Jaroszyńska[2], Anna Nagielska[1],
Alicja Niesłuchowska[1], Agnieszka Purzyńska-Pugacewicz[2]
[1]Firmenich Sp z o.o., Laboratory of GC/MS,
ul. Chrzanowska 10, 05-825 Grodzisk Maz., Poland
[2]University of Białystok, Institute of Chemistry,
Al. J. Piłsudskiego 11/4, 15-443 Białystok, Poland
jjarosz@mail.uwb.edu.pl

Among several species of walnut, two are growing in Poland: walnut *Juglans regia* L. called also king's walnut and black walnut *Juglans nigra* L. [1].

This first of them is using to prepare many precious pharmaceutical and cosmetics. Besides walnut oil is used in the confectionery business. Walnut leaves contain such compounds as tannins and flavonoids (e.g. quercetin, kaempferol) and their derivatives, phenolic acids, ascorbic acid and a little amounts of essential oils. Most characteristic component, occurring in all tissues (leaves, bark, root, fruits) is juglone, product from the shikimic acid pathway. This component is well-known since a long time but still the methods for his determination are completed [2].

The black walnut *Juglans nigra* is planted in Poland to regard his decorative values. The qualitative composition of his secondary metabolites is known less than of walnut. The black walnut shells have made to produce the active carbons. They could be used in purge and trap analysis as adsorbents [3].

In this work, the results of qualitative analysis of the compounds emitted by leaves and fruits will be introduced. The volatile components isolated from leaves and fruits have been explored by sensory and SPME-GC/FID methods. Besides the compounds contained in the green fruits were obtained by steam distillation and identified by GC/MS analysis. Comparative organoleptic evaluation of walnut supercritical CO_2 extracts [4] was also done. Additionally the content of the phenolic compounds has been investigated. The results will be published.

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P 76

Influence of Phytohormones on Growth and Essential Oil Composition of Some *Mentha* Species

Nihad Hadzifeizovic[1], Gerald Binder[2], Ahmed A. Abou-Mandour[3],
Franz-Christian Czygan[3]
[1]Department of Pharmaceutical Biology and Phytochemistry, University of Münster,
Hittorfstrasse 56, 48149 Münster
[2]Heinrich KLENK GmbH & Co KG,
Aschenhof 35, 97525 Schwebheim
[3]Department of Pharmaceutical Biology, University of Würzburg,
Julius-von-Sachs-Platz 2, 97082 Würzburg

All investigated species of the genus *Mentha* showed great conformity of growth during *in-vitro*-cultivation. Variations in morphology induced by exogenous application of phytohormones showed far extending correspondence, too. Especially the intense root formation on the surface of the nutrition medium induced by 1-naphthaleneacetic acid and the suppression of rootbranching due to abscisic acid were striking. Furthermore, an enhanced branching and development of lateral shoots was observed in all plants after treatment with the cytokinins 6-benzylaminopurine and zeatin.

An uniform effect of selected phytohormones, as observed for the development of the plants, could not be detected in the case of essential oil accumulation, the effect of phytohormones is very specific for all species.

In vitro cultivated *Mentha spicata* accumulated an essential oil comparable with the one of field-grown plants. In this case the composition of essential leaf oil could be influenced significantly by addition of phytohormones [1]. The content of monoterpene hydrocarbons increased continously with the rising addition of 6-benzylaminopurine. Also C-3-oxigenated monoterpene hydrocarbons rose in content, whereas amounts of C-6-oxigenated monoterpene hydrocarbons like carvone and dihydrocarvone and all identified sesquiterpenes were reduced. The results led to the assumption that under the influence of 6-benzylaminopurine the content and/or the activity of limonene-6-hydroxylase was reduced.

Mentha citrata accumulated an essential oil dominated by the acyclic-oxigenated monoterpenes linalool and linalooloxid. All through the year and also in *in-vitro*-culture, the plants showed a constancy in essential oil composition, even phytohormones did not influence essential oil composition. In spite of the sometimes serious influence of phytohormones on growth and morphology, all plants investigated formed an essential oil similar to that of control regarding the respective amounts of mono- and sesquiterpenes as well as the percentages of the individual essential oil compounds. Only the application of high cytokinin concentrations revealed a remarkable enhancement in essential oil content.

Under natural conditions *Mentha x piperita* accumulates an essential oil dominated by C-3-oxigenated monoterpenes like menthol, menthone, isomenthone and menthyl acetate as main components. In contrast, the essential oil of *in-vitro*-cultured plants mainly consisted of menthofuran and pulegone. With the addition of selected phytohormones, the shares of mono- and sesquiterpenes could be altered.

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P 77

Incorporation Studies on the Biosynthesis of the Sesquiterpene Germacrene D in *Solidago canadensis*

Panagiotis Steliopoulos[1], Klaus Peter Adam[2], Armin Mosandl[1]
[1]Institut für Lebensmittelchemie, Johann Wolfgang Goethe-Universität Frankfurt (Main),
Marie-Curie-Str. 9, 60439 Frankfurt (Main), Germany
Phone: +49-69-798-292-02/03
Fax: +49-69-798-292-07
mosandl@em.uni-frankfurt.de
[2]Pharmakognosie und Analytische Phytochemie der Universität des Saarlandes,
66041 Saarbrücken, Germany

The chiral hydrocarbon germacrene D is a widely spread plant constituent and is considered as an important intermediate in biosynthesis of many sesquiterpenes [1, 2]. Schmidt et al. [3] have shown that the plant *Solidago canadensis* generates both optical antipodes of this compound by enzymatic cyclization of farnesyl diphosphate using two different enantiospecific synthases.

Recent studies demonstrated that two routes can be involved in the formation of the isoprene units of sesquiterpenes: the mevalonate pathway and the deoxyxylulose pathway [4, 5]. In order to elucidate the biogenetic origin of the isoprenoid building blocks of germacrene D in *Solidago canadensis* in vivo feeding experiments were carried out with [5,5-D_2]-1-deoxy-D-xylulose-5-phosphate (D_2-DOXP), [5-^{13}C]-mevalonolactone (^{13}C MVL) and [1-^{13}C]-D-glucose.

The hydrodistillate of a cut shoot fed with D_2-DOXP was investigated by enantio-MDGC-MS, the volatile fraction of a shoot supplied with ^{13}C-MVL was examined by GC-^{13}C-IRMS. Incorporation of [1-^{13}C]-D-glucose was analyzed by quantitative ^{13}C-NMR spectroscopy after isolation of germacrene D from the essential oil.

Our labeling studies revealed that biosynthesis of the C-15 backbone of sesquiterpene germacrene D in *Solidago canadensis* proceeds predominantly via the deoxyxylulose phosphate pathway.

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P 78

Composition and Antimicrobial Activity of the Essential Oil of *Tanacetum argenteum* (Lam.) Willd. subsp. *flabellifolium* (Boiss. & Heldr.) Grierson

K. Hüsnü Can Baser, Nurhayat Tabanca, F. Demirci
Medicinal and Aromatic Plant and Drug Research Centre (TBAM),
Anadolu University, 26470-Eskisehir, Turkey
http://www.tbam.anadolu.edu.tr/

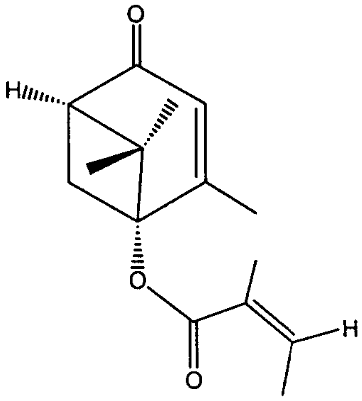
Tanacetum argenteum (Lam.) Willd. subsp. *flabellifolium* (Boiss. & Heldr.) Grierson (Compositae) is an endemic species for Turkey. Water distilled essential oil of this plant was analyzed by GC/MS and characterized with α-pinene (29.1 %), (*E*)-sesquilavandulol (15.9 %) and camphor (14.0 %) as main constituents. Enantiomeric separation of some constituents in the oil was determined by MD-GC/MS using a fused silica Lipodex E capillary column. Antimicrobial activity of the oil was also determined.

P 79

A New Monoterpene Ester from *Ferulago thirkeana* (Boiss.) Boiss. Growing in Turkey

K. Hüsnü Can Başer[1], Betül Demirci[1], Fatih Demirci[1], Toshihoro Hashimoto[2],
Yoshiaki Noma[2], Emine Akalın[3], Neriman Özhatay[3]
[1]Medicinal and Aromatic Plant and Drug Research Centre (TBAM),
Anadolu University, 26470-Eskişehir, Turkey
http://www.tbam.anadolu.edu.tr/
[2]Faculty of Domestic Sciences,
Tokushima Bunri University, Yamashiro-Cho180, Tokushima City, 770-8055, Japan
[3]Faculty of Pharmacy, Department of Pharmaceutical Botany,
Istanbul University, Üniversite, 34452, Istanbul, Turkey

The essential oils obtained by micro-distillation and hydrodistillation of the endemic *Ferulago thirkeana* (Boiss.) Boiss. of Apiaceae were analyzed by GC/MS. An unknown component in the essential oil which was isolated and characterized by chromato-spectral techniques. Its structure was elucidated as (1*S*)-2,6,6-trimethyl-4-oxobicyclo[3.1.1]hept-2-enyl(2*E*)-2-methylbut-2-enoate (trivial name: ferulagone) (**1**) was accomplished by spectral methods (1D-, 2D NMR, HRMS, IR and UV). Both the essential oil and the pure major compound (**1**) were tested against various human pathogenic microorganisms.



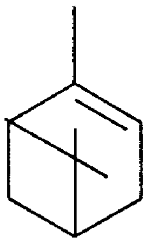
1

P 80

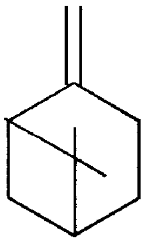
The Topological Indexes Importance in Research of α- and β-Pinene as well as α- and γ-Terpinene Separating by Means of TLC

Alina Pyka, Katarzyna Bober
Department of General and Analytical Chemistry, Faculty of Pharmacy,
Silesian Academy of Medicine, ul. Jagiellońska 4, 41-200 Sosnowiec, Poland
chemogan@infomed.slam.katowice.pl

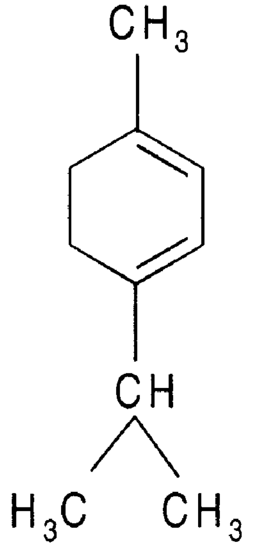
The problem of topological indexes application for estimation of chromatographic separation was subject of our publications [1–7]. The research concerning using of topological indexes chosen and electrotopological states for estimation of isomers chosen are presented in this work. The subject of investigations were isomeric α- and β-pinene as well as α- and γ-terpinene:



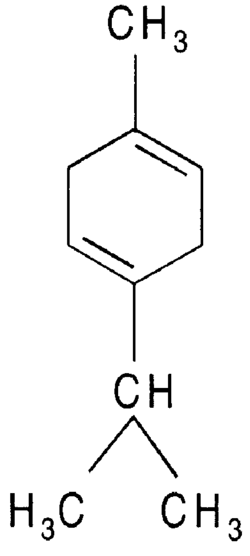
α- pinene



β-pinene



α- terpinene



γ-terpinene

They were separated by means of TLC. Chromatographic data obtained directly correlate with topological indexes and electrotopological states counted. The further researches of the above problem are continued.

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P 81

Correlation of the Chemical Composition with the Antibacterial Activities of the Essential Oils of Four Aromatic Plants from Cameroon: *Ocimum gratissimum*, *Plectranthus glandulosus*, *Cinnamomum zeylanicum* and *Xylopia aethiopica*

Jirovetz L.[1], Buchbauer G.[1], Ngassoum M. B.[2], Essia-Ngang J. J.[2], Tatsadjeu N. L.[2]
and Adjoudji O.[2]
[1]Institute of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria
[2]University of Ngaoundéré, P.O. Box 455, Ngaoundere, Cameroon

Four aromatic plants have been selected for chemical investigation on account of their antibacterial activities, *Ocimum gratissimum* (fresh leaves), *Plectranthus glandulosus* (fresh leaves), *Cinnamomum zeylanicum* (dried leaves) and *Xylopia aethiopica* (dried fruits). *P. glandulosus* is used as medicinal plant while the other species are used as common spices in many recipes in Cameroon. The essential oils of the plants have been obtained by hydrodistillation using a Clevenger type apparatus, the yields of oils are about 0.5 %, 0.3 %, 2.0 % and 2.5 % respectively.

The essential oil of *O. gratissimum* contains a higher percentage of thymol (about 50 %) whereas the one of *P. glandulosus* contains mainly β-thujone (about 31 %), p-cymen-8-ol (about 25 %) and neral (about 10 %). The essential oil of *C. zeylanicum* is characterised by a high percentage of eugenol (85 %), and the one of *X. aethiopica* contains some kaurane-derivatives and monoterpenes, such as β-pinene (about 18 %), sabinene (about 7 %), α-phellandrene (about 7 %) and terpinen-4-ol (about 9 %).

These essential oils have been tested against three strains of bacteria, namely *Pseudomonas fluorescens*, *Escherichia coli* and *Staphyllococcus aureus*; qualitative tests (diffusion through solid medium) and quantitative tests (dilution method) have been carried out. *P. fluorescens* shows a higher resistance to the four oils (MIC value not less than 5000 ppm). *E. coli* is more sensible to these oils, but the highest activities were observed with the oils of *O. gratissimum* (625 ppm) and *X. aethiopica* (1250 ppm). Against *S. aureus* the essential oil of *O. gratissimum* still shows a high activity (1000 ppm) followed by the one of *C. zeylanicum* (3500 ppm).

The antibacterial activities of the essential oils could be correlated with the components. Thymol is responsible of the activities of the oil of *O. gratissimum*, while eugenol is the active component of *C. zeylanicum*, and β-thujone and p-cymen-4-ol are the active compounds in the essential oil of *P. glandulosus*.

Key words: antibacterial activities, essential oils, *Ocimum gratissimum*, *Plectranthus glandulosus*, *Cinnamomum zeylanicum*, *Xylopia aethiopica*.

P 82

Structure Requirements for Bellpepper Aroma Impression

Gerhard Buchbauer[1], Johanna Klocker[2], Bettina Wailzer[2], Peter Wolschann[2]
[1]Institute of Pharmaceutical Chemistry, University of Vienna,
Althanstrasse 14, A-1090 Vienna, Austria
[2]Institute of Theoretical Chemistry and Molecular Biology, University of Vienna,
Währinger Strasse 17, A-1090 Vienna, Austria

The relationship between the molecular structure of flavor compounds and the intensity as well as the quality of their aroma impression has received more and more interest within the last years. This led to a better understanding of the physicochemical mechanism of both flavor and odor perception. However, up to now there is no extended information from the three-dimensional structures of the receptor binding sites availaible.

Thus, instead of receptor-based approaches ligand-based ones have to be applied to obtain more insight into the interactions between the aroma compounds and the odorant binding proteins as well as the olfactory receptors. Quantitative structure-activity relationships (QSAR) techniques are widespread and rather successful methods in modern drug design and should, therefore, also represent an important tool for the study of structure-activity relationships in olfaction. The aim of such investigations is to obtain correlations between molecular parameters with sensory properties of the aroma compounds.

