29TH INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

September 6 – 9, 1998

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Final Program

Abstracts

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L Lectures
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Lectures

SCIENTIFIC PROGRAM

1. Biosynthesis of essential oil compounds
2. Biological activity of essential oil compounds
3. Analytics – chemical aspects
4. Analytics – technical aspects
5. Analytics – structure elucidation of novel compounds
6. Production of essential oils
7. Genetics and physiology

Monday 07th September

08.30 Registration

10.00 Opening Ceremony

10:20 Break

10.50 Plenary Lecture

L 1 - 1 **W. Boland**, *Jena, Germany*
Induced biosynthesis of insect semiochemicals in plants

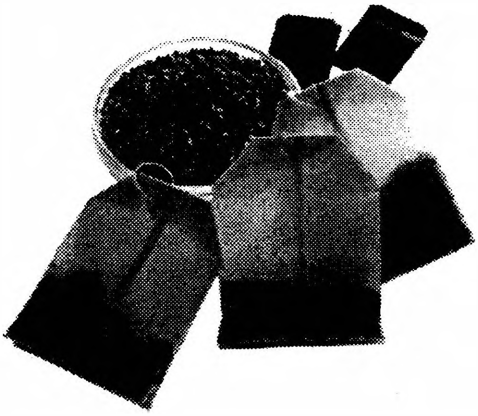
Short Communications

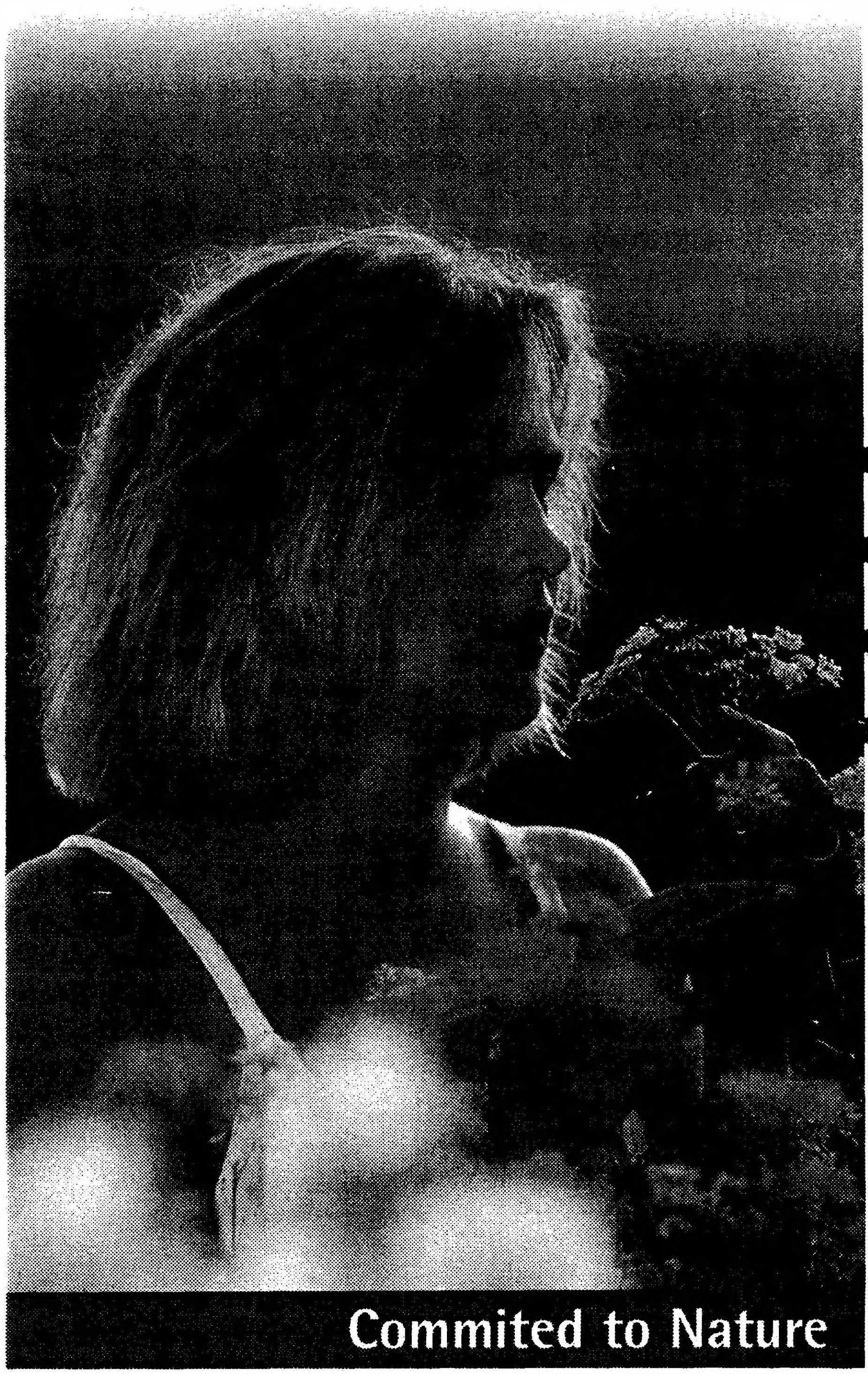
11:20 L 1 – 2 **J. Novak**, *Wien, Austria*
Biosynthesis of sabinene hydrate in *Origanum* sp.

11:40 L 1 – 3 **M. Wüst**, *Frankfurt, Germany*
On the biogenesis of rose oxide in *Pelargonium graveolens* (*Geraniaceae*)

12:00 Lunch

13:00 Poster session

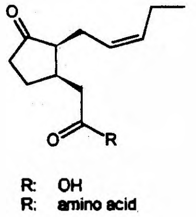



Commited to Nature

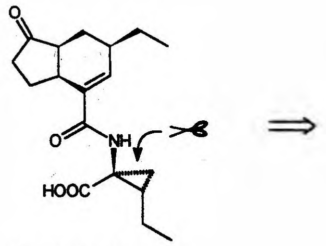
INDUCED BIOSYNTHESIS OF INSECT SEMIOCHEMICALS IN PLANTS

W. Boland, T. Krumm, T. Koch, Max-Planck-Institute for Chemical Ecology, Tatzendpromenade 1 a, D-07745 Jena, Germany.

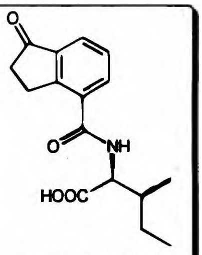
Plants under attack by a herbivore (or a micro-organism) may emit characteristic volatiles that are implicated in the attraction of the natural enemies of the herbivore. The signal cascade from the primary leaf damage to the emission of low molecular weight compounds, or other defenses, is believed to start with the enzyme mediated release of oligosaccharins from the damaged cell walls. Transduction of the signal proceeds via compounds from the octadecanoid pathway and ends with the transcription of genes and the *de novo* synthesis of proteins and their products [1] [2]. Evidence will be given that, besides jasmonic acid also some structurally non-related amino acid conjugates, like, for example, the phytotoxin coronatine **1** or the accordingly designed synthetic analogue indanoyl-isoleucin **2** act as specific signals for volatile induction. Crucially, the component parts of such conjugates are inactive, attributing the biological activity to the conjugate. The biological activity is linked to natural (S)-amino acids like (S)-Ile and (S)-Leu [3] or (S)-Gln in case of the insect-derived conjugate of 17-hydroxylinolenic acid and glutamin. Minor changes in the amino acid moiety may result in different volatile profiles (sesqui- and diterpenoids), attributing a specific role to the amino acid sub structure for the recognition and the selective induction of plant defenses. 1 and 2 are not simple mimics of the structurally related amino acid conjugates of jasmonic acid, but instead represent a new class, or mimics of a new class, of endogenous plant signalling compounds. Administration of d_5-mevalolactone and d_2-deoxy-D-xylulose clearly showed that the biosynthesis of the induced, as well as of constitutively emitted volatiles proceeds predominantly via the novel glycerinaldehyde-3-phosphat/pyruvat pathway. In the case of sesquiterpenoids both, the mevalonate-independend as well as the mevalonate pathway contribute to their biosynthesis. This finding may of importance for the plant defense by adding a greater plasticity to the defense response in case of injection of inhibitors from salivary secretion of herbivores or from enzyme cocktails of attacking pathogens [4].



epi-Jasmonic acid



Coronatin **1**



1-oxo-indanoyl-isoleucine **2**

[1] W. Boland, J. Hopke & J. Donath, J. Nüske & F. Bublitz (1995) Angew. Chem. *107, 715.*
[2] J. Piel, R. Atzorn, R. Gäbler, F. Kühnemann & W. Boland (1997) FEBS Letters, *416,* 143.
[3] T. Krumm, K. Bandemer W. Boland (1995) FEBS Lett. 377, 523.
[4] a) W. Boland, J. Hopke, J. Piel in: Natural Product Analysis, Chromatography, Spectroscopy, Testing (P. Schreier, M. Herderich, H.-U. Humpf W. Schwab, Eds., Viehweg, Braunschweig (1998) *255.*
b) J. Piel & W. Boland, Angew. Chem. (1998), in press.

L 1 - 1

BIOSYNTHESIS OF SABINENE HYDRATE IN *ORIGANUM* SP.

J. Novak[1], Ch. Bitsch[1], J. Langbehn[2], F. Pank[2], M. Skoula[3], P. Gotsiou[3] and Ch. Franz[1], [1] Institute of Applied Botany, University of Veterinary Medicine, Veterinärplatz 1, 1210 Wien, Austria, [2] Federal Center for Breeding Research, Neuer Weg 22/23, D-06484 Quedlinburg, Germany, [3] Mediterranean Agronomic Institute of Chania, Department of Natural Products, P.O.Box 85, GR-73 100 Chania, Crete, Greece

(+)-*cis*-sabinene hydrate and (+)-*trans*-sabinene hydrate are the main monoterpenes found in marjoram *(Origanum majorana)*, but can also be found in other *Origanum* species as well as in e.g. *Melaleuca alternifolia.* The synthesis of sabinene hydrate in marjoram *(Origanum majorana)* is due to sabinene hydrate synthase. Both, (+)-cis- and (+)-trans-sabinene hydrate are produced by the same enzyme in an exact ratio of 10 to 1 [1, 2]. To verify this *in vitro* results *in vivo,* we analysed single plants of 20 different genotypes *of Origanum majorana* and single-plant-descendants of 5 different populations *of Origanum microphyllum* from Crete and calculated the ratios of (+)-cis- to (+)-trans-sabinen hydrates. In *Origanum majorana* a distinct ratio could be found, whereas in *Origanum microphyllum* the ratio did not proof to be constant.

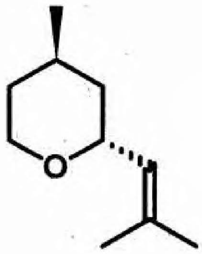
[1] T.W. Hallahan and R. Croteau, Arch Biochem Biophys., **264** (1988) 618-631.
[2] T.W. Hallahan and R. Croteau, Arch Biochem Biophys., **269** (1989) 313-326.

ON THE BIOGENESIS OF ROSE OXIDE IN *PELARGONIUM GRAVEOLENS* (GERANIACEAE)

M. Wüst, A. Mosandl, Institut für Lebensmittelchemie, Biozentrum, Johann Wolfgang Goethe-Universität, Marie-Curie Str. 9, D-60439 Frankfurt, Germany

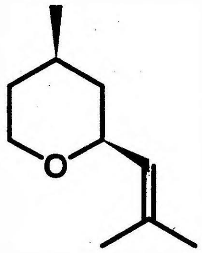
Geranium oil is one of the most important essential oils used in perfumery and aromatherapy. The plants botanically known as the genus *Pelargonium* (family Geraniaceae) are used for essential oil production by steam distillation of leaves and branches. World production of geranium oil reaches about 150 metric tons which illustrates the importance of this natural raw material.

Regioselectively deuterium and ^{18}O labelled precursors of defined stereochemistry are used in order to investigate the biogenesis of the cyclic monoterpenoid ether rose oxide (**1-4**) in the essential oil of *Pelargonium* spp. *In vivo* feeding experiments with subsequent headspace isolation and enantio-MDGC-MS analysis of the extracts allowed to elucidate the biosynthetic pathway including stereochemical and mechanistic aspects [1-4].



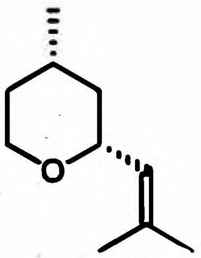
1

(2*R*,4*R*)



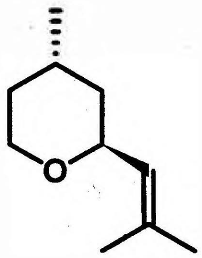
2

(2*S*,4*R*)



3

(2*R*,4*S*)



4

(2*S*,4*S*)

[1] M. Wüst, T. Beck, A. Dietrich and A. Mosandl, Enantiomer **1** (1996) 167-176
[2] M. Wüst, A. Rexroth, T. Beck and A. Mosandl, Chirality **10** (1998) 229-237
[3] M. Wüst, T. Beck and A. Mosandl, J Agric Food Chem, in press
[4] M. Wüst, T. Beck and A. Mosandl, Phytochemistry, submitted

14:00	Plenary Lecture	
	L 1 – 4	**H. Becker**, *Saarbrücken, Germany* In vitro-cultures for the production of terpenoid compounds from liverworts
	Short Communications	
14:30	L 1 - 5	**S. K. Chatterjee**, *Calcutta, India* Productivity increase of some essential-oil crops by application of herbal photo synthetic improvers
14:50	L 2 - 1	**M. Mucciarelli**, *Turin, Italy* The effect of Peppermint (*Mentha piperita*) essential oil and oil components on cucumber root respiration
15:10	Break	

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IN VITRO-CULTURES FOR THE PRODUCTION OF TERPENOID COMPOUNDS FROM LIVERWORTS

H Becker, Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, D-66041 Saarbrücken, Germany

Liverworts are a distinct class of plants within the bryophytes. Morphologically they can be grouped in species with flat thallus vegetation and those with a small Stern bearing leaves without a midrib. Most liverworts contain oilbodies within the cells. These oilbodies are rich in various terpenoid compounds [1,2]. Only a few species occur in relatively large amounts so that they can be collected for chemical investigations. Most species are rare and therefore it is difficult to get appropriate amounts of plant material.

In the latter case in vitro culture of aspetic plants can provide a powerful alternative for biomass production. During the last 12 years we have cultivated some 20 species. The cultured plantlets (best growth rate 2 1/2 days doubling time) contain the same compounds in similar concentrations than field collected plantlets.

A survey will be given about the numerous terpenoids isolated from the cultures [3]. In addition liverwort cultures offer good experimental conditions to study physiological and biosynthetic questions. Recently we investigated the isopentenyl diphosphate biosynthesis in liverworts by feeding [1-^{13}C] labelled glucose [4,5]. We found that the isoprene building blocks of the sesquiterpenes and sterols of *Ricciocarpos natans* and *Conocephalum conicum* are built up via the classical mevalonic acid pathway, whereas the isopentenyl units for monoterpenes and diterpenes are derived from the glyceraldehyde/pyruvate pathway.