For the presented work, 46 pyrazine derivatives with bellpepper flavor and known threshold values are selected in order to investigate those parameters, which are of importance for the intensity of their aroma. Therefore, different approaches of molecular modelling are applied on these molecules: Conformational Analysis, Multiple Linear Regression (MLR), Comparative Molecular Field Analysis (CoMFA), Comparative Molecular Similarity Indices Analysis (CoMSIA), Holographic QSAR (HQSAR) and Artificial Neural Network (ANN).

The models obtained from different techniques show statistical significance. The predictivity of these QSAR models is comparable and, moreover, the interpretation of the models reinforces each other to give helpful information about the structural requirements, which enhance the biological activity and, therefore, influence the aroma intensity of bellpepper aroma impression. This study is also a contribution for the investigation of the physico-chemical mechanism of odor and aroma perception.

P 83

Classification of Pyrazine-Derived Aroma Compounds by Artificial Neural Networks

Gerhard Buchbauer[1], Johanna Klocker[2], Bettina Wailzer[2], Peter Wolschann[2]
[1]Institute of Pharmaceutical Chemistry, University of Vienna,
Althanstrasse 14, A-1090 Vienna, Austria
[2]Institute of Theoretical Chemistry and Molecular Biology, University of Vienna,
Währinger Strasse 17, A-1090 Vienna, Austria

Nowadays, flavor science centers — among other interests — on a better understanding of the relationships between the molecular structure and the quality as well as the intensity of the aroma impression. As structure-activity relationships of different compounds with known biological activity can be investigated by numerous methods of molecular modelling, their application on aroma compounds opens a promising field.

One group of aroma compounds, which can be recognized in a wide range of raw and processed foods, are derived from pyrazines. These nitrogen containing heterocyclic compounds are potent flavorants and show a broad spectrum of sensory features, ranging from green, nutty, roasted and earthy to fruity and bellpepper-like.

In the presented study, structure-flavor relationships of pyrazine-based flavor molecules are analysed by the use of artificial neural networks, a method which has been applied extensively and successfully in various drug design strategies. For the calculations a set of 96 pyrazine-derived aroma compounds was selected with an aroma quality of either green, bellpepper or nutty, in order to correlate a distinct impression with molecular structures and properties and to enable, therefore, the distinction between these three groups of sensory properties.

The obtained classification model leads to a good agreement between the literature-derived aroma properties of the studied pyrazines and the calculated molecular properties, resulting in a correct classification. It will be proven, that artificial neural networks are versatile tools for the classification of aroma compounds using different molecular descriptors.

P 84

New Esters of Tertiary Alcohols

Barbara Kąkol, Anna Wiśniewska, Maria Bełdowicz, Anna Kulig-Adamiak,
Krzysztof Bajdor, Bożenna Obukowicz, Lidia Lewicka
Industrial Chemistry Research Institute, 8 Rydygiera, 01-793 Warsaw, Poland

Esters of tertiary alcohols are used as raw materials for making drugs and as components of fragrance compositions in the pharmaceutical and in the perfume and cosmetic industries, respectively. They are characterized by interesting intensive odors with a fruity-wooden note. Esters were prepared by Grignard reactions involving direct esterification of bromomagnesium alcoholates with acid anhydrides. Bromomagnesium alcoholates were prepared by the reaction of Grignard compounds with the ketones. The resulting esters were isolated by distillation or column chromatography and identified by ^{1}H NMR and GC-MS.

Ten of them most suitable for the purpose were selected for examination of odour-structure relationship with use of BRUD ODOUR PROFILES.

P 85

Chemical Composition and Antibacterial Activity Related to the Essential Oil of *Piper mollicomum* Kunth

Aluizio A. Marcondes[1], Inês Maria da Costa Brighente[2], Mirele Titton Calderari[2],
Gladys Rosane Thomé Vieira[3], Ricardo Andrade Rebelo[4]
[1]Universidade Federal do Paraná (UFPR), Department of Chemistry, Curitiba/PR, Brazil
[2]Universidade Federal de Santa Catarina (UFSC), Department of Chemistry, Florianópolis/SC, Brazil
[3]Universidade Regional de Blumenau (FURB), Medical Department, Blumenau/SC, Brazil
[4]Universidade Regional de Blumenau (FURB), Department of Chemistry, Blumenau/SC, Brazil
mireletc@hotmail.com

As part of a systematic search for natural products useful as precursors in organic synthesis, the present work describes the chemical composition and antibacterial activity of the essential oil of *Piper mollicomum* Kunth.

Leaves of *P. mollicomum* were collected during the summer season (December 2000) at São Francisco de Assis Reserve in Blumenau, state of Santa Catarina, South Brazil. The collecting place was characterized as being a Modified Ombrophila Dense Forest. The species was identified and authenticated by plant taxonomist, and a voucher specimen was deposited at the Herbarium of the Regional University of Blumenau under the number 3797.

The oil was obtained by classical procedures. Analysis was carried out by GC and GC/MS on HP 5890A/5970 instruments. GC was conducted using a fused silica capillary column of cross-linked methyl silicone (HP-PONA, 50 m x 0.21 mm, 0.50 μm film) and a programmed oven temperature from 80 °C to 250 °C. GC/MS spectrometry, 70 eV, was performed under the same analytical condition as applied to GC. Identification of components was made on matching of MS spectra and, when required, on the basis of their Retention Indices with reference to the following standards: α-pinene, limonene, trans-caryophyllene, E,E-α-farnesene, spathulenol and δ-cadinol [1]. Quantification was based on GC peaks areas, without correcting for response factors. The antibacterial activity was performed against *S. aureus*, *E. coli* and *P. aeruginosa* by agar diffusion method, quantified by measuring the inhibition halo diameter.

Piper mollicomum Kunth afforded by hidrodistillation of the fresh leaf oil in 0.51 % yield. Analysis revealed an oil rich in mono and sesquiterpenes, with eight major constituents: α-pinene (10.7 %), β-pinene (18.1 %), limonene (5.1 %), trans-caryophyllene (10.1 %), α-humulene (6.3 %), β-selinene (6.8 %), biciclogermacrene (4.8 %) and benzyl octanoate (11.6 %). The unindentified compounds accounted for about 5 %. In the antibacterial assays it showed to be a moderate inhibitor of *S. aureus*, inducing a 10 mm inhibition halo.

The identified compounds in the essential oil of *P. mollicomum* accounted for 95 % of its composition. Antibacterial bioassay showed inhibitory activity against *Staphylococcus aureus.*

Keywords: *Piperaceae*; *Piper mollicomum*; *essential oil; antibacterial activity; CG-MS.*

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P 86

Volatile Constituents of *Ehretia* Spp. and Their Virucidal Effects on HSV-1, Influenza Virus and HIV

Mostafa M. Saleh
Department of Drugs and Chemistry of Medicinal Plants National Research Center,
Dokki, Cairo, Egypt

Ehretia Americana, E. ovalifolia and *E. wallichiana* are growing in Egypt, while *E. microphylla* plant was collected from Welvelgem botanical garden, Holland. The volatile constituents of the four species were compared by capillary GC-MS. 139 components were detected. 98 of which were identified representing 92 till 99.0 % of the oil. The occurrence of some new mono and sesqui-terpenes is noteworthy.

The steam distillate prepared from the plants was found to have direct inhibitory activity for HSV-1 without showing cytotoxicity, but not against poliovirus and coxsachievirus. The loss of viral infectivity was related to the duration of drug treatment. Virucidal effects on influenza virus and HIV-1 were also studied.

P 87

Effect of Sage (*Salvia officinalis*) Essential Oil on Bionomy *Tetranychus urticae* Koch

Beata Kawka
Department of Applied Entomology, Warsaw Agricultural University
ul. Nowoursynowska 166, 02-787 Warszawa, Poland

Development of two spotted spider mite (*Tetranychus urticae*) on leaf disks after spraying with sage essential oil solutions at concentrations 1 and 2.5 % was investigated. Studies were carried out on bean cv. Złota Saxa. Water and water with Cremophor® were used as a control. Essential oil of sage did not have influence on the development time of spider mite. The mortality of young stages was however much grater on essential oil treated leaf disks as compared to control.

The fecundity of females feeding on sprayed leaf disks was also tested. It was found that sage essential oil caused a reduction in female fecundity considerably.

The direct toxicity of sage essential oil solutions at concentrations 1 % and 2.5 % to eggs, larvae, nymphs and adult females was tested. Both sage concentrations were toxic to larvae, nymphs and adult females but only 2.5 % solution was toxic to eggs.

Sage essential oil was produced by Etja Ltd. Cremophor® was used as emulgator.

P 88

Chemical Composition and Antimicrobial Activity of the Essential Oil of *Luma chequen*

M. J. Gonçalves, C. Cavaleiro, L. Salgueiro, and A. Proença da Cunha
Laboratório de Farmacognosia / CEF, Faculdade de Farmácia, Universidade de Coimbra,
Rua do Norte, 3004-534 Coimbra, Portugal

Luma chequen (Molina) A. Gray (Myrtaceae) is native tree from Peru growing sparsely on fresh and moistly soils from 2500 to 3200 m high. Leaves and twigs are used by locals for treatment of toothaches, gastrointestinal disorders and post-parturition infections.

In order to contribute for the validation of this traditional remedy, we now report on the composition of the essential oil of *Luma chequen* and its antimicrobial activity against several microorganisms.

The volatile oil, isolated by hydrodistillation from the aerial parts of the plant, was analyzed by GC and GC-MS, using two fused silica capillary columns with different stationary phases (polyethylenoglycol and polymethylsiloxane). Components were identified by comparison GC retention indexes, relative to a series of n-alkanes, and mass spectra with corresponding data of authentic compounds or components from reference oils. The oil is mainly constituted by monoterpenes (90.1 %) being α-pinene (57.1 %), 1,8-cineole (12.1 %) and linalool (5.5 %) the major compounds. Sesquiterpenic compounds attained only 3.1 %.

The microbial growth inhibitory properties of the essential oil were determined using disc diffusion method. Six bacterial strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus faecalis* CECT 795, *Pseudomonas aeruginosa* ATCC 27853 and *Proteus vulgaris* CECT 484; two yeasts, *Candida albicans* CECT 1394 and *Cryptococcus neoformans* CECT 1078; and three filamentous fungi, *Cladosporium cladosporioides* CECT 2111, *Aspergillus niger* CECT 2574 and *Aspergillus fumigatus* CECT 2071, were used to evaluate the essential oil inhibitory capacities.

The results showed high antibacterial and antifungal activity of the essential oil of *Luma chequers* against *E. coli, St. aureus, P. vulgaris, C. albicans, C. neoformans, Cl. cldosporioides* and *A. fumigatus.* Minimal inhibitory concentration was also evaluated.

The antimicrobial activity of this essential oil may justify the popular use of this plant.

P 89

Essential Oils of *Picea glauca* 'Conica' Infested by Spruce Spider Mite *(Oligonychus ununguis* Jacobi, *Acarida: Tetranychidae)*

Ewa Przygoda
Department of Applied Entomology, Warsaw Agricultural University — SGGW
ul. Nowoursynowska 166, 02-787 Warsaw, Poland

Picea glauca 'Conica' (white spruce) is one of the host plants of spruce spider mite *(Oligonychus ununguis* Jacobi). It was observed that mite infestation caused changes in the composition of essential oils of *Picea glauca* 'Conica'. Some terpenes like α-pinene, β-pinene, camphene, β-myrcene or bornyl acetate increased rapidly after mites' feeding.

It is known that these compounds are strongly toxic for some insects feeding on coniferous plants [1, 2]. It is possible therefore, that the increase of them is a defensive reaction of plant.

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P 90

Toxic Components of the Essential Oils

Irena Matławska, Wiesława Bylka
Department of Pharmacognosy,
Karol Marcinkowski University of Medical Sciences in Poznań,
Sieroca 10, 61-771 Poznań, Poland

Essential oils are abundant in plant kingdom. For medical purposes they are used because of their irritative properties — volatile oils cause skin and mucose as well as internal organ congestion.

Some of volatile oils contain harmful ingredients, therefore their usage is limited i.e. toxic effects have been proved for propenylbenzenes. Safrole, which consists 93 % of the ethereal fraction from *Sassafras albidum*, and in the smallest amounts is also present in *Myristica fragrans* is the most extensively investigated compound. It's methoxyl derivative — myristicin is the principal component of the nutmeg oil. Cinnamomum oil mainly contains cinnamaldehyde, and additionally safrole and related propenylbenzenes including anethole, estragole, isoeugenole, and methyleugenole.

Fennel oil and anise oil posses expectorant and spasmolytic properties thank to the presence of anethole. They also contain estragole, which is structurally related to safrole. Therefore, *Ol. Foeniculi* has to be examined for the presence of estragole content (no more than 5 %).

Safrole and its metabolites produce adducts with DNA and are genotoxic. Genotoxic properties have been shown for estragole, methyleugenole and elemicin, whereas myristicin is free of this action. Estragole, methyleugenol and its 1'-hydroxy derivatives, similar to safrole, are hepatocancerogenic for mice, although eugenole, miristicin and elemicin by themselves are not cancerogenic for female mice.

Anethole and its dimmers posses estrogenic activities. *Ol. Myristice*, because of its psychotropic and cancerogenic properties can't be used internally.

The principal propenylbenzenes in the essential oil from *Acorus calamus* and *Asarum* species are reported to be α-asarone, β-asarone and γ-asarone. Additionally safrole, methyleugenole, elemicin and miristicin have been found in some *Asarum* species. Cancerogenic effect of *Ol. Calami* is ascribed to β-asarone, which is present in a high concentration in the Indian tetraploid strains (up to 96 %) and in the smallest amounts in European triploid ones (5 %), whereas North American diploid strains are free of it. Essential oils reach in β-asarone cause a deep depression and hepatotoxic effects. They are also harmful for heart and abdominal organs [1].

Health hazards may result from using volatile oils containing unsaturated compounds, i.e. thujone, which causes central nervous system depression and internal organ damage as well as acts as aborative agent. Even very small dosages of thujone are absorbed and accumulated, therefore a special precaution should be paid to the raw materials containing this compound, i.e. *Salvia officinalis, Artemisia absinthium, Achillea millefolium, Tanacetum vulgare.*

Pulegon a toxic component of *Mentha pulegium* (70–90 %), an adulteration of *Mentha piperita* has also depression properties and may cause liver and kidney damages, abortion and clotting disturbances [2].

Because of the presence of toxic substances, some of the essential oils can't be used in medicine, mainly internally, what is more they have to be examined for the presence of harmful components. The other possibility is to obtain the volatile fraction from the varieties, which are free of toxic ingredients.

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Tolerance of *Pseudomonas aeruginosa* NCTC 6749 to Selected Essential Oil Components

Sean Cox and Julie Markham
Centre for Biostructural and Biomolecular Research, University of Western Sydney,
Hawkesbury Campus, Locked Bag 1797, Penrith South DC 1797, Australia
j.markham@uws.edu.au

As part of our studies into the antimicrobial effects of essential oils and their components we have examined factors that may confer tolerance or resistance to essential oils. In the case of the Gram negative bacterium *Pseudomonas aeruginosa* strain NCTC 6749 (PA6749), low outer membrane permeability was shown to contribute to its tolerance to *Melaleuca alternifolia* (tea tree) oil [1]. Here we present recent findings that compare the actions of different essential oil compounds against this tea tree oil tolerant strain.

All the compounds chosen for the study were inhibitory towards *Escherichia coli* at levels ranging from 0.015–0.125 % v/v. However, only carvacrol (MIC 0.03 %), *trans*-cinnamaldehyde (MIC 0.03 %) and terpinen-4-ol (MIC 0.5 %) inhibited growth of PA6749. α-terpineol, geraniol, linalool, eugenol and citral were unable to inhibit growth at levels as high as 4 % v/v. Eugenol and α-terpineol, but not linalool, geraniol or citral became inhibitory in the presence of 200 μM carbonyl cyanide m-chlorophenylhydrazone (CCCP), a respiratory uncoupler. CCCP has previously been shown to inhibit energy-dependent efflux systems in *Ps. aeruginosa.* Control experiments confirmed that CCCP addition both depolarised the cytoplasmic membrane (CM) of PA6749 and increased its susceptibility to the fluoroquinolone antibiotic norfloxacin, a known PA efflux transporter substrate. This absence of increased susceptibility to geraniol, linalool and citral in the presence of CCCP indicates that active efflux is unlikely to explain the tolerance of PA6749, although it is possible that eugenol and α-terpineol are efflux transporter substrates.

Time-kill assays showed that sub-inhibitory levels (0.1 % v/v) of geraniol, α-terpineol and terpinen-4-ol reduced logarithmic phase PA 6749 cell viability by between 50 to 90 % after 60 minutes. However, after 24 hours viability in treated cultures was comparable to that measured in control flasks. This indicates that the observed resistance of PA6749 is a consequence of re-growth of a tolerant sub-population of cells. The implications of these observations with respect to possible mechanisms that account for the resistance of PA6749 to essential oils will be discussed.

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Algorithm and Computer Program for the Identification of Essential Oils Components

V. Isidorov[1], U. Krajewska[1], M. Jdanova[2] and J. Jaroszyńska[1]
[1]Institute of Chemistry, Białystok University, 15-443 Białystok, Poland
[2]Institute of Chemistry, St. Peterburg University, 198904 St. Petersburg, Russia
isidorov@noc.uwb.edu.pl

The identification of essential oils components is one of the most complex analytical problems. This is due to the presence in these mixtures of a great number of compounds of different classes including isomer and isobar substances. Experience shows that even when such highly effective analytical methods as the combination of high performance capillary gas chromatography and low resolution mass-spectrometry (GC-MS) are used, identification errors are inevitable. Therefore, the search for approaches making possible to increase the reliability of analyses results are still continuing.

This paper deals with a new identification algorithm based on the simultaneous use of retention indices (RI) and partition coefficients (K_p) of mixtures components in a two phase heterogeneous system. The experimental methodology involves the introduction of 20–25 μl of an essential oil and 5–10 μl of a solution of C_7-C_{20} *n*-alkanes into a vial containing 0.5 ml of hexane and 0.5 ml of acetonitrile. After shaking the vial and phase separation, both phases are chromatigraphed separately on a capillary column. According to results of analysis the values of *RI* and $K_p = S_1 / S_2$ are calculated simultaneously.

The identification algorithm includes the calculation of the identification parameter j from the equation $j = a{\cdot}RI - \log K_p$ where a is the coefficient characteristic of a given heterogeneous system (in the case of the C_6H_{14}/CH_3CN system $a = 10^{-3}$). The numerical values of j vary over a narrow range and are, for example, 0.50±0.23 for mono- and sesquiterpenes, 1.10±0.10 for acetates of aliphatic and monoterpene alcohols, 1.35±0.15 for mono- and sesquiterpene carbonyl compounds, 1.55±0.25 for C_{10}-C_{15} terpene alcohols, and 2.0±0.2 for phenols. Hence, in the first identification stage, on the basic of the parameter j the mixture components are placed in a certain homologous series with subsequent identification within this series according to *RI* values.

A computer home-made program was developed for identification. It is supplied with database of K_p and *RI* values for more than 2000 organic compounds on column of SE-30, DB-5 and Carbowax-20M types. Experimental values of *RI* and K_p are interred into the computer by keyboard and the parameter j is calculated automatically. The efficiency of the algorithm and the program is demonstrated taking as an example the analysis of essential oils in pine, fir, and spruce as well as in buds of birch.

The values of *m/z* of the most intensive peak and the mass number of the molecular ion (M^+) in the mass spectra of a given component can be entered by keyboard as additional identification parameters. The use of these supplementary parameters considerably enhances the identification reliability.

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Enantiotaxonomical Aspects in the Species *Rosmarinus*, *Petrosilinum* and *Ocimum*

H. Krüger, F. Marthe
Federal Centre of Breeding Research on Cultivated Plants,
Neuer Weg 22/23, D-06484 Quedlinburg, Germany
h.krueger@bafz.de

In the year 1999, a *Coriandrum* accession was analysed regarding the ratio of (+) and (–) linalool in the essential oils [1]. It was found, that the ratio of both enantiomers in this species is absolutly constant — it was in all cases 87 : 13.

In 2000, accessions of *Rosmarinus officinalis*, *Petrosilinum crispum* and *Ocimum basilikum* were investigated in regard to the ratios of enantiomers of some main components in the essential oils. Here big variations were found. Following results were obtained:

Rosmarinus (80 samples)

	(–) : (+) α-pinene	(–) : (+) limonene	(–) : (+) camphene	(–) : (+) borneol
variation	8 : 92 – 54 : 46	60 : 40 – 83 : 17	54 : 46 – 83 : 17	60 : 40 – 100 : 0

Petrosilinum (19 samples)

	First cut	Second cut
variation of (+) : (–)-α-pinene	40 : 60 – 90 : 10	26 : 74 – 90 : 10
variation of (+) : (–)-limonene	28 : 72 – 43 : 57	26 : 74 – 46 : 54

The extreme examples were produced in both cuts by one and the same plants. Clearly different from all other types is *Petrosilinum crispum* var. neapolitanicum.

Ocimum

More than 280 *Ocimum* samples were analysed. In the most cases only L(–)-linalool was found. Exceptions are all samples of *Ocimum* x *citriodorum* Vis., *Ocimum campechianum* Mill. and one accession of *Ocimum americanum var. americanum* L.

	Ocimum x *citriodorum* Vis	*Ocimum campechianum* Mill.	*Ocimum americanum var. Americanum* L.
variation of (–) : (+)-linalool	83 : 17 – 88 : 12	75 : 25 – 78 : 21	25 : 75

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The Microbiological Oxidation of (–)-*β*-Pinene by Different Fungi

Atta-ur-Rahman[1], M. Iqbal Choudhary[1], Afgan Farooq[1],
Fatih Demirci[2], K. Hüsnü Can Başer[2]
[1]International Centre for Chemical Sciences, H.E.J. Research Institute of Chemistry,
University of Karachi, Karachi 75270, Pakistan
http://www.hej.edu.pk/
[2]Medicinal and Aromatic Plant and Drug Research Centre (TBAM),
Anadolu University, 26470-Eskişehir, Turkey
http://www.tbam.anadolu.edu.tr/

(–)-*β*-pinene (1), a flavour and fragrance monoterpene is an important constituent of essential oils of many aromatic plants. It was oxidised by a plant-pathogenic fungi, *Botrytis cinerea, Aspergillus niger, Fusarium culmorum,* and *Cunningheamella echninulata* to afford four new metabolites characterised as 6*α*-hydroxy-(–)-*β*-pinene (2), 4*β*,5*β*-dihydroxy-(–)-*β*-pinene (3), 2,3-dihydro-2*β*,3*β*-dihydroxy-(–)-*β*-pinane (4), and 4*β*-hydroxy-(–)-*β*-pinene-6-one (5) by detailed physical and spectroscopic studies along with other known metabolites.

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Analysis of Essential Oils Content Obtained after Irradiation and Further Selection of *Melissa officinalis* L. Callus Lines

Nadia Strashniuk
Laboratory of Ecology and Biotechnology, Ternopil State Pedagogical University,
2 M. Kryvonis St. Ternopil, 46027, Ukraine
nadya@ecolab.ternopil.ua

Melisa officinalis and its subspecies use in traditional and non-traditional medicine is caused by the fact that in its shoots and leaves significant amounts of essential oils, ascorbic acid, carotene, resins, tanning and bitter substances, organic acids (caffeic, oleic, ursolic, succinic, chlorogenic, oleanolic), resins and saponins are synthesised [1, 2].

Our previous research projects comprised analysis of essential oils content obtained from 10 *M. officinalis* samples cultivated under different conditions [3]. To increase content of essential oils in the preliminarily obtained callus lines they were exposed to the γ-rays irradiation (1.5 krad) and then subjected to selection.

Quantitative content of essential oils was determined by hydrodistillation and their composition — by gas chromatography.

After exposure to the γ-rays irradiation 8 lines were selected. All of them contained essential oils but their content and composition differed. Content of essential oils in the initial callus cultures of leaf and stem origin was correspondingly 0.18 % and 0.16 % [3]. Irradiation use resulted in the increase of essential oils content in the mentioned 8 lines and constituted 0.25–0.34 % that was even bigger than the essential oils content amount in intact plants (0.18–0.32 %).

According to GLC data citronellal, citral, linalool, neral, caryophyllene were identified as the main components of essential oil.

Linalool content in all analyzed callus cultures was bigger than in intact plants. The greatest concentration of citral was characteristic for one of 8 obtained lines of *M. offisinalis.*

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P 96

Chemotypic Variation Among Mediterranean *Pistacia lentiscus* Growing under Desert Conditions

Oz Barazani[1], Nativ Dudai[2], Avi Golan-Goldhirsh[1]
[1]Desert Plant Biotechnology Laboratory,
The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev,
Sede Boker Campus 84990, Israel
ozbar@bgumail.bgu.ac.il
[2]Aromatic, Medicinal and Spice Crops Unit, Newe Ya'ar Research Center,
Agricultural Research Organization, Ramat Yishay 30095, Israel

Pistacia lentiscus is an evergreen dioecious shrub, widely distributed in the Mediterranean basin. It contains a resin, known as mastic gum, which is found in resin ducts in bark and leaves. It is used for incense, as a chewing gum for pleasant breath, for spicing liqueurs and jams, and in the cosmetic industry. A collection of *P. lentiscus* from Israel, Cyprus, Spain and Tunisia are grown in a germplasm collection at the Jacob Blaustein Institute for Desert Research (BIRD), in the Negev desert, Israel. In this study, the chemical composition of volatiles of 16 accessions from the various collection sites was determined. GC-MS analysis of leaves extracted by tert-butyl methyl ether (MTBE), showed 20 constituents. The major compounds were α-pinene (5.1–49.0 %), sabinene (0.7–45.8 %), limonene (0.7–45.4 %) and germacrene D (3.5–45.9 %). Cluster analysis divided the 16 accessions into 3 main chemical groups according to the relative content of the major compounds: 1) Sabinene — included six Tunisian accessions, two Spanish and one accession from Cyprus; 2) Germacrene D — included two Spanish accessions only; 3) Limonene — included two Spanish accessions, two Israeli and one accession from Cyprus. Generally, the different accessions were clustered according to what appears to be chemotypic groups and not according to their gender or geographic origin.

P 97

Status of Mint Cultivation and Oil Production in India

Thennammai Valliappan
Department of Physicial Sciences, Horticulture College and Research Institute,
Periyakulam, S. India

Of known flavoring materials in the world mint is one of the most popular flavors and ranks probably third most important, after vanilla and citrus. The trade in mint oils and their derivatives is extensive and people in most countries of the world consume some mint flavored products. Although, there are several species and varieties of mint which are cultivated in one or the other parts of the world, five of them one grown in India on commercial scale. These are Japanese mint (*Mentha arvensis*), peppermint (*M. Pipperita*), native or common spearmint (*M. spicata*) bergamotmint (*M. citrata*) and Scotch spearmint (*M. gracillis*). The studies indicated that mint cultivation in all the producing regions is done on soils which are rich in organic matter and contain high nutrient status.

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Essential Oil Composition and Toxicity Test of *Levisticum officinale* Grown in Scotland

K. P. Svoboda[1], C. L. Hogg[1], J. B. Hampson[1] and S. Brocklehurst[2]
[1]Department of Plant Biology, SAC,
Auchincruive Campus, Ayr. KA6 5HW, Scotland, UK
k.svoboda@au.sac.ac.uk
[2]Biomathematics and Statistics Scotland, University of Edinburgh,
James Clerk Maxwell Building, The King's Building, Edinburgh. EH9 3JZ, Scotland, UK

Lovage (*Levisticum officinale* Koch), family Apiaceae, is clump-forming perennial, native to Central Asia and now naturalised throughout Europe. All plant parts are aromatic and were traditionally used in cooking, medicine and cosmetics.

Plant material for distillation came from two sources. On harvesting, samples were divided into stems, leaves, flowers and seed heads. Volatile oil yields obtained were between 0.04–0.2 % (v/w) for stems, 0.1–0.4 % for leaves, 0.4–0.9 % for flowers and 0.7 % for seed heads at early senescence stage. The samples were analysed by GC [1]. Main components were α-terpinyl acetate (8.8–66.6 % of total oil), β-phellandrene (8.3–69.0 %) and α-phellandrene (2.3–24.2 %). Other components between 1 and 6 % were α-pinene, camphene, β-pinene, myrcene, limonene, γ-terpinene, linalol and geranyl acetate. Variability of oil composition is dependant on plant ontogeny and the plant parts distilled [2, 3, 4].

Lovage oil and some of its constituents were tested for their toxicity using the brine shrimp bioassay [5]. Genstat 5 Release 4.1 copyright 1997, Lawes Agricultural Trust, Rothamstead Experimental Station was used for all statistical analyses. The range of concentrations was between 25 ppm to 1000 ppm. The LD_{50} for the whole oil ranged between 121 and 485 ppm, and for the individual components: α-terpinyl acetate (201–323), α-pinene (402–617), β-pinene (475–527), limonene (627–872), ocimene (576–851), and geranyl acetate (95–121).

An anatomical description of the ducts is included, for stem, leaves, flower stalks and developing seeds.

Lovage is a plant species well adapted for cultivation in the Scottish climate. It grows vigorously, gives high biomass yield, can be harvested 2–3 times per season, is not currently susceptible to pests and diseases. Currently, lovage is used as a flavouring in food, confectionery and liqueurs, by medical herbalists in teas and by the cosmetic industry. Lovage oil could be an alternative natural source of alpha-terpinyl acetate, alpha-pinene and alpha and beta-phellandrene, important synthetically-produced constituents used in cosmetics, household products and perfumery [6].

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P 99

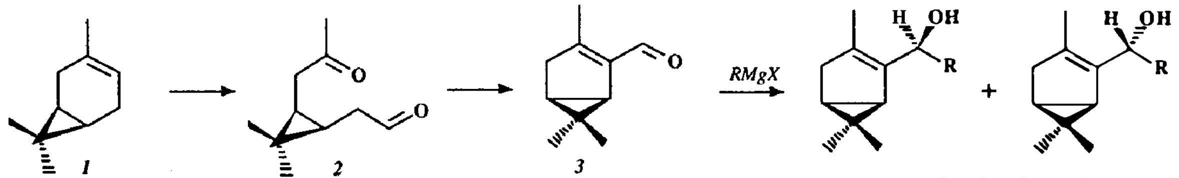
Odor-Structure Relationship of New Chiral Secondary Alcohols with 3,6,6-Trimethylbicyclo[3.1.0]hexane System

Stanisław Lochyński[1], Bożena Frąckowiak[1], Teresa Olejniczak[2]
[1]Institute of Organic Chemistry, Biochemistry and Biotechnology,
Wrocław University of Technology,
Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland
lochynski@kchf.ch.pwr.wroc.pl
[2]Department of Chemistry,
Agricultural University of Wrocław, ul. Norwida 25, 50-375 Wrocław, Poland

Our interest in synthesis of terpenoid compounds from natural sources is connected with a possibility of their application as odoriferous substances for perfumery using easy available component of polish turpentine as a starting material.