[1] Y. Asakawa, Chemical Constituents of Bryophytes in: Progress in the chemistry of Organic Natural Products [W, Herz, G.W. Kirby, R.E. Moore, W. Steglich and Ch. Tamm, eds.] 65 (1995) Springer, Wien, New York.

[2] H. D. Zinsmeister, H. Becker and Th. Eicher, Angew Chem Int Ed Engl, 30 (1991) 130.

[3] H. Becker, J. Haffori Bot Lab 76 (1994) 283.

[4] R. Thiel, KP. Adam, J. Zapp and H. Becker, Pharm Pharmacol Lett 7 (1997) 103.

[5] K. P. Adam, R. Thiel, J. Zapp and H. Becker, Arch Biochem Biophys 354 (1998), in press.

L 1 - 4

①

- leaflets → H_2O ster. → 1% NaOCl (90-120sc) → Nahr medium → H_2O

② spores → ster → open capsule → liquid → bioreactor
agar

- no disturbance of ecology
- " separate from other sp
- any amount needed
- extraction of large amount
- variat of cult med
- grown in fully differentiated form
- haploids

CH_2Cl_2 : MeOH (1:1) → 100% Hexan and 100% ethyl acetate

PRODUCTIVITY INCREASE OF SOME ESSENTIAL-OIL CROPS BY APPLICATION OF HERBAL PHOTO SYNTHETIC IMPROVERS

S. K. Chatterjee, Deputy Chairman, WBPPDCL
lila Co House (2nd floor), M.B.T Sarani, Calcutta - 700 001, India

India, possessing the natural herbarium of essential oil yielding plants, remains in an enviable position in the national and international arena of perfume and flavour industries. Though crops like *Cymbopogon, Mentha, Geranium, Pogostemon* etc. show fairly good responses towards application of inorganic fertilisers; soil environment gets very much disturbed by such entities. The present paper illustrates one very important area of essential oil crops nutrition namely application of photosynthetic improvers of herbal origin to augment crop productivity. Increase of essential oil by enhancing the rate of photosynthesis and inhibiting photorespiration has enormous scope and the present author has been able to extensively work on different important crops like *Cymbopogon winterianus, Cymbopogon flexuosus, Cymbopogon martini, Mentha arvensis, Mentha spicata* and Pan *(Piper betel)*. Foliar application of n-Tria formulation (n-triacontanol, a saturated straight-chain fatty alcohol, isolated from herbal source is 94% pure, has molecular weight 438 and melting point 78 - 82°C with a primary alcoholic functional group) in prescribed dosage has shown to increase essential oil productivity, on average, 15-16% in *Cymbopogon* and *Mentha* spp. and is 18-20% in case of Betel leaf oil. Main constituents of essential oil of these species viz. Citral, Citronellol, Geraniol and phenolic compounds also showed a distinct increase of 8-10%, on average. It was further observed that under medium water stress condition and n-Tria treatment, essential oil content could be increased significantly and in some specific crops like *C. flexuosus* and *Piper betel,* constituents of the oh also could be enhanced. Our findings indicate that commercial production of essential oil with application of herbal photosynthetic improver n-Tria, is a viable proposition keeping the crops totally free from toxicity, free from residual effect on the plants and does not create any atmospheric and soil pollution. Or in other words, ecosystem of the crop remains balanced and crop-environment remains healthy.

[1] S.K. Chatterjee, Acta Horticulturae, 390(1994)19-24
[2] YR. Chadha, Wealth of India Vol. X PID, CSIR(1976) 110-112
[3] M.L. Ghosh, D.Phil (Sc), Burdwan University, India (1979)
[4) M.L. Ghosh and S.k. Chatterjee, Indian Perfumer, 30 (1986) 315
[5] E. Guenther, The Essential Oil, Van Nostrand Inc, NewYork.(i~6O)
[6] S.K. Ries, Science, 2 (1985) 239
[7] G.M. Snedecor, Statistical Methods, The Iowa State College Press, Arnes, Iowa (1953)

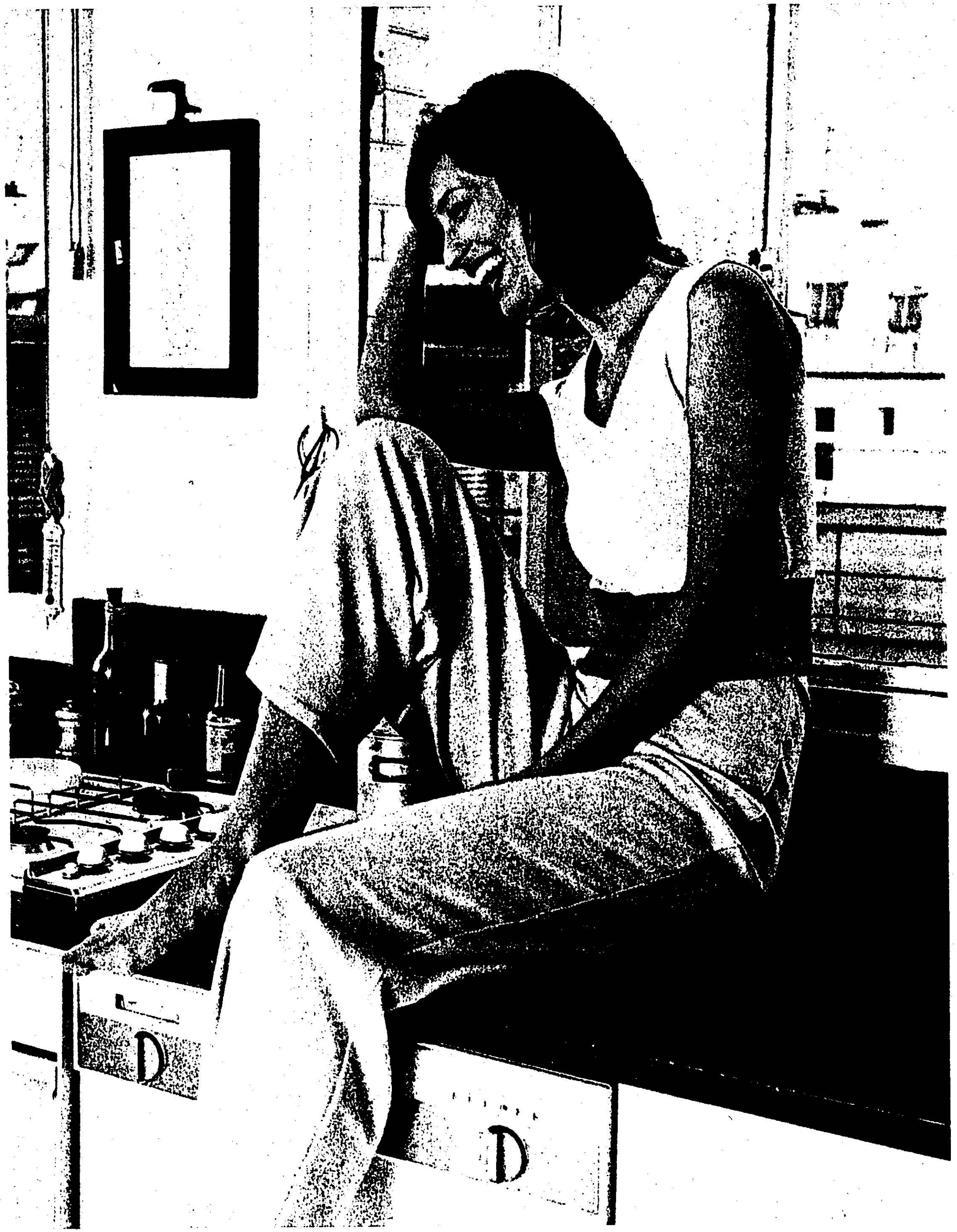
THE EFFECT OF PEPPERMINT (*MENTHA PIPERITA*) ESSENTIAL OIL AND OIL COMPONENTS ON CUCUMBER ROOT RESPIRATION

W. Camusso[1], M. Mucciarelli[2], M. Maffei[1], [1]Dept. Plant Biology, [2]Dept Morphophysiology, University of Turin, Viale Mattioli, 25 I-10125 Turin, ITALY.

Some volatile monoterpenes are potent inhibitors of O_2 uptake and inhibition is localized in that part of the Krebs cycle following succinate. The action of these substances has a dramatic effect on plant growth and developmental processes, since cellular respiration is the basic metabolism for cell life. The effect of essential oil components on growth and development of susceptible species is a branch of allelopathy, the plant-against-plant aspect of the broader field of plant chemical defence. Terpenoids produced by several aromatic plants have been found to exert a clear allelopathic effect on a number of herbaceous plants, including food/feed plants. In order to understand better the mechanism of action of terpenoids in plant-plant interaction we tested the effect of peppermint (*Mentha piperita* L.) essential oils on respiration in root segments of a classical test plant, cucumber (*Cucumis sativus* L.). The choice of peppermint was prompted by the wide application of its essential oil in food, pharmaceutical and cosmetic industries, whereas the use of cucumber was suggested by the ease of cultivation, the quick response and the wide bibliography on this plant. Cucumber (*Cucumis sativus* L. var. Piccolo di Parigi) seeds were washed for 4 hours and then laid out on 9 cm Petri dishes containing 2 paper filters moistured with 5 ml of 5 mM MES (Mes (2-[N'-morpholino]ethansulfonic acid) buffer pH 6 and 0.5 mM $CaSO_4$. Three days after ger-mination root segments were cut, weighted and placed into a 5 ml cuvette of a biological oxygen monitor (YSI-5300). After 20 min of base activity with buffer solution, root segments were tested with increasing concentrations (from 100 to 1600 ppm) of peppermint essential oil and the main oil components (menthol, menthone and pulegone). The effect of peppermint essential oil on cucumber root respiration was a 96% decrease in respiration rate (RR) when used at 900 ppm. In order to understand which of the oil components exerted the maximum effect, menthol (44.99%), menthone (20.78%) and pulegone (0.8%) were studied in the same concentration as they were present in the essential oil. Menthol treatment caused 85% decrease in RR when used at 720 ppm, menthone decreased the 84% of RR when used at 330 ppm, whereas pulegone caused a 50% decrease in the RR when used at 12.8 ppm. These results indicate that menthol is the main responsible of the essential oil decreasing effect on cucumber root RR. However, it has to be considered that when the three oil components are use at the same concentration the inhibiting action on RR follows the order pulegone > menthone > menthol.

Short communications

15:40	L 2 – 2	**M. Mayer**, *Vienna, Austria* Inhalative administration of (+)/(-)-limonene to humans: correlation between blood levels and body mass
16:00	L 2 – 3	**A. H. Gilani**, *Karachi, Pakistan* Cardiovascular actions of thymol: essential oil compound commonly present in plants
16:20	L 2 – 4	**M. Lis-Balchin**, *London, England* Antimicrobial and antioxidant properties of three strains of *Origanum* and comparison with ten antibiotics
16:40	L 2 – 5	**S. Giler**, *Tel Aviv, Israel* Combination of CO_2 laser ablation and essential oil products in the treatment of acute and chronic infected and necrotic wounds and ulcers
17:00	Poster session	



INHALATIVE ADMINISTRATION OF (+)/(-)-LIMONENE TO HUMANS: CORRELATION BETWEEN BLOOD LEVELS AND BODY MASS

M. Mayer[1], W. Jäger[1], K. Kloboucnik[2], K. Sadounig[1], G. Buchbauer[1], [1]Institute of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria, [2]Institute of Nutritional Sciences, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

The monoterpenic hydrocarbons (+)- and (-)-limonene were studied, which are commercially available in high enantiomeric purity. With concentrations from 90 to 95%, (+)-limonene (a liquid with a pleasant, lemon-like odour) is the major constituent of most citrus oils, such as orange and lemon oil. In contrast (-)-limonene, found in the essential oils of some uncommon pine species, exerts a refreshing, light odour. Since limonene is used as a flavoring compound in a number of foodstuffs, the aim of this study was to investigate the pharmacokinetics of (+)- and (-)-limonene in human subjects (average weight 61 ± 11 kg) after inhalative application.

Both fragrance compounds are well absorbed and attain the maximum concentration in blood very fast (c_{max}: 10-15 min). At a dose of 1 mg/kg differences in the plasma concentrations between the two enantiomers could be found with the (+)-limonene levels significantly higher than those for (-)-limonene (c_{max}: 206 ± 18 and 119 ± 14 ng/ml plasma).

Based on this results we further expected lower blood levels of (+)/(-)-limonene within the second experimental group (average weight of 105 ± 13 kg), because of a possible accumulation of limonene in adipose tissues. However, though the dose of both enantiomers was the same, the concentration of (+)- and (-)-limonene in the blood samples was significantly higher (c_{max}: 636 ± 41 and 433 ± 26 ng/ml plasma). These differences between the two groups of subjects should therefore always be considered when using one of these fragrance compounds. Other experimental data will be discussed.

The financial support by the Austrian Science fund (project No. P 11606-MED) is gratefully acknowledged.

CARDIOVASCULAR ACTIONS OF THYMOL: ESSENTIAL OIL COMPOUND COMMONLY PRESENT IN PLANTS

A. H. Gilani, R Shaheen and J W Herzig, Department of Physiology and Pharmacology, The Aga Khan University Medical College, Karachi-74800, Pakistan

Bradycardiac agents are considered useful in different cardiovascular complications. Currently available drugs arc usually accompanied by negative inotropic activity which is considered a harmful effect in certain situation and can sometime lead to cardiac failure. Therefore, a drug with selective bardycardiac effect would be desirable. In this investigation we report selective bradycardiac effect of thymol, a phenolic compound commonly present in plants, such as *Thymus vulgaris, Monarda punctata* and *Carum copticum.* Paired atria from guinea-pigs (400-600 g) were suspended in a 10 ml tissue bath containing Kreb's solution maintained at 37°C and aerated with 5% carbon dioxide in oxygen. Isotonic contractions were measured on a Grass Model 79 Polygraph. The results showed that thymol at the concentration range of 0.1 - 10 μM caused a dose-dependent decrease in spontaneous rate of beating, where as it has no inhibitory effect on the force of atrial contractions up to 100 μM. Pretreatment of tissue with thymol (10 μM) did not block the stimulatory effect of isoprenaline (10 - 100 nM), suggesting that the bradycardiac effect of thymol is independent of β-adrenoceptor blockade. In pentothal-anaesthetised rats, thymol caused a dose-dependent (3-10 mg/kg) fall in arterial blood pressure. These results indicate that the thymol is a highly selective bradycardiac agent with blood pressure lowering activity and the further studies on this compounds is likely to produce highly fruitful results.

ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF THREE STRAINS OF *ORIGANUM* AND COMPARISON WITH TEN ANTIBIOTICS

M. Lis-Balchin[1], *S.G. Deans*[2], *E. Eaglesham*[2] and *M. Barrett*[2] [1] *School of Applied Science, South Bank University, London SE1 0AA;* [2]*Aromatic & Medicinal Plant Group, SAC Auchincruive, Ayr KA6 5HW, Scotland.*

The antibacterial activities against 25 test bacteria and 20 strains of *Listeria monocytogenes* were determined for *Origanum vulgaris* [thymol-rich], *O. syriacum* [thymol-rich] and *O. syriacum* [carvacrol-rich] essential oils. Of the 25 test cultures, *Brevibacterium linens* was most susceptible to all three oils, while the faecal contamination indicator organism *Enterococcus faecalis* was the most resistant. The 20 strains of *Listeria monocytogenes* isolated from a variety of different foodstuffs were strongly inhibited in many cases, with the two thymol-rich oils having a greater inhibitory effect [mean diameter of zone of inhibition was 26 mm] compared with the carvacrol-rich *O. syriacum* oil [mean diamater of zone, 19 mm].

Challenging *Aspergillus niger*, *A. ochraceus* and *Fusarium culmorum* with 1 µL/mL YES broth and 10 µL/mL YES broth of oils resulted in a marked inhibition [presented as % relative to controls] of vegetative growth, especially with the two species of *Aspergillus*.

Organism	*O. vulgaris* [thymol-rich]	*O. syriacum* [thymol-rich]	*O. syriacum* [carvacrol-rich]
Aspergillus niger 1µL/mL	88	90	91
A. niger 10µL/mL	95	92	94
Aspergillus ochraceus 1µL/mL	91	92	93
A. ochraceus 10µL/mL	95	94	95
Fusarium culmorum 1µL/mL	76	76	67
F. culmorum 10µL/mL	78	81	68

The antioxidant properties of the oils were pronounced, as evaluated by the β-carotene/linoleic acid assay. *O. vulgaris* oil, rich in thymol, was the most intense in terms of colour retention, while the carvacrol-rich oil from *O. syriacum* resulted in the widest zone of colour retention.

Finally, the test organisms were exposed to a collection of 10 antibiotics and the zone of growth inhibition measured. Of these, Chloramphenicol, Streptomycin, Tetracycline, Neomycin and Ampicillin were the most effective. A number of bacteria were more inhibited by the *Origanum* oils than the antibiotics.

COMBINATION OF CO_2 LASER ABLATION AND ESSENTIAL OIL PRODUCTS IN THE TREATMENT OF ACUTE AND CHRONIC INFECTED AND NECROTIC WOUNDS AND ULCERS

S. Giler, D. Giler, H. Schloss, Laser Plastic Surgery Clinic, N.A.R.A. Medical Center, Ramat Gan, and *Tel Aviv University, Israel*

Recently, CO_2 laser ablation (resurfacing) techniques have been widely used in plastic and esthetic surgery. This technology enables the surgeon to remove tissues layer by layer with negligible thermal damage, provides excellent depth control, hemostasis and sterilizing effects, thus making this technology useful in the treatment of acute and chronic infected and necrotic wounds and ulcers. This procedure is done by ablating the affected tissues until clean wounds appear. In order to improve the postoperative wound care and enhance the healing process, we used essential oil extracts that provide therapeutic qualities and contain pure and natural extracts, unsaturated fatty acids from plants and vit E. We developed a formula as a cream, ointment or solution. In the last 18 months, 206 patients (age 25-85 yrs) with infected and necrotic acute and chronic wounds and ulcers were treated ambulatory in our Laser Clinic with a combined CO_2 laser ablation procedure and postoperative wound care using these products. Etiology included: posttraumatic, postoperative, postburn, ischemic, diabetic, decubitus, hypertensive, venous and ischemic. For ablation, we used the Kaplan PenduLaser CO_2 115 and OptoScan attachment. In most patients, treatment was done without anesthesia and, in the minority, with xylocaine spray. Following ablation, healthy tissue appeared. Two to 6 laser sessions were done in 2 to 4-day intervals (average was 3). The essential oil ointment (ETHERIN) was applied to the wound and changed twice daily. Essential oil products have antibacterial and antioxidant activity, enhance healing and relieve pain. In most patients, recovery and complete secondary healing appeared within 2 weeks to 2 months. In 12 out of 206 patients, the treatment failed (mostly in those with huge decubitus ulcers and bone involvement) and in 10, a clean granulation tissue appeared. No immediate or late complications were observed. According to our experience, improvement in the healing process is achieved by a combination of CO_2 laser ablation of necrotic tissue followed by application of essential oil products (ETHERIN).

Tuesday, 08th September

09:00 Plenary Lecture

L 3 - 1 **A. Zola**, *Marseille*, *France*
The A.F.N.O.R. (Association Française de Normalisation): some standards for the quality of essential oils

Short Communications

cancelled

09:30 L 3 – 2 **K. Jaimand**, *Tehran, Iran*
The essential oil of *Tanacetum parthenium* (L.) Schultz-Bip.

09:50 L 3 – 3 **M. B. Rezaee**, *Tehran, Iran*
Variation of the Chemical composition of essential oil of *Mentha longifolia* (L.) Hudson var. Chlorodictya from Iran

10:10 Break


25
2
2
2

THE A.F.N.O.R, (ASSOCIATION FRANÇAIS DE NORMALISATION): SOME STANDARDS FOR THE QUALITY OF ESSENTIAL OILS

Ange Zola, Adrian S.A., 15 rue de Cassis, B.P. 10, 13446 Marseille Cantini Cedex, France

There are many ways to produce an essential oil. The quality of the oil varies according to it. It also varies according to countries of origin.

It is therefore very important to set up some standards which will not only ease the international trade of those essential oils but also allow every one to evaluate their quality.

A standard is a written document which gives the specifications (odour, physical and chemical characteristics, GLC ...) of each essential oil.

The AFNOR is a group of 50 French experts from different industries. AFNOR has published about 100 standards: including not only general standards (packing, labelling, sampling) but also methods of analysis and specific standards for various essential oils.

Most of these standards have been accepted by ISO.

Some of them are given in this paper as an example, among them the standard for the essential oil of vetyver.

Huiles Essentielles (Tomes 1-2)
AFNOR
Tour Europe
92049 PARIS
La Defense
Cedex

Price = 250 libras

THE ESSENTIAL OIL OF *TANACETUM PARTHENIUM* (L.) SCHULTZ-BIP.

K. Jaimand and M.B. Rezaee, Phytochemistry group, Department of Medicinal Plants & By-Products, Research Institute of Forests & Rangelands, P.0. Box 13185-116, Tehran, Iran.

The composition of the essential oil of *Tanacetum parthenium (L.)* Schultz-Bip. (Feverfew, Asteraceae) of Iran was investigated using GC and GC-MS. Camphor and chrysanthenyl acetate were the main constituents of the sample originating from north Iran. They yielded a relatively high percentage of oil (0.65%, v/w), calculated on the dry weight. Feverfew is used over long periods of times as a migraine prophylactic agent. The sesquiterpene lactone parthenolide is held responsible for this biological activity. In addition, chrysanthenyl acetate may display an analgesic effect by inhibiting the enzyme prostaglandin synthetase. Based on the results of our investigations and literature data, a number of recommendations arc proposed with respect to the essential oil of T. parthenium.

VARIATION OF THE CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *MENTHA LONGIFOLIA* (L.) HUDSON VAR. *CHLORODICTYA* FROM IRAN

Rezaee, M. B., Jaimand, K. & Barazandeh, M. M., Phytochemistry group, Department of Medicinal plant & By-Products, Research Institute of Forests & Rangelands, P.O. Box 13185-116, Tehran, IRAN.

Abstract

The composition of the essential oil of *Mentha longifolia* (L.) Hudson Var. *Chlorodictya* collected from two localities from north Iran were isolated by steam distillation using a KAISER & W. LANG apparatus and analysed by GC and GC/MS. GC analyses were carried out using a Shimadzu GC-9A chromatograph provided with flame ionisation detector (FID) and Chromatopac C-R3A as data processor, separation was carried out on two different polarity columns (DB-1 & DB-wax, each one 60 m x 0.25 micron film thick.) composition calculated by area normalisation method neglecting response factors. GC/MS analyses were carried out on a GC Varian 3400, coupled to a Varian Saturn II mass spectrometer, with 70 eV ionisation energy on column DB-1, temp. program mode: same as GC analysis. The major constituents for sample 1 was piperitenone oxid (33.91%), isopiperitenone (11.98%) and piperitenone (8.40%), and for sample 2 was isopiperitenone (57.96%), piperitenone oxid (19,99%) and 1,8-cineole (5.49%).

Chromatograms and tables of compositions (including name of compound, its Kovats retention indices and percentage) will be presented.

Short communications

10:40	L 6 – 1	**J. Danzig**, *Oberhausen, Germany* Essential oil production by steam distillation close to the field
11:00	L 6 – 2	**A. Höner**, *Münster, Germany* Characterisation of essential oils produced by steam destillation close to the field
11:20	L 6 – 3	**P. Salehi**, *Tehran, Iran* Study of drying effect on essential oil of *Juniperus excelsa* leaves and "Berries" (cones)
11:40	L 6 – 4	**K. P. Svoboda**, *Ayr, Scotland* *Dracocephalum moldavica*, a novel aromatic plant for Scotland
12:00	Lunch	
13:00	Poster session	

ESSENTIAL OIL PRODUCTION BY STEAM DISTILLATION CLOSE TO THE FIELD

J. Danzig, M. Feseker, G. Hildebrand, J. Huebner, R. Kuemmel, Fraunhofer-Institute for Environmental, Safety, and Energy Technology UMSICHT, Osterfelder Str. 3, D-46047 Oberhausen, Germany

Essential oils from spice plants like thyme or sage are usually produced by steam distillation. For economic reasons farmers are interested not to sell the whole plants but to produce the oils by themselves close to the field. In a cooperation between Fraunhofer UMSICHT, an institute for applied research, and an agricultural company founded by farmers, corresponding investigations were carried out. The work was financially supported by the Federal State of North Rhine-Westphalia. A steam distillation plant consisting of an external steam generator, two stills for intermittend use with a volume of approximately 1.8 cubic meters, a tube condenser and a Florentine flask were set up at a farm amidst the fields with the spice plants.

In the course of the examinations the production of the essential oils was optimized in cooperation between the farmers and the scientists. According to the literature [1] parameters for optimization were the stage of plant development at the time of harvest, the duration and kind of storage of the harvested plants, filling height in the still, steam flow during distillation, temperature of condensation and total time of distillation.

The analytical examinations were carried out by the Institute for Chemo- and Biosensoric in Muenster/FRG and will be presented elsewhere.

[1] E.F.K. Denny: Field Distillation for Herbaceous Oils. Denny, McKenzie Associates, Lilydale, Tasmania 7268, Australia

CHARACTERISATION OF ESSENTIAL OILS PRODUCED BY STEAM DISTILLATION CLOSE TO THE FIELD

A. Höner, T. Theuerl, Y. Friedrich, W. Kleiböhmer, Institut für Chemo- und Biosensorik, Mendelstr. 7, D-48149 Münster, Germany

Essential oils of thyme, parsley, sage and basil for example were produced using a steam distillation plant close to the field.

The steam distillation process was developed by the Fraunhofer-Institute for Environmental, Safety, and Energy Technology UMSICHT (Oberhausen, Germany) in close co-operation with an agricultural company founded by farmers and is described elsewhere.

The maximum oil content of the herbs was determined using hydrodistillation according to DAB. Yields of hydrodistillation and steam distillation were compared.

The essential oils generated by the steam distillation process close to the field were characterised according to standard procedures and quality data sheets were set up.

The separation and identification of the individual constituents was performed using non-chiral and chiral GC methods.

In addition the essentials oils originating from the steam distillation process were compared to different oils from several suppliers available on the market.

On-going research covers the field of Supercritical Fluid Extraction (SFE) using pure carbon dioxide and Solid Phase Micro Extraction (SPME).

STUDY OF DRYING EFFECT ON ESSENTIAL OIL OF *JUNIPERUS EXCELSA* LEAVES AND "BERRIES" (CONES)

Parvin Salehi, Research Institute of Forests and Rangelands, P.O.BOX 13185-116, Tehran, IRAN

Leaves and berries (cones) samples of juniper (*J. excelsa*) were collected in summer from trees growing wild in Iran. Half of plants were dried under laboratory conditions. Then essential oils were examined by GC-MS in dried samples compared with fresh samples. That consisted of almost 23 constituents. However, yield of essential oils were significantly higher in dried berries than in fresh berries . Yield of essential oils did not change in dried and fresh leaves.

Results showed that drying affect on some of constituents but others not . For instance content of α-pinen was decreased after drying (from 88.7% in fresh berries dropped to 75.8 % in dried berries and from 44% in fresh leaves dropped to 19.8 % in dried leaves). The content of some constituents (e.g. α-elemene) in both of berries and leaves were increased after drying. Drying effect on some of constituent was variable for instance limonene content increased in dried berries but decreased in dried leaves. *Trans*-verbenol content was reversed.

DRACOCEPHALUM MOLDAVICA, A NOVEL, AROMATIC PLANT FOR SCOTLAND

K.P. Svoboda[1], J.B. Hampson[1], L. Wright[1], R. Jefferies[1], B.Galambosi[2] and Y. Asakawa[3], [1]Plant Sciences Division, SAC Auchincruive, Ayr KA6 5HW, Scotland, UK, [2]Agricultural Research Centre, South Savo Research Station, FIN-50600, Mikkeli, Finland, [3]Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan

Dracocephalum moldavica is an annual plant, distributed throughout Central Asia, Southern Siberia and Eastern Europe [1]. It has antibacterial, antiviral and antispasmodic properties. It can be used as culinary herb, for ornamental purposes and is an excellent honey-bee plant [2,3]. Geranial, geranyl acetate, neral, neryl acetate and nerol account for about 90% of the essential oil, depending on the geographical source, genotype, time of harvest and postharvest technology [4,5,6]. The objectives of these current studies were to undertake preliminary growth analysis and yield; to compare any phenological differences between various seed sources; to identify oil yield and composition of leaves and flowers (both fresh and dried material) during plant ontogenesis (GC, GC-MS); to carry out gland observation regarding the density during the main plant stages; and to test the oil for toxicity using the shrimp bioassay. The oil yield ranged between 0.1-0.8% for leaves, and 1.2-1.8% for flowers. There were no differences in oil yield between white and blue varieties. The main oil components were citral (0.6 - 73.4%), geranyl acetate (7.5 - 42.5%), geraniol (0.1 - 31.6%), citronellol (0.1 - 3.1%) and linalol (0.9 - 3.1%), depending on the ontogeny, plant source and fresh/dried material. Finland Blue and Finland White had nearly an identical oil composition. There were significantly large differences between the oil composition of leaves and flowers e.g. citral 31.2% and 75.4% respectively, and between fresh and dried material e.g. geraniol 14.1 and 9.6% respectively. All oil samples had a pleasant, distinct, light lemon scent. Growth curves were constructed for two promising sources. Plants were established sucessfully, despite the cold weather during early summer, and yields, both in Finland and Scotland, were satisfactory. The LD_{50} of the oil in the shrimp bioassay was around 320ppm.