Searching for the new synthetic analogues of naturally occurring sensorial components containing bicyclo[3.1.0]hexane system we obtained three new secondary alcohols **4**, **5** and **6** as well as diastereoisomers pairs of 2-substituted-3,6,6-trimethylbicyclo[3.1.0]hex-2-ene derivatives.

The key compound, α,β-unsaturated aldehyde **3** was synthesized in three-step procedure from monoterpene hydrocarbon-(+)-3-carene **1** *via* modified by us intramolecular condensation of ketoaldehyde **2**. Aldehyde **3** was subjected to the Grignard reaction with alkylmagnesium halides giving appropriate unsaturated secondary alcohols **4**, **5** and **6** as mixture of diastereoisomers. Enantiopure *R*- and *S*-isomers were separated by means of chromatography column. All isomers obtained possess interesting olfactory properties. Details of synthesis, odor characteristics and odor-structure relationship are presented.



4a: $R = -CH_2{\cdot}CH{=}CH_2$ *4b*
5a: $R = -CH_2{\cdot}CH_2{\cdot}CH_3$ *5b*
6a: $R = -CH(CH_3)_2$ *6b*

Supported by State Committee for Scientific Research, Grant No 3 T09B 013 14

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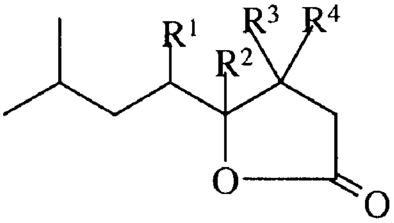
Synthesis and Odour Characteristics of Some Terpenoid γ-Lactones

Maia Szmigiel-Pieczewska, Czesław Wawrzeńczyk
Department of Chemistry,
Agricultural University of Wrocław, ul. Norwida 25, 50-375 Wrocław, Poland
c-waw@ozi.ar.wroc.pl

Terpenoid lactones are components of many essential oils gained from plants [1]. They are also present in many vegetables, fruits and other foods [2]. Our interest in synthesis of this type of lactones is connected with the possibility of their further application as feeding deterrents against insect pests or as flavourings in food production.

Lactones 1a-d were obtained in four step syntheses from isobutylmagnesium bromide and crotonaldehyde or 3-methyl-2-butenal.



1a-d

a, R^1, R^2 = H, R^2 = H, R^4 = Me
b, R^1, R^2 = –C=C–, R^3 = H, R^4 = Me
c, R^1, R^2 = H, R^3, R^4 = Me
d, R^1, R^2 = –C=C–, R^3, R^4 = Me

The key step of the synthesis the iodolactonization of corresponding acids were carried out according to procedure described by Mori [3].

The odour characteristics of compounds obtained will be presented.

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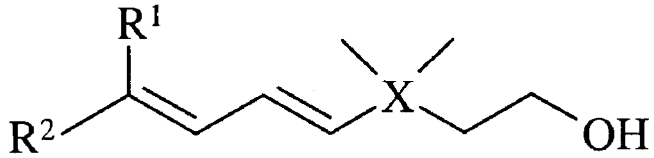
Synthesis and Odour Characteristics of Some Dienoic Alcohols and Their 3-Dimethylsila Analogues

Barbara Gawdzik[1], Józef Góra[2], Czesław Wawrzeńczyk[3]
[1]Institute of Chemistry, Świętokrzyska Academy,
ul. Chęcińska 5, 25-020 Kielce, Poland
bgawdzik@pu.kielce.pl
[2]Institute of General Food Chemistry, Technical University of Łódź,
ul. Stefanowskiego 4/10, 90-924 Łódź, Poland
[3]Department of Chemistry, Agricultural University of Wrocław,
ul. Norwida 25, 50-375 Wrocław, Poland

Continuing our studies on the influence of the presence of silicon in molecule on its odour properties [1] we carried out the comparative odour analysis of pairs (carbon and silicon) dienoic alcohols. The sila analogues (**2a-c**) of some obtained earlier [2] 4,6-dienols (**1a-c**) were prepared in five steps synthesis from propargyl chloride and chlorodimethylsilane.



1a-c, X = C
2a-c, X = Si

a, $R^1, R^1 = -CH_3$
b, $R^1 = -CH_3, R^2 = -CH_2CH_2CH{=}C(CH_3)_2$
c, $R^1, R^2 = -(CH_2)_5-$

The final step of this synthesis, Wittig-Horner reaction of corresponding ketones with 3-(hydroxyethyldimethylsila)-2-propenediphenylfosphine oxide afforded the alcohols **2a-c** in good yields. The sila analogue of sesquiterpenoid alcohol (**2b**) was obtained as (Z/E) mixture.

The odour characteristics of compounds obtained will be presented.

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Composition of the Essential Oil from *Eruca sativa*

Kohsuke Kurose, Takako Maehara and Mitsuo Miyazawa*
Department of Applied Chemistry, Faculty of Science & Engineering,
Kinki University, 3-4-1, Kowakae, Higashiosaka, Osaka, 577-8502, Japan

The components of the essential oil from the leaves, roots and seeds of *Eruca sativa* have been investigated by capillary GC and GC-MS. The essential oil of *Eruca sativa* was found to contain 70 volatile components. The main constituents were 4-methylthiobutylisothiocyanate (Leaf: 60.13 %, Root: 37.04 %, Seed: 26.52 %) and 5-methylthiopentanonitrile (Leaf: 11.25 %, Root: 44.82 %, Seed: 45.46 %). The essential oil of *E. sativa* was characterized by a high content of sulfur and nitrogen-containing compounds.

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P 103

Oxidation of 1,8-Cineole, the Monoterpene Cyclic Ether Originated from *Eucalyptus polybractea*, by Cytochrome P450 3A Enzymes in Rat and Human Liver Microsomes

Mitsuo Miyazawa[1] Masaki Shindo[1] and Tsutomu Shimada[2]
[1]Depertment of Applied Chemistry, Faculty of Science and Engineering,
Kinki University, Kowakae, Higashiosaka, Osaka 577-8502, Japan
[2]Osaka Prefectual Institute of Public Health, Nakamichi 1-chome,
Higashinari-ku, Osaka 537-0025, Japan

1,8-Cineole, the monoterpene cyclic ether and known as eucalyptol, is one of the components in essential oils from *Eucalyptus polybractea*. We investigated the metabolism of 1,8-cineole by liver microsomes of rats and humans and by recombinant cytochrome P450 (P450 or CYP) enzymes in insect cells in which human P450 and NADPH-P450 reductase cDNAs have been introduced. 1,8-Cineole was found to be oxidized at high rates to 2-exo-hydroxy-1,8-cineole by rat and human liver microsomal P450 enzymes.

In rats, pregenolone-16-β-carbonitrile (PCN) and phenobarbital induced the 1,8-cineole 2-hydroxylation activities by liver microsomes. Several lines of evidence suggested that CYP3A4 is a major enzyme involved in the oxidation of 1,8-cineole by human liver microsomes; i) 1,8-cineole hydroxylation activities by liver microsomes were inhibited very significantly by ketoconazole, a CYP3A inhibitor, and anti-CYP3A4 IgG, ii) there was a good correlation between CYP3A4 contents and 1,8-cineole 2-hydroxylation activities in liver microsomes of eighteen human samples, and iii) of various recombinant human P450 enzymes examined, CYP3A4 had the highest activities for 1,8-cineole 2-hydroxylation; the rate catalyzed by CYP3A5 was about one-forth of that catalyzed by CYP3A4.

Kinetic analysis showed that Km and Vmax values for the oxidation of 1,8-cineole by liver microsomes of human sample HL-104 and rats treated with PCN were 50 μM and 91 nmol/min/nmol P450 and 20 μM and 12 nmol/min/nmol P450, respectively.

The rates observed using human liver microsomes and recombinant CYP3A4 were very high among other CYP3A4 substrates reported so far. These results suggest that 1,8-cineole, a monoterpenoid present in nature, is one of the good substrates for CYP3A enzymes in rat and human liver microsomes.

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Plant Volatiles – A Novel Approach to Thrips Control

E. Koschier[1], K. Sedy[1] and J. Novak[2]
[1]Institute for Plant Protection, University of Agricultural Sciences (BOKU) Vienna,
Peter-Jordanstraße, A-1190 Vienna, Austria
[2]Institute for Applied Botany, University of Veterinary Medicine Vienna,
Veterinärplatz 1, A-1210 Vienna, Austria

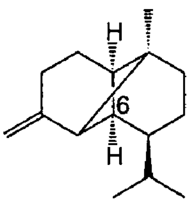
The onion thrips (*Thrips tabaci* Lindeman; Thysanoptera: Thripidae) is a serious pest in field cultures of leek in Austria. Thrips adults and larvae feed on green leaf parts, which become disfigured by white and silvery feeding marks. Essential oils from plants species within the *Lamiaceae* family have previously been demonstrated to be behaviourally active against many insect pests. We selected the essential oils of *Origanum majorana* (L.), *Rosmarinus officinalis* (L.) and *Salvia officinalis* (L.) and their monoterpene constituents terpinen-4-ol and 1,8-cineole for evaluating their antifeedant and oviposition deterrent properties against the onion thrips. In leaf disc bioassays we found marjoram oil at 1 % and 0.1 % concentration to interfere with the feeding activity of the onion thrips and to reduce its reproduction success. Furthermore, rosemary oil (*Rosmarinum officinalis* L.) application at 1 % concentration resulted in decreased feeding damage. Application of the monoterpene 1,8-cineole at both concentrations reduced the oviposition rate by about 30 %. The study of the role of volatile plant allomones in the chemical ecology of *Thrips tabaci* provides a new approach to the development of antifeedants and oviposition deterrents for use in both biological and integrated pest management strategies.

P 105

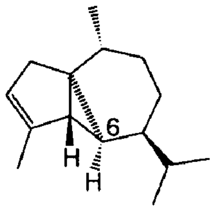
New Sesquiterpene Hydrocarbons from the *Solidago canadensis* Collected in Poland

Adeleke. A. Kasali[1,2], Olusegun Ekundayo[2], Claudia Paul[1] and Wilfried A. Koenig[1]
[1]Unversitaet Hamburg, Institut fuer Organische Chemie,
Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany
[2]Department of Chemistry, University of Ibadan, Oyo State, Nigeria

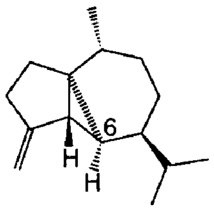
GC-MS of the essential oil prepared by hydrodistillation of the green parts of a specimen of *Solidago canadensis* collected near Katowice, Poland, revealed 2 new sesquiterpene hydrocarbons. Their EI mass spectra resembled the mass spectrum of β-ylangene (**1**) but the retention indices of the new compounds differed markedly from this known compound. After isolation of the new compounds by preparative GC their investigation by one- and two-dimensional NMR techniques resulted in identification of 6-*epi*-α-cubebene (**2**) (minor constituent, 1.5 %) and 6-*epi*-β-cubebene (**3**) (major constituent, 20.5 %).



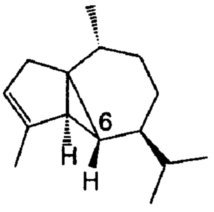
β-ylangene (**1**)



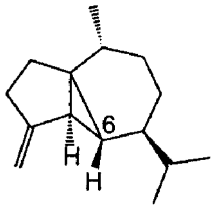
6-*epi*-α-cubebene (**2**)



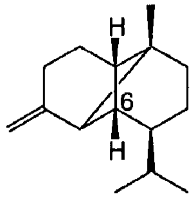
6-*epi*-β-cubebene (**3**)



α-cubebene (**4**)



β-cubebene (**5**)



β-copaene (**6**)

It is quite remarkable that the mass spectra of both new compounds are very similar to that of β-ylangene (**1**) and totally different from the mass spectra of α- and β-cubebene (**4** and **5**) [1]. Apparently β-orientation of the hydrogen in position 6 as in **1**, **2** and **3** results in a common intermediate after primary ionization and identical or very similar secondary fragmentation. The occurrence of **2** and **3** in *S. canadensis* has never before been observed. Interestingly, in addition to β-ylangene (**1**) and β-copaene (**6**), **3** was obtained as a minor product by irradiation of germacrene D at 254 nm [2].

[1] D. Joulain, W. A. Koenig, "*The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*", E. B. - Verlag, Hamburg, 1998.
[2] N. Buelow, W. A. Koenig, *Phytochemistry*, *55*, 141, 2000.

P 106

Essential Oils in First Alcoholic Perfume in History —*Aqua Reginae Hungaricae*

Iwona Konopacka-Brud, Władysław S. Brud
Pollena – Aroma Ltd., ul. Klasyków 10, 03-115 Warsaw, Poland
aroma@pollenaaroma.com.pl
w.s.brud@pollenaaroma.com.pl

„Queen of Hungary Water" – the first alcoholic perfume in history – was created by Polish born Elisabeth Queen of Hungary (1305-1381). She was the daughter of Wladyslaw Lokietek King of Poland from the Piast Royal Family. Known all over Europe she travelled a lot using life, fashions, perfumes cosmetics and men up to her old age.

There are no historical sources, which will specify date of creation of formula of "Queen of Hungary Water". According to legend it was given to Elisabeth by a mysterious monk-hermit, although most probably it was elaborated by a royal alchemist. The first public presentation of "L'eau de Reine d'Hongrie" – as we shall call it today – at a promotion show – took place in 1370 at the royal court of Charles V the Wise (1338-1380) who was known as an admirer of perfumes.

Although historical dates, legends and documents do not clarify details of the creation of "Queen of Hungary Water" it is certain that until the appearance of "Eau de Colonge" in 1727 (1742?), the Queen Elisabeth product was Nr 1 on the list of perfumes and medicaments used through centuries by all courts and rich people all over the world. As for many other products based on herbs and essential oils "Queen of Hungary Water" was and can be today not only a fragrance but also a valuable medicament. The most detailed description of its healing properties is given in "Pharmacopeia Londoniesis" in 1683.

After nearly 700 years Polish perfumers from Pollena-Aroma Ltd recreated the best similar formula of "Queen of Hungary Water". It has all outstanding properties of Queen Elisabeth creation based on our best knowledge of contemporary art of perfumery. The unique mysterious fragrance acts upon ones imagination and health, invigorates (aphrodisiac), adds vigour and heals.

The medieval form of the bottle and the Royal Piast Eagle seal on the hand made box in the form of a royal prayer book (inlaid it can be taken off making an excellent jewellery box) it can be unusual and unique gift. "Queen of Hungary Water" is XIV century Polish participation in the history of perfumery, discovered once again at the beginning of XXI century. It is a limited edition, with each piece individually made and numbered.

P 107

Composition of Chiral Monoterpenes from Bulgarian Origins of the *Achillea millefolium aggr.* and Offsprings from Crossing Experiments

Heidi Steinlesberger, Chlodwig Franz
Institute of Applied Botany, University of Veterinary Medicine Vienna, Veterinärplatz 1,
1210 Vienna, Austria
heidi.steinlesberger@vu-wien.ac.at

The polyploid *Achillea millefolium* complex (Compositae) is distributed all over the northern hemisphere and can be divided in subspecies or varieties according to ploidy and morphological characteristics. The above ground parts of the plant are widely used in folk medicine due to antiinflammatory, antiphlogistic and spasmolytic activity [1, 2]. As the crude drug mainly comes from wild growing plants in South-Eastern Europe, it is necessary to define the drugs morphologically and phytochemically.

Plants were collected from natural habitats in Bulgaria (Golo Burdo, Lozen [3]) and cultivated in the greenhouse. Crossing experiments were made with selected tetraploid Bulgarian and one Austrian origin (4x). The obtained F_1-Generation was cultivated under field conditions, flowers were harvested in full bloom. Enantioselective analysis with gaschromatography was carried out of the extract with CH_2Cl_2.

The progenies of the crossing experiments show first signs of a pattern in the compostion of chiral monoterpenes. According to previous investigations of Orth et. al. [4] the enantiospecific composition of monoterpenes of different yarrow species might serve as an additional chemical marker in Achillea taxonomy.

[1] Hänsel R., Keller K., Rimpler H., Schneider G., Hrsg., Hagers Handbuch der Pharmazeutischen Praxis., *4*, Springer-Verlag, Berlin-Heidelberg-New York-London-Paris-Tokyo-Hong Kong-Barcelona-Budapest, 1992.
[2] Kastner U., Glasl S., Jurenitsch J., „Achillea millefolium - Gallentherapeutikum?“, Z. *Phytotherapie*, *16*, 34-36. 1995.
[3] Saukel J., Rauchensteiner F., unpublished, 1998.
[4] Orth M., Czygan F. - C., Dedkov V. P., „Variation in Essential Oil Composition and Chiral Monoterpenes of Achillea millefolium s.l. from Kaliningrad.“, *J. Essent. Oil Res.*, *11*, 681-687, 1999.

Acknowledgement: Financial support by the „Fonds zur Förderung der wissenschaftlichen Forschung“ (P12322-BIO) is gratefully acknowledged.

P 108

Aroma Academy in Zagreb

Slobodanka Poštić
Aromara d.o.o., Zagreb, Croatia

A poster presents Aroma Academy in Zagreb, the first comprehensive education in aromatherapy in Croatia.

Certified with Ministry of education this course incorporates following components: Anatomy & Physiology, Essential Oil Chemistry, Essential Oil Pharmacy, Botany of Aromatic Plants, Holistic Approach, Massage, Cosmetology, Essential oils & Emotions, Stress Management, First Aid, Business Sense.

It comprises 280 in-class hours and 240 self study hours.

P109

Chemical Variation in the Essential Oil of *Hyptis suaveolens* (L.) Poit. from El Salvador

Paolo Grassi, Chlodwig Franz
Institute of Applied Botany, University of Veterinary Medicine of Vienna,
Veterinärplatz 1, 1210 Vienna, Austria
paolo.grassi@smartvia.at

Hyptis suaveolens (*Lamiaceae*), known in El Salvador as Chichinguaste, is a well known aromatic and medicinal plant of Central America. In El Salvador this plant is used as a wound remedy, as skin disinfectant, carminative and for the treatment of gastrointestinal disorders. Salvadorians also appreciate this plant for baths, especially for hair care. Similar ethnobotanical uses for other countries are known [1].

Recent studies in Brazil present the strong chemical variability of different wild growing populations within this species [2]. Ngassoum et al. point out that terpene composition depends on part of plant material used as well as on the stage of development [3]. Former studies about chemical diversity of *Hyptis suaveolens* were reviewed by Hac et al. [4].

Essential oil of *H. suaveolens* plants collected from 20 different sampling sites of El Salvador gave a oil yield from 0.2 % up to 1.3 % depending on provenance, light exponation, age and parts of material used. Three chemical varieties were obtained concerning the monoterpene composition in correspondence to geographical distribution. Chichinguaste of the southern coastal region contain fenchone and α-fenchyl alcohol as main compounds, whereas plants of the inner northern and western region accumulate 1,8-cineol and sabinene with a higher oil yield. Some populations of the South East contain α-Terpinolen as main constituent. Investigations within the "fenchone-fenchol-type" showed an increasing content of fenchone in aged tissues.

[1] Heinrich M., "Economic botany of American Labiatae", Harley R. M., Reynolds T. (eds), *Advances in Labiate Science*, Royal Botanical Gardens, Kew, 475 - 488, 1992.

[2] Azevedo N. A., Campos I. F. P., Ferreira H. D., Portes T. A., Santos S. C., Seraphin J. C., Paula J. R., Ferri P. H., "Chemical variability in the essential oil of *Hyptis suaveolens*", *Phytochemistry*, ***57***, 733 - 736, 2001.

[3] Ngassoum M. B., Jirovetz L., Buchbauer G., "Essential Oil and Headspace from *Hyptis suaveolens* (L.) Poit. Leaves and Flowers from Cameroon", *Journal of Essential Oil Research*, ***11***, 283 - 288, 1999.

[4] Hac L. V., Khoi T. T., Dung N. X., Mardarowicz M., Leclercq P. A., "New chemotype of *Hyptis suaveolens* (L.) Poit. from the Nghe An Province, Vietnam", *Journal of Essential Oil Research*, ***8***, 315 - 318, 1996.

Acknowledgement: Financial support by the "Österreichische Akademie der Wissenschaften" is gratefully acknowledged

List of Authors

ISBN 83-915199-0-2

32nd International Symposium on Essential Oils

September 9 – 12, 2001

PROGRAMME

LIST OF PARTICIPANTS

Sponsors

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Sigma – Aldrich Poznań, Poland

Avicenna – Oil Wrocław, Poland

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ISEO Records 1969 — 2000

1969	Leiden, Netherlands
1971	Freiburg i. Br., Germany
1972	Helsinki, Finland
1973	Freiburg i. Br, Germany
1974	Freiburg i. Br, Germany
1975	Leiden, Netherlands
1976	Würzburg, Germany
1977	Freiburg i. Br, Germany
1978	Münster, Germany
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1980	Groningen, Netherlands
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Location

The plenary and poster sessions are held at the Institute of Physics, in the Main Building (A-1) of Wrocław University of Technology, Wybrzeże Wyspiańskiego 27.

Registration

The registration desk is located in the lounge of the lecture hall 322 of the Institute of Physics, Main Building (A-1), Wybrzeże Wyspiańskiego 27. It is open on Sunday, September 9 from 14.00 to 19.00. From Monday to Wednesday it is open from 8.30 onwards and it closes on Wednesday, September 12 at 12.00.

Oral Presentations

The speakers are requested to label the slides adequately and hand them (or other materials) over to the operator before the beginning of each session.

Poster Sessions

The poster boards (110 x 110 cm) are located in the passage way on the first flour at the Institute of Physics. They are labelled. The number corresponds to the number listed in the programme. You are kindly requested to attach your poster on Sunday, September 9 or in the Monday morning, September 10. You are pleased to be present at your poster during the indicated poster session for discussion of your results. Please take care that the poster should be removed before the end of ISEO 2001 (it will be around 13.00 on Wednesday, September 12). Affixing materials are available at the registration desk.

Lunch

Lunches are served in academic canteen (about 250 metres from Main Building) from 12.30 to 14.30.

Social Events

Sunday, September 9, 2001

19.00 Get-together party will be arranged in the student club (A-1), Wrocław University of Technology, Wybrzeże Wyspiańskiego 27.

Monday, September 10, 2001

19.00 An informal meeting for ISEO 2001 participants (prices 15 % off) is proposed in Restauracja Złoty Pies (Gold Dog Restaurant), Rynek 41 (Market Square).

Tuesday, September 11, 2001

19.00 Symposium Dinner
Museum of Architecture (ul. Bernardyńska 3, close the Mercure Panorama Hotel)

Wednesday, September 12, 2001

13.45 City guided tour and visit in the Panorama of the Battle of Racławice (finish about 18.00)

Scientific Programme

Sunday, September 9, 2001

14.00 - 19.00		Registration at the Symposium Office
19.00		Get-together party

Monday, September 10, 2001

8.30		Registration at the Symposium Office
9.30		Opening
		Chairman: A. Zabża
10.00	PL 1	Kenji Mori, *Tokyo, Japan* **Terpenoids as Semiochemicals and Bioregulators**
11.00		Coffee
		Chairman: P. Weyerstahl
11.20	L 1	Alexey V. Tkachev, *Novosibirsk, Russia* **From Natural Terpenes Towards Chiral Auxiliary: New Ideas and New Approach**
12.00	L 2	Mugio Nishizawa, Yoshihiro Iwamoto, Hiroko Takao, Hiroshi Imagawa, and Takumichi Sugihara, *Tukushima, Japan* **TiCl$_4$-Induced Rearrangement into the Anti-Markovnikov Cation that Observed in the Sterol Biosynthesis**
12.20	L 3	Carlo Bicchi, Claudio Brunelli, Patrizia Rubiolo, Mario Galli, Albino Sironi, *Torino, Italy* **Conventional Inner Diameter Short Capillary Columns: an Approach to Speeding Up GC Analysis of Medium Complexity Samples**
12.40	L 4	Mercedes G. López, Lourdes Macías-Rodríguez, *Guanajuato, Mexico* **Volatile Polymorphism in Agave Plants by SPME-GC-MS**

13.00 Lunch

Chairman: K. H. C. Baser

14.00 PL 2 Alain Chaintreau, *Geneva, Switzerland*
Quantitative Gas Chromatography - Olfactometry – Review

15.00 L 5 Shamai Giler, Dina Giler, Haim Schloss, Avraham Weinberger, *Tel Aviv, Israel*
The Use of Essential Oils Ointment Combined with Minor Surgical Debridement in the Treatment of Infected Wounds and Ulcers

L 6 Shamai Giler, Dina Giler, Haim Schloss, *Tel Aviv, Israel*
Photo—Aromatherapy — A Novel Combined Treatment Using Low Intensity Non-Coherent Light Source and Essential Oils Ointment for Prompt Healing of Sporadic Infected Facial Lesions

15.30 L 7 Renata Zawirska-Wojtasiak, Przemysław Czarnomski, Erwin Wąsowicz, *Poznań, Poland*
GC-SPME as a Tool for Rapid Estimation of Dill Seed Aroma Compounds and Their Enantiomers

→ 15.50 L 8 Abhijit Talukdar, G. U. Ahmed and S. K. Dutta, *Guwahati, India*
Gas Chromatography — Mass Spectrometric Analysis of the Components of Agarwood (*Aquilaria agallocha* Roxb.) Oil from Assam, India

16.10 Coffee and 1st poster session (posters with odd numbers)

Tuesday, September 11, 2001

Chairman: B. Lawrence

9.00 PL 3 Peter Wolschann, *Vienna, Austria*
Theoretical Methods in Aroma and Fragrance Research

10.00 L 9 Braja D. Mookherjee, Subha M. Patel, Robert W.

		Trenkle and Boaping Jin, *Union Beach, New Jersey, USA* **Aroma of Living Flowers and Fruits™ from Earth to Heaven (MicroGravity)**
10.40	L 10	K. Hüsnü Can Başer, Nese Kirimer, Gülendam Tümen, *Eskisehir, Turkey* **The Essential Oils of *Thymus* species of Turkey: An Update**
11.00		Coffee
		Chairman: K. – H. Kubeczka
11.20	L 11	Joseph Casanova, *Ajaccio, France* **Aromatic Plants from Corsica: Composition and Chemical Variability of Their Essential Oils**
← 11.50	L 12	Y. Ramachandra Rao and Prashant K. Rout, *Bhubaneswar, India* **The Fragrance Components of Flowers of Quisqualis Indica and the Composition of the Essential Oil**
12.10	L 13	Johannes Novak, Henrich Grausgruber, Carla Vender, Leon van Niekerk, Wolfram Junghanns, Chlodwig Franz, *Vienna, Austria* **Stability of Essential Oil Composition**
12.30	L 14	M. M. Barazandeh, *Tehran, Iran* **Essential Oil Composition of the Eight Aroma Plant Species from Iran**
	L 15	M. M. Barazandeh, *Tehran, Iran* **Determination of Gas Chromatographic Retention Indices by Computer Programs**
13.00		Lunch
		Chairman: W. A. König
14.00	PL 4	J. Góra, A. Lis, J. Kula, M. Staniszewska, A. Wołoszyn, *Łódź, Poland* **Chemical Composition Variability of Essential Oils in Ontogenesis of Some Plants**

15.00 L 16 Maria Lis-Balchin, Suzanne Groisse, SandraVollmaier and Stephen Hart, *London, UK*
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15.40 L 18 Karmen Voda, Margareta Vrtačnik, Bojana Boh, Franc Pohleven, *Ljubljana, Slovenija*
Antifungal Properties of Some Oxygenated Aromatic Essential Oil Compounds against White-Rot and Brown-Rot Fungi

16.00 Coffee and 2nd poster session (posters with even numbers)

19.00 Symposium Dinner

Wednesday, September 12, 2001

Assoc. Editor of many journals

full Prof since 1981 *Chairman: G. Buchbauer* [illegible]

9.00 PL 5 Y. Asakawa, *Tokushima, J*
Volatile Components of [illegible] Some Oriental Medicinal Plants and Southern Hemispheric Liverworts

He has studied over 1000 sp of bryophytes and publ over 420 papers

10.00 L 19 Claudia Paul, Wilfried A. König, Chia-Li Wu, *Hamburg, Germany* Institute of Organic Chemistry
New Sesquiterpene Derivatives from the Liverworts *Lepidozia fauriana* and *Lepidozia vitrea*

10.20 L 20 Horng-Shing Shy, Chia-Li Wu, Claudia Paul and Wilfried A. König, *Tamsui, Taiwan* Dep. Chem, Tamkang Univ.
A Linear Dihydrodichromene Derivative from the Liverwort *Metacalypogeia alternifolia*

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Dep. Phytochem./Medic & Aromatic Plants Research Institute

Constituents of the Essential Oil of *Cymbopogon nervatus* Inflorescences from Sudan

11.00 Coffee

G. Buchbauer

Chairman: ~~A. Ch. S. Figueiredo~~

11.20 L 22 Jan C. R. Demyttenaere, An Adams, Jan Vanoverschelde, Norbert De Kimpe, *Ghent, Belgium*
Biotransformation of (S)-(+)-Linalool and Natural Coriander Oil by *Aspergillus niger*

11.40 L 23 Yoshiaki Noma, Toshihiro Hashimoto, Chie Murakami, Mai Furusawa, Yoshinori Asakawa, *Tokushima, Japan*
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12.00 L 24 J. George, K. Udaya Sankar, J. Pereira, N. Keshava, S. Divakar, M. D. Laing, S. E. Drewes and G. A. Ravishankar, *Pietermaritzburg, South Africa*
Supercritical Fluid Extraction of *Decalepis hamiltonii* W. & A. and Novel Insecticidal and Antimicrobial Activity of 2-Hydroxy-4-Methoxybenzaldehyde from the Supercritical Extract

12.20 L 25 F. Sefidkon, S. A. Mirmostafa and F. Askari, *Tehran, Iran*
Essential Oil Content and Composition of Five *Thymus* Species from Iran and Study of Their Anti-Microbial Effects

12.40 L 26 Susana Carolina Nuñez Montoya, Alicia Mariel Agnese, José Luis Cabrera, *Córdoba, Argentina*
The Chemical Composition of *Senecio oreophyton* Essential Oil

13.00 Closing

13.15 Lunch

13.45 City guided tour and visit in the Panorama of the Battle of Racławice (finish about 18.00)

Poster Presentations

On Monday, September 10, posters with odd numbers and on Tuesday, September 11, posters with even numbers are presented.

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Comparative Chemical Composition of the Essential Oil of Common Juniper Berries Produced by Liquid CO_2 Extraction and Hydrodistillation

P 2
S. Shatar, TS. Chimiddulam, L. Amarjargal, SH. Darjima
The Essential Oil Components of some Species of Genus Artemisia Growing in Western Mongolia

P 3
S. Shatar, S. Altantsetseg, L. Odsuren
The Essential Oil of *Bergenia crassifolia* (L.) Fritsch in Mongolia

P 4
S. Altantsetseg, S. Shatar
The Essential Oil Composition of *Ocimum basalicum* L. Depending on Mongolian Climate

P 5
S. Ebrahim Sajjadi and M. Khatamsaz
Essential Oil Composition of *Salvia rhytidea* Benth.