[1] G. Hegi, Illustriette Flora Von Mittel-Europa, Band V, (1964) 2360 - 2362.
[2] I. Koch-Heitzmann and W. Schultze, Dtsch. Apoth. Ztg., **124** (1984) 2137.
[3] K. Suchorska, Z. Starck and E. Osińska, Herba Polonica (Tome XL), **3** (1994) 83 - 94.
[4] M.P. Kolalite, Rast. Resursy, **30** (1994) 120 -125.
[5] Y. Holm, R. Hiltunen and I. Nykänen, Flav. & Frag. J. **3** (1988) 109 - 112.
[6] Y. Holm, B. Galambosi and R. Hiltunen, Flav. & Frag. J. **3** (1988) 113 - 115.

14:00 Plenary lecture

L 4 – 1 **G. J. Martin**, *Nantes, France*
A comprehensive pattern of deuterium
distribution in aromatic molecules

Short communications

14:30 L 4 – 2 **C. Bicchi**, *Torino, Italy*
Multidimensional GC in Essential oil analysis:
preliminary results with a new system for
multidimensional and micropreparative GC

14:50 L 4 – 3 **M. M. Barazandeh**, *Tehran, Iran*
Determination of Kovats retention indices by
computer softwares

15:10 Break

KNEIPP WERKE

Focus and Approach

The KNEIPP-WERKE focus on

- Establishing herbal medicinal products as an alternative to synthetics, thus delivering products well-suited for self-medication, self-treatment and prevention - entirely in line with Sebastian Kneipp´s philosophy.
- Internationalising phytocompetence.
- Supporting and disseminating the Kneipp approach as the original form of Wellness: Kneippness as an holistic approach to modern day life.

The KNEIPP-WERKE are the representative of phytotherapy within the context of Kneipp's 5 pillars (Hydrotherapy, Phytotherapy, Kinesitherapy, Dietetics Therapy, Regulative Therapy) and the pioneer in the development and expansion of the phyto-idea in general medicine.

The KNEIPP-WERKE are committed to the heritage of Sebastian Kneipp. Kneipp's original formulas are the foundation for our continued development of herbal medicinal products according to modern day requirements, both natural and scientific: We strive to integrate the best of nature with the best of science.

This translates into

- Developing and maintaining an extensive range of products which adhere to the principles of Kneipp's 5 pillars and which integrate state-of-the-art pharmaceutical know-how with traditional, well-proven methods.
- Upholding the highest standard of quality.
- Promoting the concepts of self-medication and preventive medicine among the general public.

KNEIPP-Werke, D-97064 Würzburg

A COMPREHENSIVE PATTERN OF DEUTERIUM DISTRIBUTION IN AROMATIC MOLECULES

G. J. Martin, CEAIS-Université de Nantes, Site de la Géraudière, Rue P.A. Bobierre, BP 72304, 44323 Nantes cedex 3, France

The Site-specific Natural Isotopic Fractionation of hydrogen studied by ^{2}H-Nuclear Magnetic Resonance (SNIF-NMR) is a powerful tool for the authentication of a molecule in terms of occurrence or origin of its precursors and nature of its fabrication process.

A great number of aromatic molecules has been investigated in order to obtain a comprehensive map of ^{2}H-distribution in flavours and perfumes as a function of the metabolic or synthetic pathways. Isotopic fractionations observed in natural and synthetic *[1]* products are tentatively rationalised by considering formal analogies between chemical reactions and some biochemical steps occurring in the mevalonic [2], phenyl propanoid [3] and lipidic [4] pathways.

[1] G. Heck, C. Mileham and G.J. Martin, Analusis, 25, *(1997)*, 202-206.

[2] S. Hanneguelle, J.N. Thibault, N. Naulet and G.J. Martin, J. Agricult. Food Chem., 40, (1992), 81-87.

[3] R. Zainal, PhD dissertation, Nantes (1992).

[4] A. Royer, J. AOCS, (1998), submitted for publication.

MULTIDIMENSIONAL GC IN ESSENTIAL OIL ANALYSIS: PRELIMINARY RESULTS WITH A NEW SYSTEM FOR MULTIDIMENSIONAL AND MICROPREPARATIVE GC.

Carlo Bicchi, Angela D'Amato, Patrizia Rubiolo; Dipartimento di Scienza e Tecnologia del Farmaco; via Pietro Giuria 9 - I-10125 Torino (Italy)

Multidimensional GC (MDGC) is very successful for the analysis of complex mixtures such as essential oils. This communication reports on a new system for both multidimensional and micropreparative GC, which is based on the principle of that described by Claude and Tabacchi in 1988 [1]. Heart-cut is operated by varying the pressure at the four way point interface, thus affording a fully metal-contactless transfer. The heart-cut fraction is trapped in a cold trap and then reinjected through flash vaporisation in the second column for further separation. Electronic pressure control makes heart-cut pressure conditions independent of temperature. The performance of the system is illustrated through some examples in the field of essential oil analysis and enantiomer separation.

[1] S.G. Claude and R. Tabacchi, Journal of HRC & CC , **11**, (1988), 187-190

DETERMINATION OF KOVATS RETENTION INDICES BY COMPUTER SOFTWARES

M.M. Barazandeh, Research Institute of Forests & Range/ands, P.O.Box:13185-116

Essential oils of Iranian aromatic plants are being analysed by a chemist team in the phytochemistry lab of Research Institute of Forests and Rangelands. Up to now about 80 species have been analysed and their main constituents identified by the aim of GC and GC/MS methods. In this way we had a problem and it was the lack of an accurate method for calculating Kovats retention indices which are useful for qualitative analyses. For solving this problem, a computer program was written in basic language and applied to some species which had been previously analysed by GC and GG/MS. For example this method was applied to evaluating Kovats retention indices of essential oil constituents of two species of *Lavandula* (*L. angustifolia* P.Miller and *L. spica* L.), three species of *Mentha* (*M. longifolia* L., *M. x piperita* L. and *M. aquatica* L.), five species of *Salvia (S. officinalis* L., *S. sclarea* L., *S. santolinifolia* Boiss., *S. syriaca* L. and *S. virgata* Jacq.), two species *of Calamintha (C. officinalis* Moench and *C. grandiflora* (L.) Moench), two species of *Thymus (T. kotchyanus* Boiss. & Hohen. and *T. serphyllum* L.) and also *Matricaria chamomilla* L., *Pimpinella anisum* L. and *Ferula assa-feotida* L. These species had been previously steam distillated in the lab and their essential oils injected to GC on two columns of different polarities (i.e. DB-1 and DB-WAX, 60m. x 0.25mm. ID, 0.25 micron film thick.). Using this method, main constituents of these species identified and then confirmed by their mass spectra.

Two basic programs which have been written for both columns as well as table of percentage composition for some species will be presented.

Short communications

15:40 L 3 – 4 **F. Tateo**, *Milano, Italy*
The current situation and updated in respect of the quantification of ß-asarone, safrole and hypericin in flavourings and other flavoured food matrices

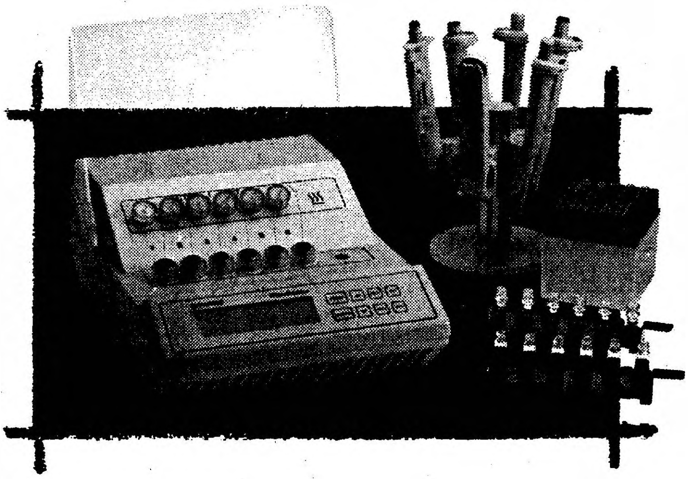
16:00 L 3 – 5 **K. H. C. Başer**, *Eskişehir, Turkey*
Essential oils of *Salvia* species growing in Turkey

16:20 L 3 – 6 **A. Ilcim**, *Eskişehir, Turkey*
The essential oil composition *of Salvia vermifolia* Hedge & Hub. Mor.

16:40 L 3 – 7 **S. Nacar**, *Eskişehir, Turkey*
The essential oil composition of *Origanum amanum* Post.

17:00 Poster session

19:30 Congress Dinner



THE CURRENT SITUATION AND UPDATED IN RESPECT OF THE QUANTIFICATION OF β-ASARONE, SAFROLE AND HYPERICIN IN FLAVOURINGS AND OTHER FLAVOURED FOOD MATRICES.

M. Bononi, E. Lubian, S. Martello, F. Tateo, DIFCA - Sezione di Chimica Analitica Agroalimentare ed Ambientale - Università degli Studi di Milano, Via Celoria 2 - 20133- Milano

Limitation of the content of certain compounds present in extracts derived from aromatic plants is governed by EEC Directives N. 88/388 and N. 91/71. These limited compounds include safrole, β-asarone and hypericin.

The paper presents certain critical considerations regarding the analytical methods available nowadays for determining the aforementioned compounds, and criteria are proposed that have been optimized through the use of the DAD detector by HPLC. The use of HPLC/MS co-ordinated methods is also suggested.

Detectable limits are defined, and the possibility of simplifying the methods of extraction from food matrices is also verified. In addition, a basis is established for verifying the applicability of analytical methods other than those already referred to.

ESSENTIAL OILS OF *SALVIA* SPECIES GROWING IN TURKEY

K.H.C Başer. M.Kürkçüoğlu and N.Kırımer, Medicinal and Aromatic Plant and Drug Research Centre (TBAM), Anadolu University, 26470 Eskişehir, Turkey

The genus *Salvia* L. (Labiatae) is represented by eighty eight species and altogether ninety three taxa in the flora of Turkey, over *50%* of the species being endemic. Dried leaves of several *Salvia* species are used as herbal tea in Turkey. Dried leaves and essential oil of *S. fruticosa* Miller (syn. *S.triloba* L. fil.) are export commodities. This presentation summarises the results of current research at TBAM into the essential oils of thirty four taxa of *Salvia* growing in Turkey using capillary GC and GC/MS. The results are presented in a comparative manner according to major chemical constituents.

Most data are communicated for the first time in this presentation.

1997 - 721 Tonel. S. fruticosa

THE ESSENTIAL OIL COMPOSITION OF *SALVIA VERMIFOLIA* HEDGE & HUB.MOR.

A.Ilcim, S.Nacar, K.S.U Faculty of Science and Letters, Kahramanmaras /TURKEY

The genus Salvia is represented in Turkey by more than 80 species of which 50% are endemic. *Salvia vermifolia* Hedge&Hub.Mor. a narrow endemic from Sivas city (Central Anatolia). The plant was collected from Kabaktepe village. The volatile oil of Salvia *vermifolia* Hedge&Hub.Mor. was isolated clavenger type apparatus. Ratio of essential oil was obtained 0.5%. The essential oil components were analysed by GC/MS. Thirty-nine component were determined in oil. The main components of this volatile oil was as fallows α-pinen (9.25%), camphene (6.69%), β-pinen (8.61%), 1.8 cineol (5.84%), β-caryophyllen (10.85%) and α- caryophyllen (8.69%).

Reference

1- P.H.Davis, Flora of Turkey and East Aegean Islands. Vol.7. University Press, Edinburgh (1982)

THE ESSENTIAL OIL COMPOSITION OF *ORIGANUM AMANUM* POST.

S.Nacar , A.Ilcim K.S.U Faculty of Science and Letters
Kahramanmaras /TURKEY

Origanum amanum Post. a narrow endemic from Mediterranean Region of Turkey. The sample was collected from K.Maras Cimendagi (1800- 1900 m).The volatile oil of flowering parts of *Origanum amanum* Post. was isolated by hydrodistillation for 3h using Clevenger-type apparatus to produce oils in 0.7-0.5%. The essential oil components were analysed by GC/MS. Thirty compounds were identified in oils, of which,
Thymol (5.98%), Carvacrol (5.98%), α-terpineol (11.02%), 1-borneol (12.8%)

Reference
1- P.H.Davis, Flora of Turkey and East Aegean Islands. Vol.7. University Press, Edinburgh (1982)

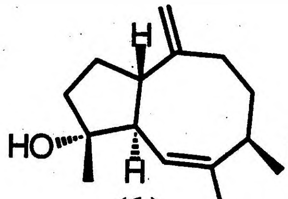
Wednesday, 09th September

09:00	Plenary lecture	
	L 5 – 1	**Y. Asakawa,** *Tokushima, Japan* Novel sesqui- and diterpenoids from selected liverworts
	Short communications	
09:30	L 5 – 2	**W. A. König,** *Hamburg, Germany* New sesquiterpenes from *Valeriana officinalis* and their biogenetic relationship to constituents of the liverwort *Frullania tamarisci*
9:50	L 5 – 3	**K. Schultz,** *Dübendorf, Switzerland* Cyclanthone, a new natural product in the flower scent of *Cyclanthus bipartitius*
10:10	L 5 – 4	**R. Näf,** *Geneva, Switzerland* Phenols and lactones in italo-mitcham peppermint oil
10:30	Break	

NOVEL SESQUI- AND DITERPENOIDS FROM SOME SELECTED LIVERWORTS

Y. Asakawa, T. Hashimoto, M. Toyota, F. Nagashima, H. Irita, M. Suzuki, M. Yoshida, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan

Liverworts are rich sources of mono-, sesqui- and diterpenoids and phenolic compounds several of which show interesting biological activity [1]. Fractionation of the ether extracts of *Dumortiera hirsuta* (**1**,**2**), *Jungermannia infusca* (**3**,**4**), *Porella acutifolia* subsp. *tosana* (**5**,**14**), *P. japonica* (**6**,**7**) *Frullania hamatiloba* **(8,9)**, *F. tamarisci* (**15**) and *Odontoschisma denudatum* **(10-13)** resulted *in* the isolation of a number of novel sesqui- and diterpenoids. The isolation, structure characterisation and biological activity of several new compounds and identification of the characteristic fragrance of liverworts will be discussed.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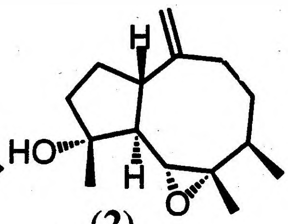
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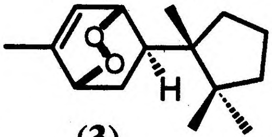
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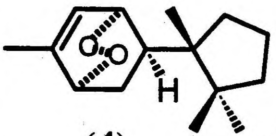
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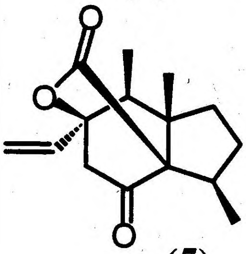
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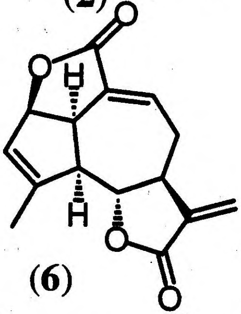
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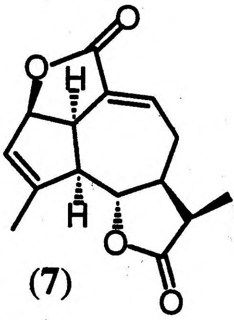
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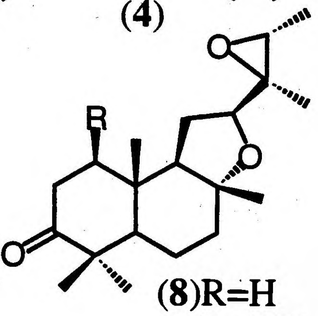
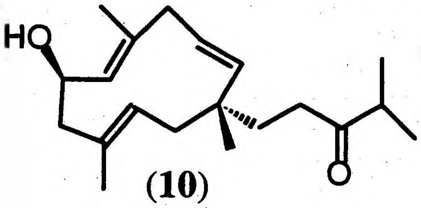