P 6
S. Masoudi, A. Rustaiyan
Comparative Study of the Essential Oils of Five Salvia Species Grown Wild in Iran

P 7
S. Sedaghat, A. Rustaiyan, M. Khosravi
Chemical Composition of Essential Oils from the Umbelliferae Family: *Ferulago angulata* Boiss., *Ducrosia assadi* Alva. and *Lomatopodium khorassanicum* Mozaffarian Species Growing in Iran

P 8
S. Vaverkova, M. Holla, J. Tekel, V. Ostrovska, M. Haban
Study of Qualitative Properties of Some Sorts of the *Echinacea* Moench Genus

P 9
S. Vaverkova, M. Holla, J. Tekel
The Influence of Prometrex 50 WP on the Content and Quality of the Essence of Garden Sage *Salvia officinalis* L.

P 10
A. C. Figueiredo, J. G. Barroso, L. G. Pedro, S. S. Fontinha, M. Sim-Sim, J. J. C. Scheffer
Composition of the Essential Oil from the Liverwort *Asterella africana* (Mont.) Evans Grown on Madeira

P 11 P. A. G. Santos, J. G. Barroso, A. C. Figueiredo, L. G. Pedro, L. R. Salgueiro, S. S. Fontinha, S. G. Deans, J. J. C. Scheffer
Chemical Polymorphism of the Essential Oils from *Thymus caespititius* Brot. Grown on the Islands São Miguel and Terceira (Azores) and on Madeira

P 12 L. G. Pedro, C. S. Duarte, A. C. Figueiredo, J. G. Barroso, J. J. C. Scheffer
Composition of the Essential Oils from Nine Populations of *Crithmum maritimum* L. Grown on Four Azorean Islands

P 13 D. J. Charles
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List of Participants

Prof. **Aasen Arne Jorgen**
University of Oslo
P. O. Box 1068, Blindern
0316 Oslo
Norway
ajaasen@farmasi.unio.no

Dr **Agnese Mariel**
Fac. De Ciencias Quimicas -
Universidad Nacional de Córdoba
Edificio de Ciencias 2 - Ciudad
Universitaria
5000 Córdoba
Argentina
magnese@dqo.fcg.unc.edu.ar

Mr **Akrout Ahmed**
Institut des Régions Arides
4119 Medenine
Tunisia
ahmed.akrout@ira.rnrt.tn

Dr **Aniol Mirosław**
Department of Chemistry,
Agricultural University
Norwida 25
50-375 Wrocław
Poland
aniol@ozi.ar.wroc.pl

Mrs **Antosik Kamila**
Firmenich Sp. z.o.o.
Chrzanowska 10, 05-825 Grodzisk
Mazowiecki
Poland
kamila.antosik.@firmenich.com

Prof. **Asakawa Yoshinori**
Faculty of Pharmaceutical Sciences
Tokushima Bunri University
Yamashiro-cho
770-8514 Tokushima
Japan
asakawa@ph.bunri-u.ac.jp

Mr **Asfaw Nigist**
University of Oslo 1068
N-0316 Oslo
Norway
nigist.asfaw@farmasi.uio.no

Dr **Asghari Gholamreza**
School of Pharmacy,
Isfahan University
of Medical Sciences
Hazarjereb 81745-359
Isfahan
Iran
dr_asghari@yahoo.com

Ms **Ayoub Nahla**
Ain-Shams-University Faculty of
Pharmacy Cairo
Cairo
Egypt
ayoub.n@link.com.eg

Dr **Bal Karol P.**
Firmenich Sp. z.o.o
Chrzanowska 10
05-825 Grodzisk Mazowiecki
Poland
karol.bal@firmenich.com

Dr **Barazani Oz**
The Jacob Blaustein Institute
for Desert Research,
Ben-Gurion University of the Negev
84990 Sede Boker
Israel
ozbar@bgumail.bgu.ac.il

Mr **Barazandeh Mohammad Mahdi**
Medicinal Plants Division
P.O. Box: 13185-116
Tehran
Iran
m_baraz@hotmail.com

Prof. **Barroso José G.**
Faculdade Ciencias
Lisboa Dep. Biologia Vegetal
P. O. Box C2, Piso 1, Campo Grande
1749-016 Lisboa
Portugal
jgbarroso@fc.ul.pt

Ms **Basar Simla**
Institut für Organische Chemie,
Universität Hamburg
Martin-Luther-King-Platz 6
D-20146 Hamburg
Germany
simlabasar@hotmail.com

Prof. **Baser K. Hűsnű Can**
TBAM, Anadolu University
Yunusemre Campus
26470 Eskisehir
Turkey
khcbaser@anadolu.edu.tr

Mrs **Bauermann Ulrike**
IGV Institut für Getreideverarbeitung
GmbH
Arthur-Scheunert-Allee 40/41
14558 Bergholz-Rehbrücke
Germany
u- bauermann@igv-gmbh.de

Prof. **Bełdowicz Maria**
Industrial Chemistry Research
Institute
Rydygiera 8
01-793 Warsaw
Poland
maria.beldowicz@ichp.waw.pl

Dr **Bernotiene Genovaite**
Institute of Chemistry
Gostauto Str. 9
LT-2600 Vilnius
Lithuania
albmal@takas.lt

Mr **Bhgwat Madhav**
Mas Perfumery PVT LTD.
7TH Cross, 3RD Main, Chamarajpet
Bangalore - 560 018
India
masenterprises@vsnl.com

Prof. **Biavati Bruno**
Department of Agro-Environmental
Science and Technology
Filippo Re, 6
40126 Bologna
Italy
biavati@agrsci.unibo.it

Prof. **Bicchi Carlo**
Dipartimento di Scienza e Tecnologia
del Farmaco
Via Petro Giuria 9
I-10125 Torino
Italy
carlo.bicchi@pharm.unito.it

Dr **Binder Gerald**
Heinrich Klenk GmbH & Co, KG
Aschenhof 35
97525 Schwebheim
Germany
binder@chemie.uni-wuerzburg.de

Dr **Biniyaz Tayebeh**
Shahid Beheshti University Evin,
Department of Chemistry,
Tehran
Iran
zohrel1340@hotmail.com

Ms **Bober Katarzyna**
Department of General and
Analytical Chemistry Faculty
of Pharmacy,
Silesian Academy of Medicine
Jagiellońska 4
41-200 Sosnowiec
Poland
chemogan@infomed.slam.katowice.pl

Mr **Bosilcov Alin**
Brüder Unterweger GMBH
Thal-Aue 13
9911 Thal-Assling
Austria
labor.bu-oils@tirol.com

Mr **Boydag Ilhan**
TBAM, Anadolu University
Yunus Emre Campus
26470 Eskisehir
Turkey
iboydag@anadolu.edu.tr

Dr **Brud Władysław, Stanisław**
„Pollena-Aroma" Ltd
Klasyków 10
03-15 Warszawa
Poland
w.s.brud@pollenaaroma.com.pl

Prof. **Buchbauer Gerhard**
Institut für Pharmazeutische Chemie
der Universität Wien
Althanstrasse 14
A-1090 Vienna
Austria
gerhard.buchbauer@univie.ac.at

Dr **Bylka Wiesława**
Department of Pharmacognosy,
Karol Marcinkowski University
of Medicinal Science
Sieroca 10
61-771 Poznań
Poland

Mr **Cadariu Traiam**
Brüder Unterweger GMBH
Thal-Aue 13
9911 Thal-Assling
Austria
labor.bu-oils@tirol.com

Mrs **Calderari Mirele Titton**
FURB
Mal. Deodoro, 1647 - apto 02
80050-010 Curitiba
Brazil
mirele@furb.br

Prof. **Casanova Joseph**
Casanova Paula
Université de Corse
Route des Sanguinaires
20000 Ajaccio
France
casanova@vignola.univ-corse.fr

Dr **Chaintreau Alain**
Firmenich SA
1 Route des Jeunes
CH-1211 Geneve 8
Switzerland
alain.chaintreau@firmenich.com

Dr **Charles Denys**
Frontier Natural Product Co-op
3021 78th Street, P. O. Box 299
52318 Norway, Iowa
USA
denys.charles@frontiercoop.com

Prof. Dr **Cisowski Wojciech**
Department of Pharmacognosy,
Wrocław Medical University
Nankiera 1
50-140 Wrocław
Poland

Mrs **Ćwikowska Beata**
Firmenich Sp. z.o.o
Chrzanowska 10
05-825 Grodzisk Mazowiecki
Poland
beata.cwikowska@firmenich.com

Mrs **Damjanovic Biliana**
University of Montenegro,
Faculty of Metallurgy
and Technology
Centijski put bb
81000 Podgorica
Montenegro,
Yugoslavia
biljanad@cg.ac.yu

Dr **Demirci Betül**
Anadolu University
Faculty Pharmacy
TBAM
26470 Eskisehir
Turkey
bdemirca@anadolu.edu.tr

Dr **Demirci Fatih**
Anadolu University
Faculty Pharmacy
TBAM
26470 Eskisehir
Turkey
fdemirci@anadolu.edu.tr

Dr **Demyttenaere Jan**
Ghent University – Deptartment
of Organic Chemistry,
Faculty of Agricultural and Applied
Biological Sciences
Coupure Links 653
9000 Ghent
Belgium
jan.demyttenaere@rug.ac.be

Mr **Dillenséger Daniel**
ADRIAN (Germany) GmbH
Moorhof 2b
22399 Hamburg
Germany
adrian.de@adrian.fr

Dr **Dorman Damien**
University of Helsinki,
Department of Pharmacy
P. O. Box 56 (Viikinkaari 5E)
FIN-00014 Helsinki
Finland
damien.dorman@helsinki.fi

Dr **Eberhardt Reinhild**
Kellergasse 166
A-2103 Langenzersdorf
Austria
re.eberhardt@kabsi.at

Mr **El-Kamali Hatil**
Omdurman Islamic University
P. O. Box 382 Omdurman
Sudan
mustafa_mmm@hotmail.com

Prof. **Figueiredo Ana Cristina**
Faculdade Ciencias Lisboa
Department Biologia Vegetal
P. O. Box C2, Piso 1, Campo Grande
1749-016 Lisboa
Portugal
acsf@fc.ul.pt

Mrs **Filipowicz Natalia**
Department of Biology
and Pharmaceutical Biology,
Medical University of Gdańsk
al. Hallera 107
80-416 Gdańsk
Poland
nata@biology.pl

Prof. **Franz Chlodwig**
Institut for Applied Botany,
University of Veterinary Medicine
Vienna
Veterinärplatz 1
1210 Vienna
Austria
chlodwig.franz@vu-wien.ac.at

Ms **Frąckowiak Bożena**
Institute of Organic Chemistry
Biochemistry and Biotechnology
Wybrzeże Wyspiańskiego 27
50-370 Wrocław
Poland
frackowiak@kchf.pwr.wroc.pl

Prof. **Gaca Jerzy**
Technical and Agricultural
University,
Department of Chemical Technology
and Engineering
Seminaryjna 3
85-326 Bydgoszcz
Poland
gaca@atr.bydgoszcz.pl

Dr **Gawdzik Barbara**
Institute of Chemistry,
Świętokrzyska Academy
Chęcińska 5
25-020 Kielce
Poland
bgawdzik@pu.kielce.pl

Dr **George Jacob**
Department of Chemistry,
University of Natal Pietermaritzburg
P Bag X01, Scottsville
3209 Pietermaritzburg
South Africa
georgej@nu.ac.za

Dr **Giler Shamai**
Sackler School of Medicine,
Tel Aviv University
26 Dakar St.
Petah Tikva
Israel
sharrjoyce@netscape.net

Prof. **Głowniak Kazimierz**
Department of Pharmakognosy,
Medical University of Lublin
Peowiaków 12
20-007 Lublin
Poland

Mr **Goerig Axel**
Bell Flavors & Fragrances Duft
und Aroma GmbH
Schimmelstrasse 1
D-04205 Leipzig-Miltitz
Germany
a.goerig@bell-europe.com

Prof. **Góra Józef**
Institute of General Food Chemistry,
Technical University of Łódź
Stefanowskiego 4/10
90-924 Łódź
Poland

Mrs **Groiss Susanne**
Pharmazeutische Chemie University
Vienna
Althanstrasse
1090 Vienna
Austria

Dr **Habibi Zohreh**
Abedini Rad/Amir
Shahid Beheshti University Evin,
Department of Chemistry,
Tehran
Iran
zohreh340@hotmail.com

Mr **Hadzifeizovic Nihad**
Department of Pharmaceutical
Biology and Phytochemistry
Hittorfstrasse 56
48149 Münster
Germany
hadzinihad@gmx.de

Mr **Harlalka Ramakant**
Sanjay Harlalka
Nishant Aromas
424, Milan Ind. Estate, off T. J. Road,
Cotton Green
400033 Mumbai
India
nishantaromas@vsnl.com

Prof. **Hiltunen Raimo V. K.**
University of Helsinki,
Department of Pharmacy,
P. O. Box 56 (Viikinkaari 5E)
FIN-00014 Helsinki
Finland
raimo.hiltunen@helsinki.fi

Mr **Hudewenz Volker**
Drom Fragrances International
Oberdiller Str. 18
82065 Baierbrunn
Germany
hud@drom.com

Dr **Jakobsen Henrik**
Vibevadgaard
Blaesenborg Ved 1
4320 Lejre
Denmark
henrik.byrial@get2net.dk

Dr **Jaroszyńska-Turulska Jadwiga**
Institute of Chemistry,
Białystok University
al. J. Piłsudskiego 11/4
15-443 Białystok
Poland
jjarosz@noc.uwb.edu.pl

Mr **Jaimand Kamkar**
Research Institute of Forest
and Ranglands
13185-116 Tehran
Iran
kjaimand@hotmail.com

Dr **Jeleń Henryk**
Institute of Food Technology,
Agricultural University of Poznań
Wojska Polskiego 31
60-624 Poznań
Poland
henrykj@owl.au.poznan.pl

Dr **Joulain Daniel**
Roberted S.A.
B. 52100
06131 Grasse Cedex
France
daniel.joulain@robertet.fr

Prof. **Kafarski Pawel**
Institute of Organic Chemistry
Biochemistry and Biotechnology
Wybrzeże Wyspiańskiego 27
50-370 Wrocław
Poland
kafarski@kchf.ch.pwr.wroc.pl

Prof. **Kalemba Danuta**
Institute of General Food Chemistry,
Technical University of Łódź
Stefanowskiego 4/10
90-924 Łódź
Poland
dakal@snack.p.lodz.pl

Prof. **Kamiński Edward**
Food Technology Institute
of Plant Origin,
University of Agriculture
Wojska Polskiego 31
60-624 Poznań
Poland

Mr **Kasali Adebisi Adeleke**
Organische Chemie,
Universität Hamburg
Martin Luther King-Platz 6
20146 Hamburg
Germany
alekekasali@hotmail.com

Mrs **Kawka Beata**
Department of Applied Entomology,
Warsaw Agricultural University
Nowoursynowska 166
02-787 Warszawa
Poland

Mr **Kaya Durmus Alpaslan**
University of Cukurova
Faculty of Agriculture
Department of Filed Crops
01330 Adana
Turkey
dkaya@mku.edu.tr
dakaya@mail.cu.edu.tr

Mrs **Kąkol Barbara**
Industrial Chemistry Research
Institute
Rydygiera 8
01-793 Warsaw
Poland
barbara.kąkol@ichp.waw.pl

Dr **Khalid Hassan El-Subki**
(Awadalla Amal, Hatil Mohamed)
Medicinal and Aromatic Plants
Research Institute
P. O. Box 2404
Khartoum
Sudan
hsubi@hotmail.com

Prof. **Kirimer Nese**
TBAM, Anadolu University
Yunusemre Campus
26470 Eskisehir
Turkey
nkirimer@anadolu.edu.tr

Ms **Klocker Johanna**
Institute of Theoretical Chemistry
and Molecular Structural Biology
Waehringerstrasse 17/6/601
1090 Vienna
Austria
j.klocker@gmx.at

Dr **Konopacka-Brud Iwona**
„Pollena-Aroma" Ltd
Klasyków 10
03-115 Warszawa
Poland
aroma@pollenaaroma.com.pl

Dr **Korolyuk Elena**
Novosibirsk Institute
of Organic Chemistry
9 Academician Lavrentjev Ave.
630090 Novosibirsk
Russia
koroljuk@nioch.nsc.ru

Ms **Kovatcheva Assia**
Institute of Theoretical Chemistry
and Molecular Structural Biology
Waehringerstrasse 17/6/601
1090 Vienna
Austria
a9308849@unet.univie.ac.at

Ms **Krajewska Urszula**
Institute of Chemistry,
University Białystok
al. J. Piłsudskiego 11/4
15-443 Białystok
Poland
ukrajew@noc.uwb.edu.pl

Mrs **Kraszewska Renata**
Avicenna - Oil
Opolska 11-19
52-010 Wrocław
Poland
avi@avicenna.com.pl
www.avicenna.com.pl

Dr **Krűger Hans**
Krűger Barbel
Bundesanstalt
für Zűchtungsforschung
an Kulturpflanzen
(Federal Centre of Breeding Research
on Cultivated Plants)
Neuer Weg 22/23
D-06484 Quedlinburg
Germany
h.krueger@bafz.de

Prof. **Kubeczka Karl-Heinz**
Kubeczka Christa
Department of Pharm. Biol.
Hamburg University
Untere Steigstr. 12b
D-97276 Masrgetshoechheim
Germany
kubeczka@gmx.de

Prof. **Kőnig Wilfred A.**
Universität Hamburg
Martin-Luther-King-Platz 6
20146 Hamburg
Germany
wkoenig@chemie.uni-hamburg.de

Mr **Kohsuke Kurose**
Department of Applied Chemistry
Faculty of Science & Engineering
Kinki University
3-4-1, Kowakae, Higashiosaka,
Osaka, 577-8502
Japan
miyazawa@apch.kindai.ac.jp

Prof. **Kula Józef**
Institute of General Food Chemistry,
Technical University of Łódź
Stefanowskiego 4/10
90-924 Łódź
Poland
jkula@snack.p.lodz.pl

Ms **Kułdo Joanna**
Department of Chemistry,
Agricultural University
Norwida 25
50-375 Wrocław
Poland
joanna.kuldo@ehobby.pl

Dr **Kurkcuoglu Mine**
Anadolu University
TBAM
Yunus Emre Kampus
Eskisehir
Turkey
mkurkcuoglu@anadolu.edu.tr

Dr **Laleh Ahmad**
Shahid Beheshti University
Department of Chemistry,
Shahid Beheshti University Evin
Tehran
Iran
zohreh340@hotmail.com

Mrs **Latacz Bogna**
Department of Chemistry,
Agricultural University
Norwida 25
50-375 Wrocław
Poland
latacz@poland.com

Dr **Lawrence Brian**
Lawrence Judith
R I Reynolds Tobacco Co.
Journal Essential Oil Research
110 Staffordshire CE
NL 27104 Winston Salem
USA
nitena@rirt.com

Dr **Lechner Marion**
Akras Flavours AG
P. O. Box 104
A-2340 Moedling
Austria
marion.lechner@akras.at

Dr **Lis-Balchin Maria**
South Bank University
Borough Road SE1 OAA
London
UK
lisbalmt@sbu.ac.uk

Dr **Lochyński Stanisław**
Institute of Organic Chemistry
Biochemistry and Biotechnology
Wybrzeże Wyspiańskiego 27
50-370 Wrocław
Poland
lochynski@kchf.ch.pwr.wroc.pl

Dr **Lopes Daise**
EMBRAPA Food Technology
Av. das Américas, 29501
23020-470 Rio de Janeiro
Brazil
dlopes@ctaa.embrapa.br

Dr **Lopez Mercedes**
Centro De Investigation Y Estudios
Avanzados U-Irapuato
KM. 9.6 Libramiento Nte. Carr.
Irapuato-Leon
36500 Irapuato, Guanajuato
Mexico
mlopez@ira.cinvestav.mx

Mrs **Manteuffel Elfriede**
Institute of Food Chemistry
Technical University of Berlin
Germany
e.manteuffel@snafu.de

Dr **Masoudi Shiva**
Azad Islamic University,
Central Tehran Branch
19585/936 Tehran
Iran
shmasoudi@yahoo.com

Ms **Markham Julie**
University of Western Sydney,
Hawkesbury Campus
Locked Bag 1797
1797 Penrith South DC
Australia
j.markham@uws.edu.au

Ms **Mączka Wanda**
Department of Chemistry,
Agricultural University of Wrocław
Norwida 25
50-375 Wrocław
Poland
maczka@ozi.ar.wroc.pl

Prof. **Miguel Graca**
FERN, Univ. Algarve
Campus dr Gambelas
8000-117 Faro
Portugal
mgmiguel@ualg.pt

Prof. **Miyazawa Mitsuo**
Department of Applied Chemistry
Faculty of Science & Engineering
Kinki University
3-4-1, Kowakae, Higashiosaka,
Osaka, 577-8502
Japan
miyazawa@apch.kindai.ac.jp

Dr **Moein Mahmood Reza**
Shaheed Beheshti School
of Pharmacy
Vali-asr Ave. 6153
14155 Tehran
Iran
mr_moein@yahoo.com

Dr **Mookherjee Braja**
Mookherjee Lila
International Flavors & Fragrances
(IFF)
1515 Highway 36
07735 Union Beach
New Jersey, USA
braja.mookherjee@iff.com

Prof. **Mori Kenji**
Mori Keiko
Department of Chemistry Science
University of Tokyo
Kagurazaka 1-3, Shinjuku-ku
162-8601 Tokyo
Japan
htakukaw@ch.kagu.sut.ac.jp

Mrs **Nagielska Anna**
Firmenich Sp. z.o.o
Chrzanowska 10
05-825 Grodzisk Mazowiecki
Poland
anna.nagielska@firmenich.com

Mrs **Niesłuchowska Alicja**
Firmenich Sp. z.o.o
Chrzanowska 10
05-825 Grodzisk Mazowiecki
Poland
alicja.niesluchowska@firmenich.com

Mrs **Niestroj Eva**
Givaudan Deutschland
Giselherstr. 11
44319 Dortmund
Germany
eva.niestroj@givaudan.com

Prof. **Nishizawa Mugio**
Faculty of Pharmaceutical Sciences,
Tokushima Bunri University
180 Yamashiro-cho
770-8514 Tokushima
Japan
mugi@ph.bunri-u.ac.jp

Prof. **Noma Yoshiaki**
Tokushima Bunri University
Yamashiro-cho 186
770-8514 Tokushima-shi
Japan
ynoma@tokushima.bunri-u.ac.jp

Dr **Novak Johannes**
Institute for Applied Botany,
University of Veterinary Medicine
Veterinaerplatz 1
A-1210 Vienna
Austria
johannes.novak@vu-wien.ac.at

Dr **Olejniczak Teresa**
Department of Chemistry,
Agricultural University
Norwida 25
50-375 Wrocław
Poland
olej@ozi.ar.wroc.pl

Dr **Omidbaigi Reza**
Department of Horticulture,
College of Agriculture,
Tarbiat Modares University
P. O. Box 16415-381
Tehran
Iran
omidba-r@net1cs.modares.ac.ir

Mr **Özek Temel**
Medicinal and Aromatic Plant
and Drug Research Centre (TBAM)
Anadolu University,
26470 Eskisehir
Turkey
tozek@anadolu.edu.tr

Dr **Pacut Ryszard**
Department of Chemistry,
Agricultural University
Norwida 25
50-375 Wrocław
Poland
pacut@ozi.ar.wroc.pl

Mrs **Patel Subha**
International Flavors & Fragrances
(IFF)
1515 Highway 36
07735 Union Beach
New Jersey, USA
subha.patel@iff.com

Mrs **Paul Claudia**
Universität Hamburg
Martin-Luther-King-Platz 6
20146 Hamburg
Germany
paul@cip.chemie.uni-hamburg.de

Dr **Pawłowicz Paweł**
Department of Chemistry,
Agricultural University
Norwida 25
50-375 Wrocław
Poland
paw-p@ozi.ar.wroc.pl

Prof. **Pedro Luis**
Faculdade Ciencias Lisboa
Dep. Biologia Vegetal
P. O. Box C2, Piso 1, Campo Grande
1749-016 Lisboa
Portugal
luis.pedro@fc.ul.pt

Dr **Peleah Elena**
Peleah Daria
Moldavian State University
Mateevich, 60
MD2012 Kishinev
Moldova
epeleah@mail.ru

Mr **Pisarski Bartłomiej**
Department of Chemistry,
Agricultural University
Norwida 25
50-375 Wrocław
Poland
bartek@ozi.ar.wroc.pl

Dr **Pop Eugenia**
Dr, Ioan Mesota National College,
Brasov, Roman
Tomis 42
2200 Brasov
Romania
plantessoils@hotmail.com

Ms **Poštić Slobodanka**
Aromara d.o.o.
Grada Mainza 6/III
10000 Zagreb
Croatia
aromara@aromara.com

Prof. **Proença da Cunha António**
Lab. Pharmacognosy/CEF,
Faculty of Pharmacy U.C.,
Rua do Norte, 3000 Coimbra
Portugal
pdacunha@ci.uc.pt

Mr **Protzen Klaus-Dieter**
Paul Kaders GmbH
Eschelsweg 27
22767 Hamburg
Germany
paul@kaders.de

Mrs **Przygoda Ewa**
Department of Applied Entomology,
Agricultural University
Nowoursynowska 166,
02-787 Warsaw
Poland
przygoda@alpha.sggw.waw.pl

Dr **Rao Y. Ramachandra**
Regional Research Laboratory
(CSIR)
RRL Post office
751 013 Bhubaneswar
India
yerramillirrao@yahoo.com

Dr **Raynaud Christine**
LCA/CATAR, ENSIACET
118 Route de Narbonne
31077 Toulouse, Cedex 4
France
craynaud@ensct.fr

Mr **Regetzki Sőren**
Hertz-Selck European Frutarome
Corporation
Belle Alliance str. 58
D-20259 Hamburg
Germany
hertz-selck@t-online.de

Mr **Rezaee Mohammad Bagher**
Research Institute of Forest
and Ranglands
P. O. Box 13185-116
Tehran
Iran
mrezaee@mail.rifr-ac.org

Dr **Rogzue Odete**
Faculty of Pharmacy
R. do Norte
3000 Coimbra
Portugal
odete@ci.uc.pt

Prof. **Rubiolo Patrizia**
University of Turin
Via P. Giuria 9
10125 Torino
Italy
rubiolo@pharm.unito.it

Prof. **Sajjadi Jazi Seyed Ebrahim**
School of Pharmacy,
Isfahan University
of Medical Sciences
Hezar Jerib
81745 Isfahan
Iran
sesajjadi@yahoo.com

Prof. **Saleh Mustafa M.**
Mrs El-Hagrasy Inas
DRUG Dept. National
Research Céntre
Tahrir street
11301 Cairo
Egypt

Dr **Schloss Haim**
Sackler School of Medicine,
Tel Aviv University
26 Dakar St.
Petah Tikva
Israel
sharrjoyce@netscape.net

Dr **Schmidt Wolfgang**
Bell Flavors & Fragrances Duft
und Aroma GmbH
Schimmelstrasse 1
D-04205 Leipzig-Miltitz
Germany
a.goerig@bell-europe.com

Mr **Schmidt Peter**
Kurt Kitzing GmbH
Hinterm Alten Schloß 21
86757 Wallerstein
Germany
erich.schmidt@kurtkitzing.de

Mr **Schmidt Erich**
Kurt Kitzing GmbH
Hinterm Alten Schloß 21
86757 Wallerstein
Germany
erich.schmidt@kurtkitzing.de

Prof. **Schulz Hartwig**
BAZ, Institute for Quality Analysis
Neuer Weg 22-23
D-06484 Quedlinburg
Germany
h.schulz@bafz.de

Dr **Schütz Erwin**
Consultant
Drosselweg 2
D-83308 Trostberg
Germany
erwinschuetz@t-online.de

Dr **Sedaghat Soheila**
Azad Islamic University
- North Tehran Branch
15815-3575 Tehran
Iran
soheila@kavosh.net

Dr **Sefidkon Fatemeh**
Research Insitute of Forest
and Rangelands
13185-116 Tehran
Iran
frsef@yahoo.com

Mr **Shah Kamlesh**
Anant Fragrance Private Limited
1-2, Everest Industrial Estate,
Nr Keiwal Weigh Bridge, Rakhial
380023 Ahmedabad
India
anantfpl@ad1.vsnl.net.in

Prof. **Shatar Sanduin**
Luvsandash Odsuren
Institute of Chemistry
and Chimical Technology
of the Mongolian Academy
of Sciences
Ulaan-Baatar 51
211051Ulaan-Baatar
Mongolia
sshatar@yohoo.com

Ms **Slavkovska Violeta**
Institute of Botany,
Faculty of Pharmacy
Vojvode Stepe 450
11000 Belgrade
Yugoslavia
vikisl@eunet.yu

Mrs **Soylu E. Mine**
University of Mustafa Kemal Faculty
of Agriculture Department
of Plant Protection
Alahan Campus
31034 Antakya/HATAY
Turkey
soylu@mku.edu.tr

Mrs **Steinlesberger Heidi**
Institut for Applied Botany,
University of Veterinary Medicine
Vienna
Veterinärplatz 1
1210 Vienna
Austria
heidi.steinlesberger@vu-wien.ac.at

Mr **Steliopoulos Panagiotis**
Institut für Lebensmittelchemie,
Johann Wolfgang Goethe-Universität
Frankfurt
Marie-Curie-Str. 9
60439 Frankfurt
Germany
steliopoulos@em.uni-frankfurt.de

Dr **Storesund Hans Johan**
University of Oslo,
School of Chemistry
P. O. Box 1068 Blindern
0316 Oslo
Norway
h.j.storesund@farmasi.uio.no

Dr **Strashnjuk Nadia**
Ternopil State Pedagogical
University,
Laboratory of Ecology and
Biotechnology
P. O. Box 2, M. Kryvonis str.
46027 Ternopil
Ukraine
nadya@ecolab.ternopil.ua

Dr **Svoboda Katerina**
Sac. Auchincruive Department
of Plant Biology
KA6 5HW, Ayr
Scotland
UK
k.svoboda@au.sac.ac.uk

Mr **Szadura Tomasz**
Urząd Celny we Wrocławiu,
Dział Laboratorium Celne
Skarżyńskiego 36
54-530 Wrocław
Poland
lcpl@airport.wroc.pl

Mr **Szumny Antoni**
Department of Chemistry,
Agricultural University
Norwida 25
50-375 Wrocław
Poland
szumny@ozi.ar.wroc.pl

Mrs **Świąder Krystyna**
Department of Pharmaceutical
Biology and Botany,
Medical Academy
Al. J. Kochanowskiego 10
51-601 Wrocław
Poland