(8)R=H
(9)R=OH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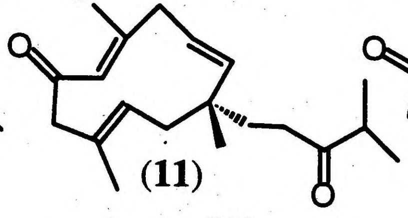
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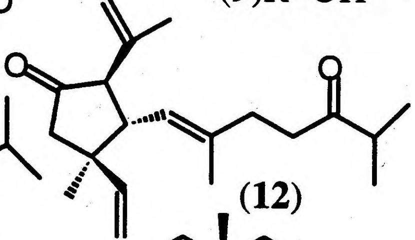
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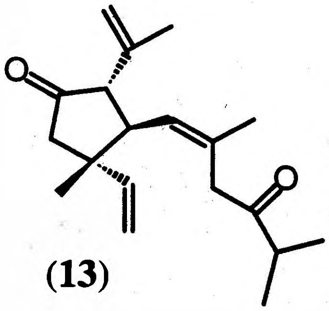
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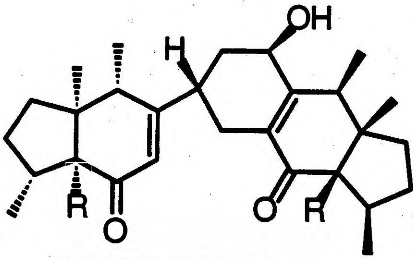
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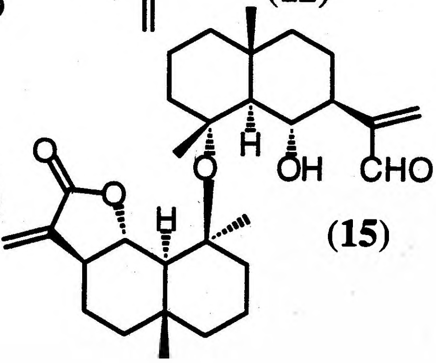
(15)

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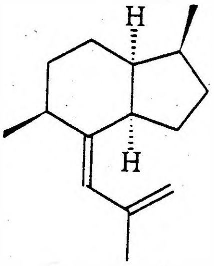
NEW SESQUITERPENES FROM *VALERIANA OFFICINALIS* AND THEIR BIOGENETIC RELATIONSHIP TO CONSTITUENTS OF THE LIVERWORT *FRULLANIA TAMARISCI*

Wilfried A. König and Claudia Paul, Institut für Organische Chemie, Universität Hamburg, D-20146 Hamburg, Germany

The investigation of the essential oil of *Valeriana officinalis* roots and of the liverwort *Frullania tamarisci* by gas chromatography and mass spectrometry revealed the presence of some sesquiterpene hydrocarbons with identical spectroscopic properties. 1- and 2-dimensional NMR studies showed that the unknown constituents (1 - 5) belong to the rare pacifigorgiane skeleton, which is also present in the known sesquiterpene alcohols pacifigorgiol (6) from V *officinalis* [1] and tamariscol (7), a highly fragrant constituent of F. *tamarisci* [2]. Not unexpectedly, the sesquiterpene hydrocarbons in both species were enantiomeric to each other, as proved by enantioselective gas chromatography. In addition, another compound 8 was identified in V. *officinalis* with a valerenane skeleton, which is closely related to the pacifigorgiane backbone. This puzzling fact could be explained after detection of compound 9 (named „Tamariscene") with a new sesquiterpene skeleton. Enantiomers of 9 are constituents of both V. *officinalis* and *F. tamarisci* and rearrange under mild acidic conditions to the pacifigorgiadienes 3 and 4, however, rearrangement to the valerenane system appears possible, too. Thus, it can be speculated that tamariscene (9) is the biogenetic precursor of both pacifigorgiane and valerenane sesquiterpenes.



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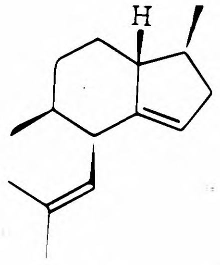

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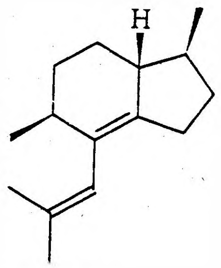

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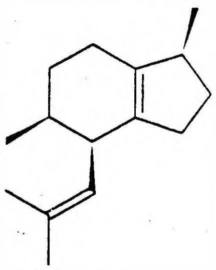

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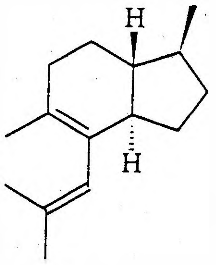

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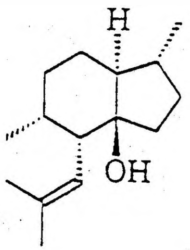

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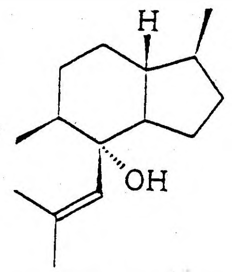

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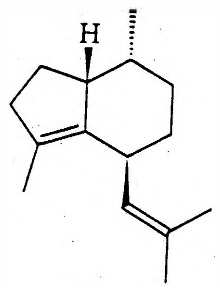

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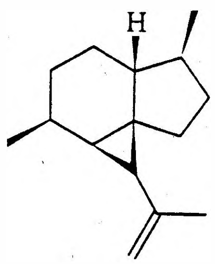

(9)

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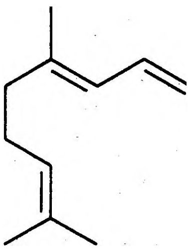
CYCLANTHONE, A NEW NATURAL PRODUCT IN THE FLOWER SCENT OF *CYCLANTHUS BIPARTITIUS*

K. Schultz[1], R. Kaiser[1], J. T. Knudsen[2], [1] Givaudan Roure Research Ltd. CH-8600 Dübendorf, Switzerland, [2] Chemical Ecology/ Botanical Institute of the University of Göteborg, Box 461, SE-40530 Göteborg, Sweden.

In continuation of our search for new or uncommon compounds in the most diverse natural scents we investigated the flower volatiles of *Cyclanthus bipartitius*, a species of the neotropic Cyclantaceae. The structure of the main constituent representing over 80% of the trapped headspace sample could be elucidated as the (*E*)-2,6-dimethyl-nona-6,8-dien-4-one (**3**), a derivative of the biologically highly interesting C_{11}-homoterpene **1**. The unambiguous structure proof for this new natural product was given by synthesis involving a Reformatzki reaction of isovaleraldehyde and ethyl 4-bromo-3-methylcrotonate to yield the intermediate **2**.

Because of the uniqueness of **3** to *Cyclanthus bipartitius* we suggest the name Cyclanthone.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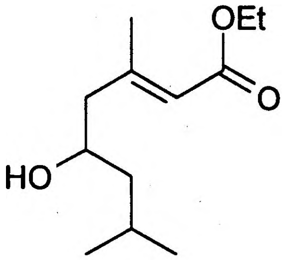

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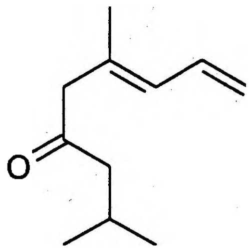
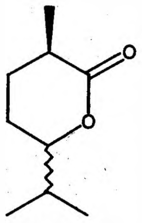


3

PHENOLS AND LACTONES IN ITALO-MITCHAM PEPPERMINT OIL

Regula Näf, Alain Velluz, Firmenich SA, Corporate Research Division, P.O.B. 239, CH-1211 Geneva 8, Switzerland*

In the acidic fractions of Italo-Mitcham black peppermint oil (*Mentha piperita* var. *vulgaris*), some uncommon phenols, carboxylic acids and lactones have been identified, mostly related to menthone. The two isomers of 2,6-dimethyl-heptan-4-olide (**1**) and 5,6-dihydro-3,6-dimethyl-benzo[*b*]furan-7(4*H*)-one (**2**) are new compounds, their structures are proved by syntheses. Three isomers of perhydro-3,6-dimethyl-benzo[*b*]furan-7(4*H*)-one (**3**) as well as 8,9-dehydro-thymol (**4**) have not been reported in a natural raw material before.



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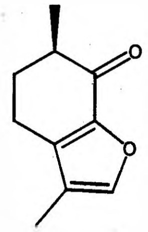

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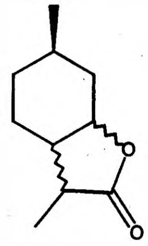

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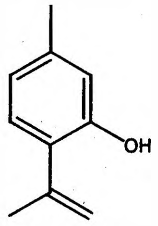

4

Short communications

10:50	L 7 – 1	**É. Németh**, *Budapest, Hungary* Genetical evaluation of the accumulation of essential oil components in *Achillea crithmifolia* W. et K.
11:10	L 7 – 2	**Z. Fleisher**, *Carlstadt, NJ, USA* Chemovariations of *Artemisia herba alba* in the holy land
11:30	L 7 – 3	**M. Maffei**, *Turin, Italy* Photomorphogenetic and chemical responses to blue, UV-A and UV-B irradiations in *Mentha piperita*
11.50	L 7 – 4	**F. Sefidkon**, *Tehran, Iran* Analysis of the oil from six *Nepeta* species from Iran
12:10	Closing remarks	
12:30	Lunch and Departure	

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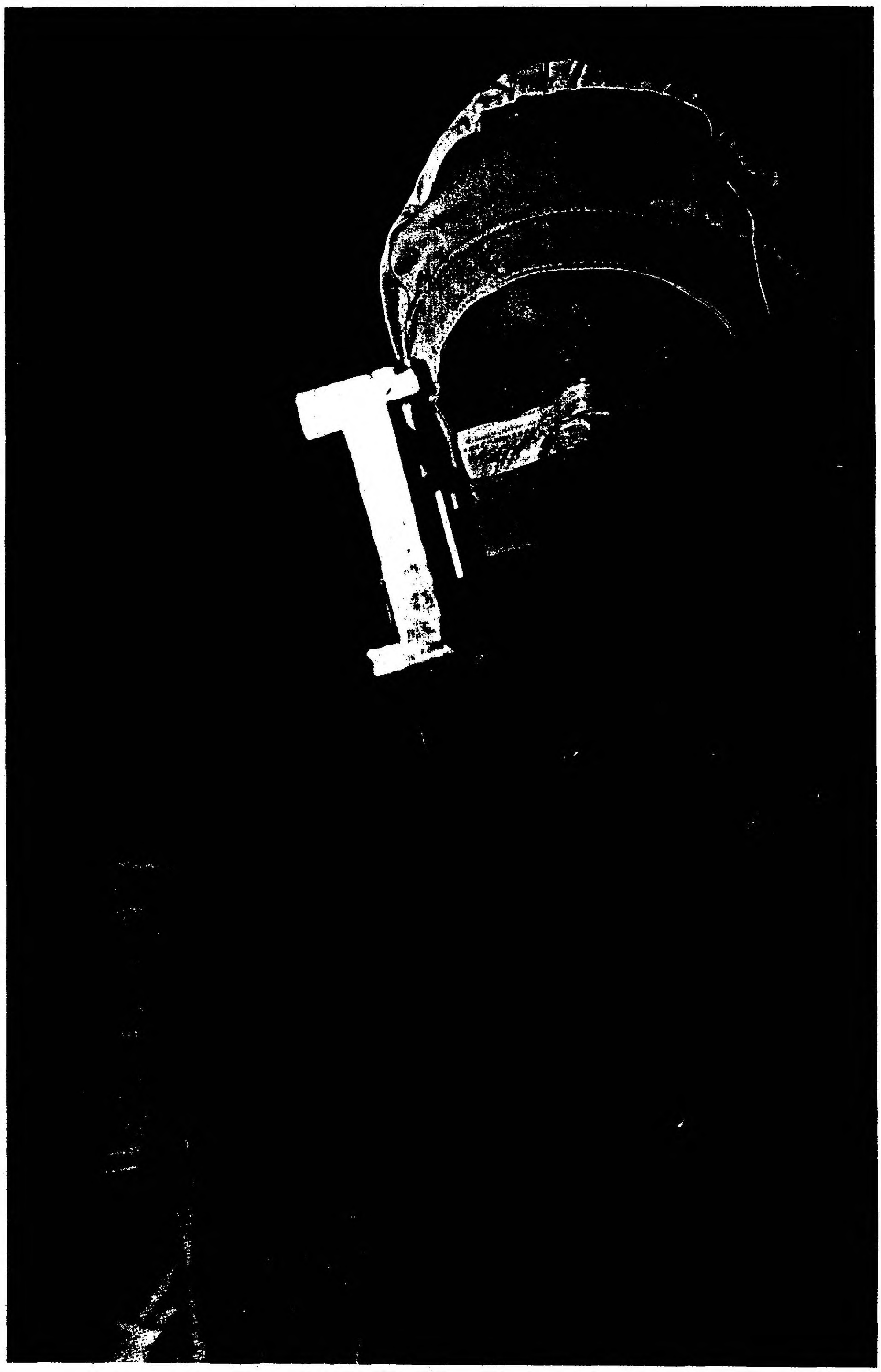

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GENETICAL EVALUATION OF THE ACCUMULATION OF ESSENTIAL OIL COMPONENTS IN ACHILLEA CRITHMIFOLIA W. et K.

Éva Németh, Jenô Bemáth, Éva Héthelyi
University of Horticulture and Food Industry, P.O. Box 53, 1518 Budapest, Hungary

The genetical relationships of the chemical diversity in *Achillea crithmifolia* were studied. After a former study describing ecological and onthogenetical factors on the main components (camphor, borneol, 1,8-cineole) of the essential oil [1], now we characterized their genetical background and heritability. Beside scientific significance of this study, practically we intended to get information for effective breeding and for ensurance of standard quality of wild growing medicinal plant drugs.

In the years 1994-97 25 combinations in 71 progenies of 18 mother plants were investigated. Besides, 48 self-pollinated lines had also been studied. Basic data were gained by GC analysis of the distilled essential oil.

12 compounds were identified in the oil. According to quantitative differences of the main components, tree chemotypes were found in the populations: a./ camphor-type (camphor above 50%, borneol around 10%, cineole under 15%); b./ cineole-type (camphor 20-30%, cineole above 30%, borneol about or under 5%); c./ mixed-type (camphor is the main component, but under 50%, cineole more than 15%, borneol 5-10%). Correlation is weak between borneol and camphor, but it is strong, negative among these ones and cineole.