Mrs **Tabanca Nurhayat**
Tabanca Ibrahim
Anadolu University
Faculty Pharmacy
TBAM
26470 Eskisehir
Turkey
nermin@anadolu.edu.tr

Mr **Talukdar Abhijit**
Gauhati University
781014 Guwahati
India
abhijit-angriv@usa.net

Dr **Thennammai Valliappan**
Assoc. Prof. Dept.
of Physical Sciences,
Horticulture College
and Research Institute
Periyakulam
S. India
valliappa78@yahoo.com

Dr **Thomann Ralph**
IGV Institut für Getreideverarbeitung
GmbH
Arthur - Scheunert - Allee 40/41
14558 Bergholz-Rehbrücke
Germany
r_thomann@igv.gmbh.de

Mr **Tigges Wolfgang**
Givaudan Deutschland
Giselherstr. 11
44319 Dortmund
Germany
eva.niestroj@givaudan.com

Prof. **Tkachev Alexey V.**
Head of The Terpenoids Laboratory,
Novosibirsk Institue
of Organic Chemistry
Acad. Lavrentjev Ave. 9
630090 Novosibirsk
Russia
atkachev@nioch.nsc.ru

Prof. **Tűmen Gűlendam**
Balikesir Üniversitesi,
Faculty of Science and Letters
P. O. Box 1068, Blindern
Balikesir
Turkey
gtumen@balikesir.edu.tr

Prof. **Vaverkova Stefani**
Faculty of Pharmacy
Comenius University
Odbojarov 10
832 32 Bratislava
Slovakia
vaverkova@fpharm.uniba.sl

Ms **Voda Karmen**
Department of Chemical Education
and Informatics,
Faculty of Natural Sciences
and Engineering,
University of Ljubljana
Vegova 4, P. O. Box 18/1
1001 Ljubljana
Slovenia
karmen.voda@uni-lj.si

Mrs **Voumaier Sandra**
Pharmazeutische Chemie
University Vienna
Althanstrasse
1090 Vienna
Austria
voumaiersa@hotmail.com

Ms **Wailzer Bettina**
Institute of Theoretical Chemistry
and Molecular Structural Biology
Waehringerstrasse 17/6/601
1090 Vienna
Austria
bettina@tbi.univie.ac.at

Prof. **Wawrzeńczyk Czesław**
Department of Chemistry,
Agricultural University
Norwida 25
50-375 Wrocław
Poland
c-waw@ozi.ar.wroc.pl

Prof. **Wąsowicz Erwin**
Agricultural University of Poznań
Wojska Polskiego 31
60-624 Poznań
Poland
erwinwas@owl.au.poznan.pl

Mr **Weber Jőrg**
University of Applied Science
Carl-Zeiss-Str. 2
07745 Jena
Germany
joerg.weber@fh-jena.de

Prof. **Weyerstahl Peter**
Technische Universität Berlin
Str. des 17. Juni 135
10623 Berlin
Germany
wey.p@chem.tu-berlin.de

Prof. **Wolschann Karl Peter**
Institute of Theoretical Chemistry
and Molecular Biology University
of Vienna
Waehringerstrasse 17
A-1090 Vienna
Austria
karl.peter.wolschann@univie.ac.at

Prof. **Wu Chia-Li**
Department of Chemistry,
Tamkang University
251 Tamsui
Taiwan
clwu@mail.tku.edu.tw

Prof. **Zabża Andrzej**
Institute of Organic Chemistry
Biochemistry and Biotechnology
Wybrzeże Wyspiańskiego 27
50-375 Wrocław
Poland
zabza@kchf.ch.pwr.wroc.pl

Dr **Zawirska-Wojtasiak Renata**
Agricultural University of Poznań
Wojska Polskiego 31
60-624 Poznań
Poland
renazaw@owl.au.poznan.pl

Mr **Zola Ange**
ADRIAN S.A.
385, Avenue A. Fresnel, La Duranne
13591 Aix en Provence
France

Prof. **Davenne Dominique**
Faculty of Sciences Le Havre
5 impasse de la poissonnerie
06130 Grasse
France
labo.davenne@wanadoo.fr

Mr **Liddle Peter**
Bacardi-Martini
B.P. 50
93401 Saint-Ouen
France
peliddle@bacardi.com

Mr **Milovanov Eugene**
Mr **Ivashchenko Andrey**
"Ukragrofin" LTD
Kreshchatik 7/9
01001 Kiev
Ukraine
andiva@i.com.ua

Dr **Nendel Maja**
Frey & Lau GmbH
Immenhacken 12
24558 Henstedt-Ulzburg
Germany

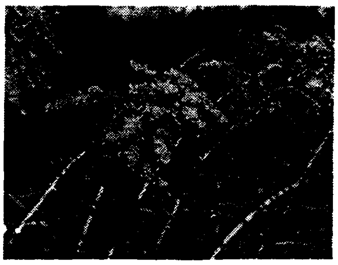
Mrs. **Maia Szmigiel-Pieczewska**
Department of Chemistry,
Agricultural University
Norwida 25
50-375 Wrocław
Poland

Mr **Wenzl Hans-Juergen**
Firmenich
Floridsdorfer Hauptstrasse 34
A-1210 Vienna
Austria
hans.juergen.wenzl@firmenich.com

New Sesquiterpene Hydrocarbons from *Solidago canadensis* collected in Poland

Adeleke A. Kasali[1,2], Olusegun Ekundayo[2], Claudia Paul[1] and Wilfried A. König[1]

[1] *Unversität Hamburg, Institut für Organische Chemie, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany*
[2] *Department of Chemistry, University of Ibadan, Oyo State, Nigeria*



1. Introduction .

The essential oils of several *Solidago* species have been investigated before (Kalemba et al., 2001; Bülow and König, 2000; Weyerstahl et al., 1993; Niwa et al., 1980). In most cases germacrene D was detected as the predominant constituent in *Solidago* species and the occurrence of both enantiomers of this sesquiterpene hydrocarbon has found particular interest (Niwa et al., 1980; Schmidt et al, 1998, Bülow and König, 2000). In this investigation of the green parts of a specimen of *Solidago canadensis* collected near Katowice, Poland, also (-)-germacrene D was identified as the main constituent, however, another abundant sesquiterpene hydrocarbon with a mass spectrum with m/z 120 as base peak very similar to β-ylangene (1) (Joulain and König, 1998), but with a different retention index, was detected by GC-MS of the essential oil.

In addition, another minor component, also with a mass spectrum very close to β-ylangene but again with a different retention time was detected in this sample. After isolation of the new compounds by preparative GC their investigation by one- and two-dimensional NMR techniques resulted in the identification of the minor component as 6-*epi*-α-cubebene (2) and the major component as 6-*epi*-β-cubebene (3).

2. Results and Discussion

The analysis of the essential oil of *Solidago canadensis* by GC and GC-MS (see **Fig. 1**) allowed the identification of (relative concentrations given in parentheses) *trans*-2-hexenol (0.3 %), α-pinene (2.9 %), camphene (0.4 %), β-pinene (0.5 %), myrcene (5.1 %), limonene (2.7 %), bornyl acetate (1.8 %), α-copaene (1.2 %), β-elemene (1.4 %), β-caryophyllene (1.5 %), α-humulene (0.3 %), germacrene D (23.8 %), β-selinene (0.5 %), bicyclogermacrene (5.0 %), δ-amorphene (0.9 %), γ-cadinene (4.1 %), δ-cadinene (0.4 %), α-cadinene (0.6 %), gemacrene B (6.3 %), 6-*epi*-cubenol (2.9 %) by camparison with a spectral library established under identical experimental conditions (Joulain and König, 1998).

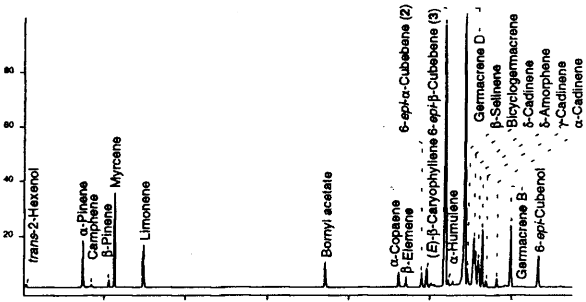


Fig. 1: Chromatogramm of the essential oil from Solidago canadensis (CpSil 5, 25 m, 250 µm i.D., 80 °C / 2 min, 10 °C/min, 270 °C).

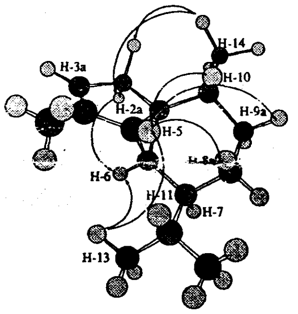


Fig. 2: Important NOE couplings observed for 6-*epi*-α-cubebene (2).

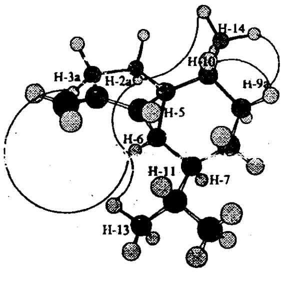
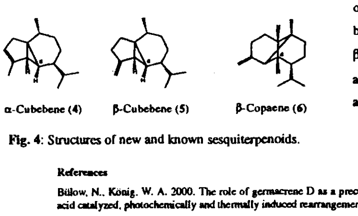


Fig. 3: Important NOE couplings observed for 6-*epi*-β-cubebene (3).

However, two compounds 2 (1.5 %) and 3 (20.5 %) could not be identified by their mass spectra and retention times and were isolated for NMR investigation.

Their two-dimensional NMR correlations are given in **Table 1** and **Table 2**. The interpretation of detected dipolar couplings (NOE) is depicted in **Fig. 2** and **Fig. 3**.



β-Ylangene (1) 6-*epi*-α-Cubebene (2) 6-*epi*-β-Cubebene (3)

α-Cubebene (4) β-Cubebene (5) β-Copaene (6)

Fig. 4: Structures of new and known sesquiterpenoids.

It is quite remarkable that the mass spectra (see **Fig. 5**) of both new compounds are practically undistinguishable from that of β-ylangene (1) and totally different from the mass spectra of α- and β-cubebene (4 and 5) (Joulain and König, 1998). This again shows that one should be very cautious in identifying unknown compounds only by mass spectral library search. Apparently β-orientation of the hydrogen in position 6 in 1, 2 and 3 results in a common intermediate after primary ionization and identical or very similar secondary fragmentation in the mass spectrometer. The occurrence of 2 and 3 in *S. canadensis* has never before been reported. Interestingly, in addition to β-ylangene (1) and β-copaene (6), 3 was obtained as a minor product by irradiation of germacrene D at 254 nm (Bülow and König, 2000).

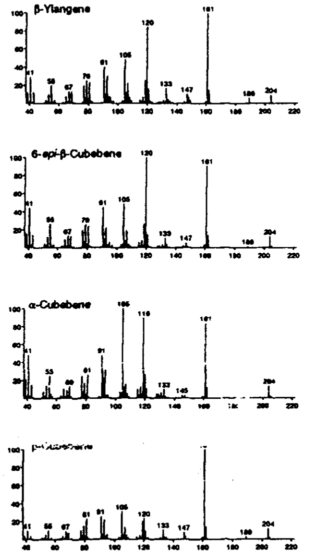


Fig. 5: Mass spectra of β-ylangene, 6-*epi*-β-cubebene, α-cubebene, and β-cubebene

Proton and / or Carbon	$^1H^1H$-COSY-Couplings	HMBC-Couplings
C-1 / C-9	-	H-2b, H-3, H-6, H-9b, H-10, H-14
H-2a / C-2	H-2b, H-5, H-15	H-3, H-6
H-2b	H-2a, H-3, H-15	-
H-3 / C-3	H-2a, H-2b, H-15	H-2b, H-5, H-15
C-4	-	H-2b, H-6, H-15
H-5 / C-5	H-2a, H-6	H-3, H-15
H-6 / C-6	H-5, H-7/H-8b/H-11	H-7/H-8b/H-11, H-8a
H-7/H-8b/H-11	H-6, H-12, H-13	-
C-7	-	H-5, H-7/H-8b/H-11, H-8a, H-9b, H-12, H-13
H-8a / C-8	H-9a, H-9b	H-6, H-7/H-8b/H-11, H-9b
H-9a	H-8a, H-9b, H-10, H-14	-
H-9b	H-8a, H-9a	-
H-10 / C-10	H-9a, H-9b, H-14	H-5, H-9b
C-11	-	H-7/H-8b/H-11, H-8a, H-12, H-13
H-12 / C-12/C-13	H-7/H-8b/H-11	H-7/H-8b/H-11, H-12, H-13,
H-13	H-7/H-8b/H-11	-
H-14 / C-14	H-9a, H-10	H-9b, H-10
H-15 / C-15	H-2a, H-2b, H-3	-

Table 1: Two dimensional correlations observed for 6-*epi*-α-cubebene (2)

Proton and / or Carbon	$^1H^1H$-COSY-Couplings	HMBC-Couplings
C-1	-	H-2a, H-2b/H-10, H-3b, H-5/H-9b, H-9a, H-12/H-14
H-2a / C-2/C-10	H-2b, H-3a, H-3b	H-2a, H-2b/H-10, H-3a, H-3b, H-5/H-9b, H-6, H-7/H-8b/H-11, H-8a, H-9a, H-12/H-14
H-2b/H-10	H-2a, H-3a, H-3b, H-5/H-9b, H-6, H-9a, H-12/H-14	-
H-3a / C-3	H-2a, H-3a, H-2b/H-10, H-5/H-9b	H-2a, H-2b/H-10, H-5/H-9b, H-15a, H-15b
H-3b	H-2a, H-3a, H-2b/H-10, H-5/H-9b	-
C-4	-	H-2a, H-3a, H-3b, H-5/H-9b, H-6
H-5/H-9b / C-5	H-2b/H-10, H-3a, H-3b, H-6, H-8a, H-9a,	H-2a, H-2b/H-10, H-3b, H-6, H-15a, H-15b
H-6 / C-6	H-5/H-9b, H-7/H-8b/H-11	H-2a, H-2b/H-10, H-5/H-9b, H-7/H-8b/H-11, H-8a
H-7/H-8b/H-11	H-6, H-8a, H-9a, H-12/H-14, H-13	-
C-7	-	H-5/H-9b, H-7/H-8b/H-11, H-8a, H-9a, H-12/H-14, H-13
H-8a / C-8	H-5/H-9b, H-7/H-8b/H-11, H-9a	H-2b/H-10, H-5/H-9b, H-6, H-7/H-8b/H-11, H-9a
H-9a / C-9/C-11	H-2b/H-10, H-5/H-9b, H-7/H-8b/H-11, H-8a	H-2b/H-10, H-7/H-8b/H-11, H-8a, H-12/H-14, H-13
H-12/H-14 / C-12/C-13	H-2b/H-10, H-7/H-8b/H-11, H-13	H-7/H-8b/H-11, H-12/H-14, H-13,
H-13	H-7/H-8b/H-11, H-12/H-14	-
C-14	-	H-2b/H-10, H-5/H-9b, H-9a
H-15a / C-15	H-2a, H-3a, H-3b, H-5/H-9b, H-6, H-15b	H-3a, H-3b, H-5/H-9b
H-15b	H-2a, H-3a, H-3b, H-5/H-9b, H-15a	-

Table 2: Two dimensional correlations observed for 6-*epi*-β-cubebene (3)

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Adeleke A. Kasali email address :- alekekasali@hotmail.com

Claudia Paul email address :- paul@chemie.uni-hamburg.de