In the progenies, segregation was described in I1, I2 and in F1 populations. In hybrid generations, differences appeared even in similar parental combinations. A strong hetroyzgotic structure of the plants in the original population is supposed.

Polygenic effects are hypotized in the synthesis of the main monoterpenes: 2-4 loci for camphor, 2-3 loci for borneol and at least 4 loci in case of cineol. Heritability (narrow sense h^2) is high for camphor (0.52), middle for borneol (0.28) and low for cineol (0.12).

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The work was supported by OTKA found (Nr. F 012846).

CHEMOVARIATIONS OF *ARTEMISIA HERBA ALBA* IN THE HOLY LAND

Zhenia Fleisher[1], A. Fleisher [2], [1]Cultor Food Science, 430 Saw Mill River Road, Ardsley, NY 10502 USA, [2]Artech International, 700 Gotham Parkway, Carlstadt, NJ 07072 USA

In continuation of our investigation of aromatic flora of the Holy land, the systematic study *of Artemisia herba alba* essential oils is being conducted. Our study shows that the population of *A. herba alba* in Israel consists of much greater number of chemovarieties than was previously believed. Though chemovarieties are unevenly distributed in different geographical areas, no clear relation between the plant type and environmental conditions could be established. Therefore, in every geographical area the analysis of large number of individual plants was conducted. In the present report the detailed compositions of five relatively rare chemovarieties of A.herba alba are presented. In accordance with the major constituent of the essential oilthese distinctive chemovarieties are defined as camphor-type, β-thujon-, α- and β- thujon-, chrisantenyl acetate- and chrisantenol-types.

PHOTOMORPHOGENETIC AND CHEMICAL RESPONSES TO BLUE, UV-A AND UV-B IRRADIATIONS IN *MENTHA PIPERITA*

Massimo Maffei, Dept Plant Biology, University of Turin, Viale Mattioli, 25 I-10125 Turin, ITALY.

The effect of UV light on photomorphogenesis and biochemistry of crop plants is well known. However, few information is available on the effect of such irradiations on medicinal and aromatic plants, which produce an array of secondary metabolites, partly as a response mechanism. One of the most important oil crops is peppermint (*Mentha x piperita*) which is the major aromatic crop cultivated in North-Western Italy. In order to evaluate the effect of UV and blue radiations on the photomorphogenesis and oil biochemistry, *Mentha piperita* plants were irradiated with three different wavelengths: Blue (B, 450 nm); UV-A (360 nm); and UV-B (310 nm). The irradiation was performed with white lights (W) or without W (night). Plant height, number of nodes, internode length and total area of leaves per node were daily recorded. Leaf area was calculated by image analysis. Protein content, total phenols and total chlorophylls were also determined. Essential oils from irradiated aerial parts were distilled and analyzed by GC/MS. CO_2 assimilation and stomatal conductance measurements were also performed using an Infra Red Gas Analizer equipped with a Parkinson leaf cuvette, whereas total soluble proteins of UV-A irradiated tissues were subjected to SDS-PAGE analyses. A clear photomorphogenetic and chemical response was observed in plants irradiated with the different wavelengths; moreover, night and day irradiations always generated different responses in treated plants with respect to controls. A significant difference was evident in the CO_2 assimilation between W and the irradiations B + W, UV-A + W and UV-B + W. A general increase in the stomatal conductance was observed by treating plants with W, B + W, UV-A + W and UV-B + W, as well as in the night irradiations from night B, night UV-A up to night UV-B. The oil chemical composition was strongly affected by the different UV irradiations, with evident changes in the menthol and menthyl acetate contents. Leaf extracts from UV-A irradiated plants showed the general absence of 101 and 126 kDa bands which were present in the controls, however night UV-A irradiated leaves showed a clear band corresponding to 56 kDa, which was absent in the controls.

ANALYSIS OF THE OILS OF SIX *NEPETA* SPECIES FROM IRAN

F. Sefidkon, Z. Jamzad and H. Rangbari; Research Institute of Forests and Rangelands PO.Box: 13185-116, Tehran, Iran.

The genus *Kepeta* (Lamiaceae) contains more than 280 species, which are spread out over most of central and southern Europe, the near East, and central and southern Asia [1]. Remarkably, about half of the existing species are found in the Iranian highlands [2]. Because of the interesting biological activities of the *Nepeta* species, their use in folk medicine[3-5] and keeping in mind that very few of them have already been studied, we became interested to investigate about the essential oil composition of *Nepeta* species. In this study, the aerial parts of *Nepeta asterotricha, N. binaloudensis, N. cataria, N. crispa, N. menthoides* and *N. pogonosperma* growing wild in Iran were collected at flowering stage. The oils obtained by steam distillation were investigated by GC and GC/MS. The principal components included nepetalactone 2 *(59.2%)* in *N. asterotricha,* nepetalactone 1 (33.0%) in *N. binaloudensis,* nepetalactone 2 (61.3%) in *N. cataria,* 1,8-cineole (51.7%) in *N. crispa,* nepetalactone 2 (44.3%) in *N. menthoides* and nepetalactone 1 (59.4%) in *N. pogonosperma.* The percentage composition of the oils will be presented.

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[5] O. P. Agarwal, D. S. Khanna and R. B. Arora, Artery, (1978) 487.

Poster

SCIENTIFIC PROGRAM

1. Biosynthesis of essential oil compounds
2. Biological activity of essential oil compounds
3. Analytics – chemical aspects
4. Analytics – technical aspects
5. Analytics – structure elucidation of novel compounds
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VARIABILITY IN ESSENTIAL OILS FROM THE GENUS MENTHA CULTIVATED IN DEPARTMENT OF BRASOV, ROMANIA

Pop E G


TASTE THE TREND
Committed to Nature

BIOSYNTHESIS OF GERMACRENE D ENANTIOMERS IN *SOLIDAGO CANADENSIS*: ENZYME ISOLATION AND MECHANISTIC STUDIES

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Sesquiterpene-synthases (cyclases) are enzymes which catalyze the biosynthesis of sesquiterpenes. The substrate for cyclases is farnesyldiphosphate (FDP) which is enantiospecifically transformed to a sesquiterpene [1]. Due to the enantiospecific character of an enzymatically catalyzed reaction usually only one of the two possible sesquiterpene enantiomers is found in the essential oil of a plant. An exception is the plant *Solidago canadensis* (goldenrod) which contains the sesquiterpene hydrocarbon enantiomers (+)- and (-)-germacrene D in similar amounts, as shown by enantioselective gas chromatography [2].

We were interested in the pathways of the (+)- and (-)-germacrene D biosynthesis in *S. canadensis.*

Using different protein purification methods we succeeded in isolating two different cyclases from a crude enzyme extract of *S. canadensis.* The cyclases were incubated with radioactively labeled FDP and the product formation was monitored by radio gas chromatography using chiral stationary phases. One cyclase produces exclusively (+)-germacrene D while the other cyclase generates enantiomerically pure (-)-germacrene D. Furthermore, biochemical properties of both cyclases were determined (K_M, optimum pH, molecular mass) [3].

To establish mechanisms for the cyclization of FDP to (+)- and (-)-germacrene D, we synthesized farnesyldiphosphates with deuterium labels in different positions. These substrates were fed to the cyclases and the obtained products were analyzed by enantioselective gas chromatography combined with different mass spectrometric techniques.

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A FOUR-ARM OLFACTOMETER TO EVALUATE THE REPELLENT PROPERTIES OF ESSENTIAL OILS AGAINST INSECTS

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The repellent properties of some essential oils make them a useful tool to control insect pests in greenhouses. In the laboratory, using a four-armed olfactometer, we tested the repellent effects of essential oil of tansy, *Tanacetum vulgare*, white cedar, *Thuya occidentalis*, balsam fir, *Abies balsamea*, and rosemary, *Rosmarinus officinalis*, and the D-limonene against two greenhouse pests: the two-spotted spider mites, *Tetranychus urticae* and the greenhouse whitefly, *Trialeurodes vaporariorum*.

The two treated fields contained the volatile components of 100 µL of essential oil; the two remaining fields had only humidified air. The degree of repellency was evaluated by recording the amount of time an insect pest spent in each field.

Results indicate that the five essential oils or components could be classified in three categories depending on their properties to repel insects. The essential oil of tansy and white cedar showed a high level of repellency against the two pests. The essential oil volatile of these two plants reduced the time the insects spent in the field by as much as 17 times. The essential oil of balsam fir and the D-limonene repulsed only the two-spotted spider mites which spent up to 10 times less time in the treated than the control fields. The essential oil of rosemary showed less repellency on the two-spotted spider mites and did not affect the greenhouse whitefly. The essential oil of the tansy or the white cedar could be used in plant protection to control simultaneously the two-spotted spider mites and the greenhouse whitefly.

SECRETOLYTIC AND ANTIOXIDATIVE PROPERTIES OF MYRTOL STAND.

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Myrtol stand. (MYS), an essential oil standardized on α-pinene, d-limonene and 1,8-cineol is the active principle of Gelomyrtol® forte used for treatment of acute and chronic inflammatory diseases of the lower and upper respiratory tract. The secretolytic properties of MYS orally administered were tested in a mouse model, which allowed the determination of excreted phenol red via the trachea as indicator, after its intraperitonal administration. MYS as well as eucalyptus and orange oil increased the secretion in the range of 20 - 40%, comparable to the chemically defined compounds Ambroxol and N-acetyl-L-cysteine (40 and 18%). Because pathological situations like inflammation of the respiratory tract are accompanied by the production of reactive oxygen species (ROS) MYS was tested on its antioxidative properties. The immigration and degranulation of leukocytes is of special importance in this context. Degranulating leukocytes produce superoxide, hydrogen peroxide, OH-type oxidants and hypochlorite. In whole blood the degranulation of stimulated leukocytes could be followed in a model reaction, which measures the decomposition of the indicator substance ACC (1 -amino-cyclopropane-1 -carboxylic acid) by hypochlorite yielding ethene [1, 2]. Following the ethene formation over a period of 30 minutes, an IC_{50} of 0,14% for MYS in the reaction mixture, for the inhibition of the ethene formation, could be calculated. The corresponding IC_{50} values for eucalyptus and orange oil were 0,18% and 0,19%,

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BIOLOGICALLY ACTIVE PLANT SECONDARY METABOLITES FROM *ORIGANUM* SPECIES.

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The essential oils from three species of *Origanum* grown in Chania were chemically defined and tested for their biological activities in antibacterial and antioxidant assays. The five test bacteria were *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Yersinia enterocolitica*, all organisms of significance in terms of food poisoning. Plant volatile oils from the leaves and flowers of *Origanum x intercedens*, *O. onites* and *O. vulgare* were extracted by hydrodistillation and analysed by GC-MS. The antioxidative properties were determined by the β-carotene / linoleic acid agar diffusion technique.

The major components [%] in the volatile oils and their bioactivities are given below :

	O. intercedens		*O. onites*		*O. vulgare*	
Oil component	Leaf	Flower	Leaf	Flower	Leaf	Flower
α-Thujene	0.98	1.59	0.99	1.06	0.84	1.20
α-Pinene	0.45	0.48	0.90	0.61	0.55	0.59
β-Myrcene	1.21	1.97	1.46	1.89	1.45	1.83
α-Terpinene	1.24	2.31	1.71	1.42	0.79	1.36
p-Cymene	21.97	6.27	9.67	4.16	7.66	2.99
cis-β-Ocimene	1.59	0.95				
γ-Terpinene	13.24	15.99	13.53	6.71	6.96	8.08
Borneol	1.16	0.64	1.89	0.88	0.18	0.13
Terpinen-4-ol	0.91	0.72	0.91	0.80	0.43	0.45
Carvacrol	49.95	62.74	59.02	74.73	76.42	78.57
Antibacterial and antioxidant activity of volatile oils : diameter of zone of bacterial inhibition and colour retention in antioxidant assay						
B. cereus	58	21	38	28	50	43
E. coli	52	39	38	33	51	41
S. typhimurium	47	32	37	28	50	36
Staph. aureus	25	39	35	27	42	36
Y. enterocolitica	33	38	35	28	41	38
Antioxidant value	65	77	65	64	59	61

The antibacterial and antioxidant properties associated with these volatile oils is often related to the carvacrol content, although synergistic reactions may also take place.

This study was supported by the EU FAIR 3 Program.

ESSENTIAL OIL BLENDS ENHANCE THE HEALING PROCESS IN CO_2 LASER SURGERY AND SKIN RESURFACING - EXPERIMENTAL AND CLINICAL STUDIES

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$C0_2$ laser surgery and skin resurfacing are well-accepted modalities in esthetic and plastic surgery. Various treatments have been used following these procedures either by application of topical ointments or occlusive semipermeable dressings. In order to improve the patient's postoperative care and enhance the healing process, we developed formulas containing pure and natural essential oils (cream or ointment), unsaturated fatty acids from plants and large amounts of natural vitamin E. Previous studies reported that these ingredients have antibacterial and anti-oxidant activities which enhance wound healing and reduce further tissue damage caused by oxygen-free radicals and pain relief effect. In order to study the effect of essential oil extracts, we conducted experimental and clinical studies. Comparative experimental study was performed on rats: $C0_2$ laser surgical wounds were induced on the backs of rats. OptoScan $C0_2$ laser resurfacing of the wounds was performed on the 2nd and 4th postoperative days. One group served as a control and the others were treated 4 times a day with various antibiotic creams, vaseline and essential oil creams. Macroscopic observations revealed that up to 28 days, there was enhancement in the healing of the wounds in the group treated with these essential oil creams when compared to the other groups. Histology (H & E) confirmed this observation. Picrosirius Red staining and polarized light of the histologic sections revealed maturation of collagen fibers in the healed wounds treated with essential oil creams when compared to partial maturation in the other groups. In clinical use, volunteer patients following OptoScan $C0_2$ laser skin resurfacing (indications: facial skin rejuvenation, wrinkles, actinic changes, pigmentation) were treated with essential oil products several times a day on one side, and on the other side, with antibiotic cream or vaseline. The patients were examined and photographed daily. Improvement in the healing process in the areas treated with essential oil creams was observed when compared to the other areas, with no immediate or late complications. The cosmetic results were superior. We conclude that pure and natural essential oil products are highly effective in tissue repair.

ANALYSIS OF TUBEROSE VOLATILES BY HEAD-SPACE ENRICHMENT ON TENAX AND THEIR NON SUB-ACUTE DERMAL TOXICITY

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GC/MS of volatiles of *Polianthes tuberosa* L. produced by head-space enrichment on Tenax giving seventeen components, the major of them are benzyl benzoate, transmethyl isoeugenol and ethyl myristate constituting about (16.76%, 15.3% and 14.0% respectively) [1]. The presence of N_2-containing compound, methyl anthranilate constituting 7.1% was identified. Volatiles of *Polianthes tuberosa* L. [2] showed no significant increase in blood glucose, blood urea nitrogen, glutamic oxalo acetic transaminase (SGOT) and pyrovic transaminase (SGPT) when applied to the skin which indicate that it has no any sub-acute dermal toxicity [3].

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EFFECTS OF FRAGRANCE COMPOUNDS ON PHYSIOLOGICAL AND NEURO-PSYCHOLOGICAL PROCESSES: EVIDENCE FOR A PSYCHOLOGICAL MECHANISM

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The scientific knowledge about the activating and sedative effects which are attributed to many essential oils in the popular literature on aromatherapy is rather poor. The results of a former investigation [1] carried out by our group indicated that these effects are due to a psychological mechanism. However, since the effect occurred only some time after the onset of inhalation, a pharmacological way of action cannot be ruled out. The aim of the present study was to examine whether stimulating / sedative effects of fragrances can be observed in terms of both neuropsychological and physiological parameters after inhalative application of the substances.

In the present investigation two components of essential oils (1,8-cineol, activating [2], and linalool, sedative [3]) and a placebo substance (pure air) were administered by inhalation from an olfactometer for 20 minutes while the physiological data were recorded. Afterwards subjects had to perform a visual vigilance task for 30 minutes. In addition, subjects had to give ratings of the inhaled substances and on their mental and emotional conditions at the beginning and at the end of inhalation.

Preliminary data analyses revealed no group effects on either vigilance performance or physiological measures, but some of the subjective ratings differed when male and female subjects were examined seperately. Also, intra-group analyses showed complex interactions between sex, subjective ratings, objective performance and physiological measures. These results rather support the hypothesis of a psychological than that of a pharmacological mechanism for the effects of fragrances applied by inhalation.

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[2] K. A. Kovar, B. Gropper, D. Friess and H. P. T. Ammon, Planta Med. **53** (1987) 315-318

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MEANS, POSSESSING TUBERCULOSTATIC ACTIVITY

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The test of some essential oils (EO-L1,L2,L3,L4) as the most effective means against a wide spectrum of pathogenic Mycobacterium tuberculosis have been conducted.

The essential oils have been produced according to the traditional technology, described in the state

Pharmacopoeia of the USSR, edition II. The essential oils, giving by "Misitano and Strakussi" company (Italy, Messina) have been used. To prove efficiency of the offering means their tests on Mycobacterium tuberculosis cultures resew directly from patients, having heavy destructive forms of lung tuberculosis were carried out. The test were conducted according to the conventional method (Order N 558, June 8, 1978, MH, USSR).

The clinic confirmation of the efficiency of the essential oils was registered during treatment of patients

(volunteers) in a surgical department of Municipal specialized pulmonary surgical hospital N 1, Novosibirsk.

STRUCTURE – ODOR RELATIONSHIP OF SUBSTITUTED *gem*-DIMETHYLCYCLOHEXANE DERIVATIVES

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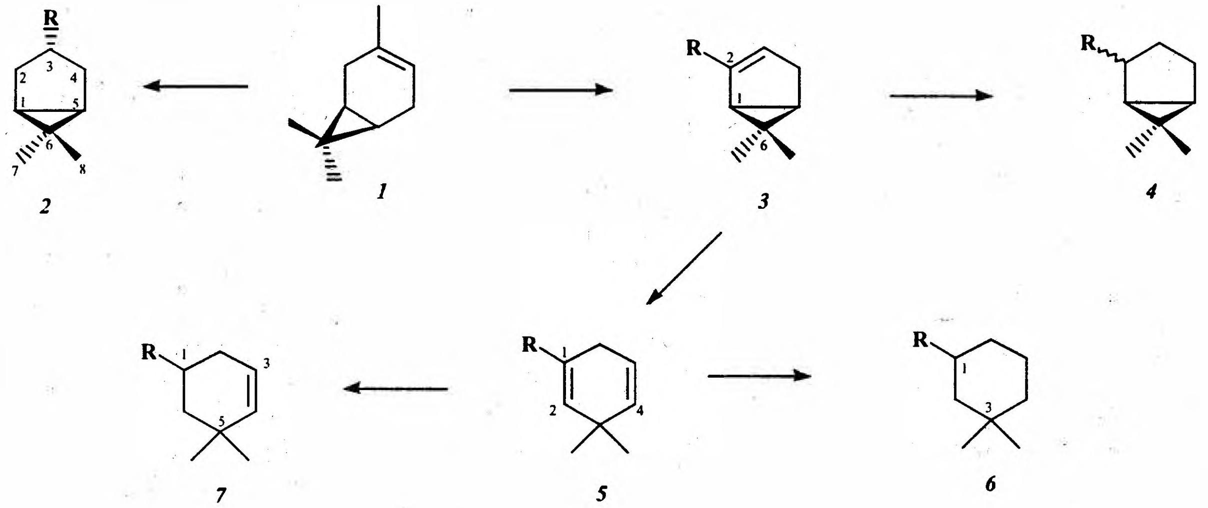
Structure – odor relationships for the floral note were studied using a set of ca. 50 compounds, derivatives of *trans*-3-substituted-6,6-dimethylbicyclo[3.1.0]hexane (**2**), 2-substituted-6,6-dimethylbicyclo[3.10]hex-2-ene (**3**), 2-substituted-6,6-dimethylbicyclo[3.1.0]hexane (**4**), 1-substituted-3,3-dimethylocyclohexane (**6**) and 1-substituted-5,5-dimethylo-3-cyclohexene (**7**) obtained from easy available component of polish turpentine (+)-3-carene (**1**). Olfactory properties and chemical structure of all presented compounds were earlier described [1,2].



We have tried to find common structure factor (SF) responsible to their olfactory properties. The low-energy conformations of studied compounds have been obtained using CVFF force field in Insight II program. Superimposition of analysed compounds with the most likely conformations on reference structures has been made using MSI molecular modelling packages.

These comparisons showed that the differences of location and nature of substituents in studied compounds are the most important factor for their olfactory properties.

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[2] S. Lochyński, Pollena TPŚK, **10** (1997) 427-431

THE EFFECT OF ESSENTIAL OILS ON CHILDHOOD ATOPIC ECZEMA

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A preliminary study of a group of eight children from the middle-socio-economic class, born to professional working mothers was undertaken to test the hypothesis that essential oils would alleviate the symptoms of childhood atopic eczema. The children were counselled and massaged with essential oils by both the therapist and the mother over a period of eight weeks. The essential oils, chosen by the mothers from 36 presented, were: sweet marjoram, frankinsence, German chamomile, myrrh, thyme, benzoin, spike lavender and Litsea cubeba. A control group of children received the same treatment without essential oils. The treatments were evaluated by means of day-time irritation scores and night time disturbance scores, determined by the mother before and during the treatment; finally general improvement scores were allocated two weeks after the treatment by the therapist, the general practitioner and the mother.

The results showed a statistically significant improvement in the eczema in the two groups of children following therapy, but there was no significant improvement shown between the experimental and control group. There is therefore proof that counselling and tactile contact between mother and child benefits the symptoms of atopic eczema but no proof that essential oils are more beneficial than massage alone.

Further studies on the experimental group showed a deterioration in the eczematous condition after two further eight week periods of essential oil massage therapy. This may have been due to the decrease in maternal-child interaction after the initial experimental period, or possibly allergic contact dermatitis provoked by the essential oils.

ACTIVITY OF ESSENTIAL OILS AGAINST CUCURBIT POWDERY MILDEW (*SPHAEROTHECA FULIGINEA*) ON ZUCCHINI

Brunelli, A.[1]*, Flori, P.*[1]*, Marotti, M.*[2] *and Piccaglia, R.*[2]*,* [1] *Dipartimento di Protezione e Valorizzazione Agroalimentare,* [2] *Dipartimento di Agronomia - Università di Bologna, via Filippo Re, 8, 40126 Bologna, Italy.*

Some essential oils were tested against powdery mildew caused by Sphaeroteca fuliginea under greenhouse and field conditions on zucchini.

The oils, belonging to thyme (Thymus spp.), basil (Ocymum basilicum), clove (Eugenia caryophyllus), oregano (Origanum vulgare), winter savory (Satureja montana) and camel hay (Andropogon schoenanthus) were purchased from different suppliers.

The treatments with essential oils were performed in greenhose on cotyledons artificially infected with conidia of S. fuliginea and, in field, on naturally infected plants.

All the oils, in greenhouse trials, showed a good control of powdery mildew comparable to that of sulphur (standard treatment) whereas, in field, they showed a limited persistence due to their volatility.

Most of the oils were characterized by GC/MS and several components having a well known biological activity were found.

SYNTHESIS AND ODOR OF CHIRAL PARTIAL STRUCTURES OF KHUSIMONE

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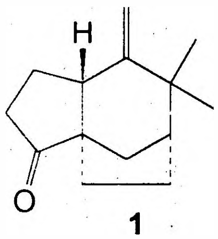
(-)-Khusimone (**1**), one of the main odor-donating compounds of vetiver oil, is subject of the following study on structure/odor relationship. Vetiver oil is widely used in the cosmetic industry and its odor has been described as heavy-sweet, woody, earthy, reminiscent of roots and wet soil with a rich undertone of precious wood.

The correlation between the complex tricyclic structure of (-)-khusimone and its characteristic odor-properties has been examined. Therefore the following chiral bicyclic partialstructures **2**, **3**, **4**, **5** and **6** were synthesized enantioselectively and the olfactory consequences were studied.



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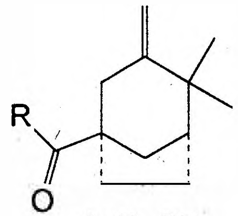
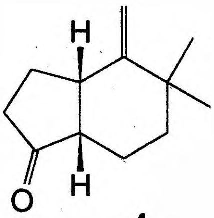


2: R=CH_3
3: R=C_2H_5



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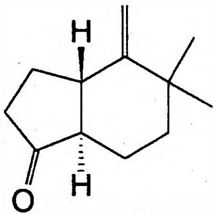

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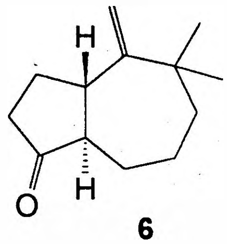

6

The opening of the cyclopentanone ring led to the seco compounds **2** and **3**. Their synthetic approach started from an enantiomerically pure enone ester which is easily obtained from camphorsulfonic acid.

The hydrindane derivatives **4** and **5** were obtained by omitting the ethano bridge. The stereochemical key steps of the synthesis of these epimeric compounds were based on a highly diastereoselective conjugate addition to a chiral oxocyclopent-1-ene-2-carboxylate.

The omittance of the methylene bridge led to a hydrazulene nucleus. Following the procedure described for the synthesis of the hydrindane derivatives resulted exclusively in the formation of the trans-anellated product **6**.

None of these chiral partial structures exhibited olfactory characteristics similar to the typical vetiver odor descriptors. Thus the degradation of the tricyclic (-)-khusimone led to a loss of the typical odor.

SYNTHESIS AND ODOR OF CHIRAL PARTIAL STRUCTURES OF β-VETIVONE

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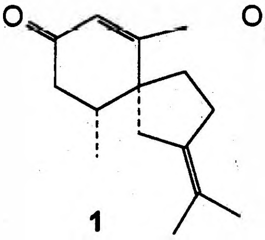
Althanstraße 14, A-1090 Vienna*

(-)-β-Vetivone (**1**) is one of the main constituents of vetiver-oil, an extract of the roots of *Vetiveria zizanoides*. Because of it's heavy sweet-woody, earthy odor this essential oil is widely used in perfume industry. Synthesis of the chiral partial structures **2**, **3**, **4** and **5** of (-)-β-Vetivone (**1**) should give information about the possibility of synthetic reconstruction of the typical vetiver-aroma by such kind of structures. The absolute configurations of these enantiomerically pure compounds also should be determined.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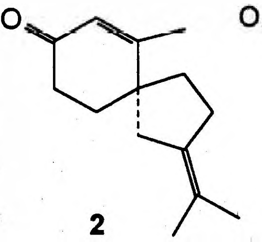
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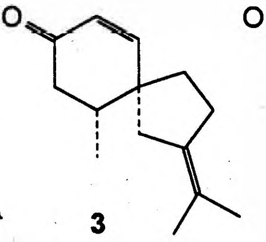
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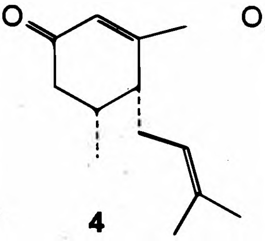
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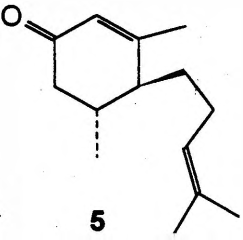
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Stereoselective spiroanellation [1] (**1** - **3**) or alkylation (**4**, **5**) of the corresponding alkoxycyclohexenones followed by a short sequence of reactions resulted in the formation of the desired structures **1** - **5** as racemic mixtures. Treatment of the racemates with lithiated (-)-N,S-Dimethyl-S-phenylsulfoximine proceeded with excellent facial selectivity affording chromatographically separable mixtures of diastereomeric β-hydroxy-sulfoximines (de: >99%), respectively [2]. Thermolysis in refluxing toluene allowed regeneration of the ketones. The absolute configurations of all prepared enantiomerically pure partial structures **2** - **5** were determined by comparing their CD-data with those of the natural substance (-)-β-Vetivone (**1**), thats absolute configuration is known.

None of the degraded structures of (-)-β-Vetivone (**1**) exhibited olfactory-characteristics typical to the odor of vetiver-oil. Surprisingly even the odor of (-)-β-Vetivone (**1**) - as mostly maintained - does not present the typical vetiver-character.

[1] G. Stork, R.L. Danheiser, B. Ganem, J Am Chem Soc **95** (1973), 3414-3415.
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TOXICITY TESTING OF VOLATILE OILS USING THE BRINE SHRIMP (*Artemia salina*)

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The brine shrimp is a crustacean belonging to the subclass *Branchiopoda* of the order *Anostraca.* The hatching rate of the eggs after exposure to petroleum oil, pesticides, carcinogens and other environmental contaminants has been used as a criterion for toxicity, but the most commonly used stage is 24-48 hours after hatching [1,2]. The acute LD_{50} is often used to assess the toxicity of whole plant extracts, or the various fractions of these extracts [3,4]. Although there are no references available regarding volatile oils, terpinen-4-ol, carvone, camphor, limonene, menthone and citral have all been tested and showed relatively low toxicity if used between 500-1800 ppm [5]. The significance of the test lies mainly as an indicator of the possible antitumour activity of compounds (ideal LD_{50} being <40ppm) or for use as an insecticide (ideal LD_{50} being around I ppm). Five oils were used in the preliminary tests; Roman and German chamomile, thyme, melissa (true) and fennel, and all analyzed for their major components by GC and GC-MS. The oils were initially used at concentrations of 1000ppm, followed by 480, 240, 200, 150, 100, 60 and 33ppm. Four replicates were prepared for each dose level and each experiment was repeated three times with two controls. Concentrations of over 500 ppm proved lethal for all the oils. The logit of the mortality was related to the logarithm (to base 10) of the concentration of oil [6]. The model was parammeterised in terms of the LD_{50} slope. The LD_{50} and the confidence interval have also been backtransformed to give an estimate and 95% confidence interval on the original scale i.e. concentration of oil in ppm (German chamomile, 260ppm, Roman chamomile 192ppm, fennel 100ppm, melissa 170ppm, thyme 123ppm) .

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[2] B.N. Meyer, J.E. Ferriigni, L.B. Putnam, D.E. Jacobsen, D.E.Nichols and J.L. McLaughlan, *Plant Med.* **45** (1982) 31 - 34.
[3] S. Doganca, E. Gurkan, F. Hurlak, O.T. Tuzan and E. Tuzlaci, *Fitoterapia*, **68** (1) (1997) 80.
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ANTIOXIDATIVE ACTIVITIES OF FLAVOR COMPONENTS FROM GRAPE SEED OIL MADE FROM ARGENTINA

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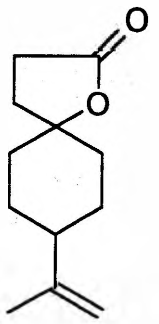
Abstract : Grape seed oil in Argentina is producing for vegetable oil. Recently, this oil became familiar for one of the healthy food in Japan. In the present paper, the flavour oil was isolated from grape seed oil made from Argentina. The components of flavour oil have been investigated by capillary GC, GC-MS. The oil contained more than 40 volatile components of which mainly components were fatty acids, its derivatives and terpenoids. The flavour oil was also examined for antioxidative activity.

ODORIFEROUS BICYCLIC TERPENOID LACTONES

Edyta Paruch, Czesław Wawrzeńczyk, Department of Chemistry, Agricultural University, Norwida 25, 53-357 Wrocław, Poland.

Terpene and sesquiterpene lactones are constituents of many essential oils. They are also widely spread in the food of animal origin and alcoholic beverages.

Continuing our syntheses of bicyclic terpenoid lactones as potential insect antifeedants we have obtained several compounds with the limonene system. They exhibit not only quite good feeding deterrency but also are characterized by interesting odours.



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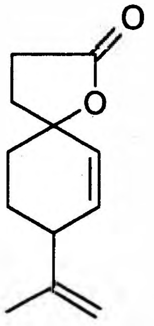
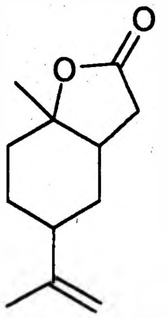


2a 5R, 8S
b 5S, 8S



3a 1R, 4R, 6R
1S, 4S, 6S

Spirolactones **1** and **2** were obtained in four step synthesis from (+) or (-)-limonene. Both enantiomers of lactone **3** were synthesized from (+) or (-) perillyl alcohol via their Claisen rearrangement and iodololactonisation of corresponding γ,δ-unsaturated acids.

Odour characteristics of compounds obtained will be also presented.

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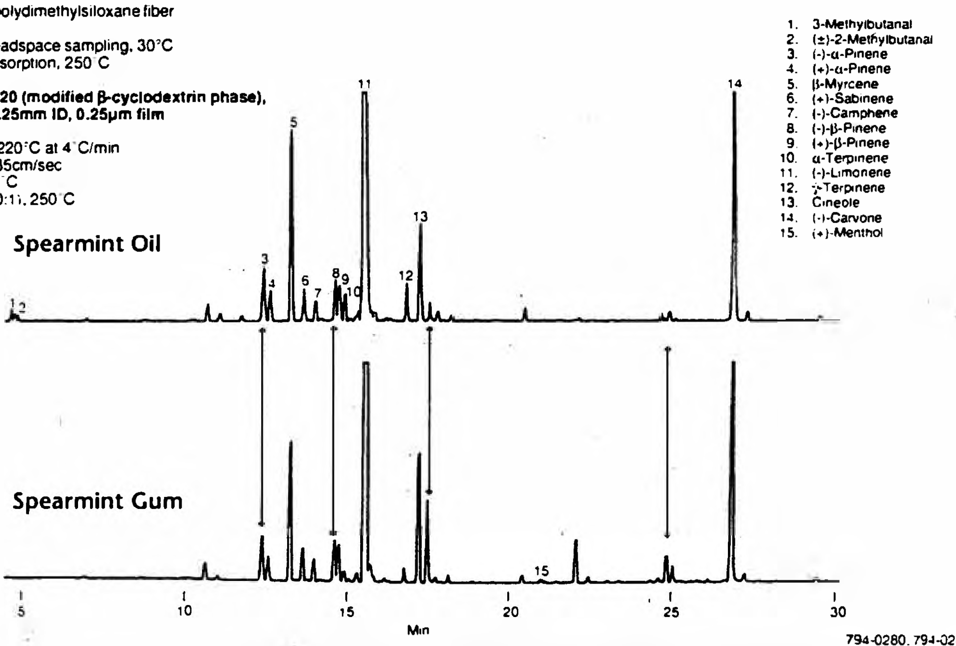
Carrier: helium, 35cm/sec
Det.: FID, 250°C
Inj.: split (100:1), 250°C



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Volatile Constituents of *Coriandrum sativum* L. from Isfahan

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Coriandrum sativum L. (Coriander) is a culinary-medicinal plant from Umbelliferae family that cultivating wide-spread in Iran. Coriander fruits are used as carminative, spasmolytic and galactagogue in Iran(1). In this study, the chemical composition of the volatile oil of fruits of *C. sativum* L. , collected in August 1997 near Isfahan was examined. The amount of the oil was determined after hydrodistillation and it's composition was investigated by TLC, GC and GC/MS (2,3).

The essential oil yield was 0.7% . The major component of the oil was D-(+)-Linalool. In contrast to the literature (4-6), no alpha-Pinene, Limonene, 1, 8 - Cineole and Camphor were detected in the volatile oil of the Isfahanian *C. sativum* L.

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COMPARISON OF THE ESSENTIAL OIL COMPOSITION OF VITEX AGNUS-CASTUS L. AND V. AGNUS-CASTUS F. ROSEA RADIĆ F. NOVA

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Vitex agnus-castus is growing wild along the Croatian Adriatic coast. In the region of Makarska we have found some shrubs with rosy flowers, cited in the literature [1] as *Vitex agnus-castus f. rosea Radić f. nova.* The plants with violet compaired to those with rosy flowers have some morphological difference as well as the content of Δ^4-3-ketosteroids [2].

Therefore, we decided to analyze the content and composition of the essential oils of the flowers, leaves and fruits to find possible difference. The compositions of the oils were found qualitatively similar but quantitatively different.

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QUANTITATIVE AND QUALITATIVE ANALYSES OF THE *PIMPINELLA ANISUM* L. OIL FROM IRAN

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Anis is a very important plant which is used in food and medicinal industries [1,2]. Dry fruits of Anis *(Pimpinella anisum* L.) were collected from provinces Isfahan and Fars in summer and the essential oils obtained by steam distillation were examined by GC and GC/MS. Among 11 compounds identified, the major component was *trans*-anthrole, 89% in Fars sample and 91.7% in Esfahan sample. Other important constituents in Fars sample and Esfahan sample were as follow respectively: γ-gurjunene (3.27%, 1.85%), estragol (2.38%, 1.04%), eugenyl-acetate (1.43%, 2.0%), α-zigiberene (1.01%, 0.92%).

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COMPOSITION OF THE ESSENTIAL OILS FROM DIFFERENT PARTS OF *ERYNGIUM MARITIMUM* L. (APIACEAE)

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Eryngium maritimum L. (Sea Holly) is an intensely glaucous perennial plant belonging to the apiaceae family with coriaceous, lobed leaves and large spinose teath, growing on maritime sands at the coasts of Europe, North Africa, and S.W. Asia.

In course of our investigations of essential oils from Apiaceae we have analyzed the essential oils of the different parts from *Eryngium maritimum* collected near Alicante (Spain) by GC, GC/MS, HPLC and NMR spectroscopic techniques.

Main constituents of the investigated oils are sesquiterpene hydrocarbons and oxigenated sesquiterpenoids while monoterpene derivatives were found only in small amounts. In addition, 2 different substituted trimethylbenzaldehydes, which exhibit the highest proportion in the root oil, could be identified in the oils from all parts of the plant originating from ferulol and isoferulol esters and formed during hydrodistillation [1,2].

In the green parts of the plant germacrene-d was the most abundant component with 46% in the herb and 45% in green fruits. During fruit ripening the proportion of oxygenated sesquiterpenoids increases from 35 to 72% at the expense of hydrocarbons, especially of germacrene-d, which is present in the oil of ripe fruits with only 10%.

The root oil with a comparable high portion of sesquiterpene hydrocarbons (55%) is characterized by guaiane derivatives, whereas germacrene-d decreased to ca. 6 %. In addition, the polyacetylene derivative falcarinol, which was found in the above ground parts only in traces, could be detected in significant higher proportions (2.5%).

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ESSENTIAL OIL COMPOSITION OF *MENTHA AQUATICA* L. FROM IRAN

M. M. Barazandeh, Research Institute of Forests & Rangelands, P.O. Box:13185-116, Tehran, Iran

Mentha aquatica L. was collected from research & experimental forests of Vaz in the north of Iran in Sept.1996. The essential oil of the plant was produced by steam distillation of its dry leaves with the yield of 1⁰/o (based on dry leaves) and then analysed by the aim of capillary gas chromatography method using two columns of different polarities (DB-1 and DB-WAX, each one 60m. x 0.25mm. id, 0.25 micron film thick.). Sixteen compounds were identified in this essential oil which consist of about 83% of total oil. It was found that menthofuran (52.6%), beta-caryophyllene(11 %), germacrene D (7.5%) and 1,8-cineole (4.2%) were the major constituents.

ESSENTIAL OILS COMPOSITION OF *LAVANDULA ANGUSTIFOLIA* P. MILLER FROM FRANCE AND *LAVANDULA SPICA* FROM IRAN

M.M. Barazandeh, Research Institute of Forests & Rangelands, P.O.Box:13185-116

The essential oils of *Lavandula spica* L. from Iran and *Lavandula angustifolia* P.Miller from France were analysed by capillay gas chromatography (cGC) using two columns of different polarities (DB-1 and DB-WAX). The essential oil of Iranian *Lavandula* was produced in Aug.1996 by hydrodistillation of flowers *of Lavandula spica* L. which had been brought from Europe and cultured in the field of Research Institute of Forests and Rangelands (R.I.F.R) about 25 years ago. French essential oil of *Lavandula* was brought by Dr. J. Touche from France to Iran in Aug.1996 in relation to a common project with F.A.O. Twenty eight compounds were identified in each essential oil. It was found that Iranian essential oil to contain linalool (38. 9%), 1,8-cineole (14.6%), borneol (11.5%), camphor (4.2%), linalyl acetate (3.7%), terpinene-4-ol (3.2%), alpha-humulene (3.1%) and (Z)-beta-ocimene(1.7%) while in French essential oil linalool (39.5%), linalyl acetate (32.5%), terpinene-4-ol (3.8%), beta-caryophyllene (3.02%), lavandulyl-acetate (2.8%), (Z)-beta-ocimene (2.6%) and 3-octanone (1.3%) were the major constituents.

COMPOSITION OF THE ESSENTIAL OILS OF *ORIGANUM x INTERCEDENS* RECHINGER AND ITS PARENTS FROM TURKEY

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Origanum x intercedens Rechinger is a hybrid of *Origanum onites* L. and *Origanum vulgare* L. subsp. hirtum (Link) letswaart. Water distilled essential oils of the hybrid and its parents were analyzed by GC/MS. Carvacrol was found as the main constituent in the ojis.

COMPOSITION OF THE ESSENTIAL OILS OF *SIDERITIS TAURICA* AND *S. ARMENIACA*

N. Kırımer [1], N. Tabanca [1], K. H. C Başer [1], T. Özek[1], H. Duman[2], [1]Medicinal and Aromatic Plant and Drug Research Centre (TBAM), Anadolu University, 26470 Eskişehir, Turkey , [2]Department of Biology, Faculty of Science and Letters, Gazi University, 06500 Ankara, Turkey

Sideritis taurica Stephan ex Willd. and *S. armeniaca* Bornm. are endemic species in Turkey. Water distilled essential oils from the aerial parts of these plants were analysed by GC/MS. The oils from both samples were found to contain β-pinene and α-pinene as major constituents.

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL AND EXTRACT FROM WILD *ACORUS CALAMUS* FROM QUEBEC REGION IN CANADA

André Bélanger[1], F. Boudreau[1], D. Rahman[2], E. Schausberger[2] and G. Buchbauer[2]
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Acorus calamus is native of India and the diploid variety grows in North of America. Acorus calamus, commonly named sweet flag, is a perenniel herbaceous plant growing wild on the edges of swamps, on the banks of rivers and ponds in North America, Europe and Asia. The dried rhizomes has long been employed in medicinal preparations and for the flavoring of liqueurs. It contains a volatile oil with a pleasant slightly sweetish odor, wich can be isolated by steam distillation.

The essential oil and extract compositions of Acorus calamus were analysed by capillary gas chromatography (GC). Compounds were identified by their mass-spectra and retention indices relatives to n-alkanes on polar and apolar capillary column. The highest oil yield of 12% was found in the dried crunched rhizomes when extracted with solvants. The steam distillation give a yield of 7% in essential oil.

The major compounds of the essential oil are iso-shyobunone, 6-epi-shyobunone, preisocalamendiol, acorenone, calamendiol, acorone, iso-acorone and crypto-acorone. This diploid variety that grows in Quebec does not contain □-asarone.

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THE ESSENTIAL OILS AND THEIR HEAVY METAL CONTENTS

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Changes of environment, especially chemical, industry, water and atmoshere pollution with all complex of anthropogenic affects, have unambigons effect on growth plant association in nature phytocenosis and culture monocenosis. Sufficient curable composition and the highest quality (a low content of heavy metals) of essential oil are the main condition for their further production and aplication in aromatheraphy.

This contribution is presenting the heavy metals determination in several herb essential oils (table), which are sold in the Slovak aromatheraphy market. The AAS results were otained by the Shimadzu Model 660.

Table : The contents of heavy metals in herb essential oils

ESSENTIAL OILS	CONTENT OF HEAVY METALS [mg.kg^{-1}]				
	CADMIUM	LEAD	CHROME	NICKEL	MERCURY
CARAWAY	0.005	0.010	0.011	0.016	0.001
CHAMOMILE	0.010	0.116	0.031	0.024	0.002
PEPPERMINT	0.003	0.010	0.020	0.019	0.001
JUNIPER	0.004	0.016	0.034	0.018	0.001
FENNEL	0.003	0.018	0.050	0.030	0.002
SCOTS PINE	0.006	0.150	0.060	0.054	0.002

The values of heavy metal contents in these essential oils were compared with the highest permissible concentrations according to the Regulation No. 2/1994 of the Ministry of Public Health of Slovak Republic. In regard to these results we can conclude that these heavy metal contents had lower values than is the highest permissible contents (into herb teas).

THE COMPOSITION OF COMMERCIAL MANUKA OILS FROM NEW ZEALAND

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Manuka oil, a product of increasing commercial interest, is exclusively produced on the North Island of New Zealand, namely in the area from the Coromandel Peninsula to the East Cape. From there Manuka oil penetrates the world market. In New Zealand the trade name is Manex oil; in Germany it is known as „Neuseeländisches Teebaumöl". The botanical source of Manuka oil, *Leptospermum scoparium* J.R.et G.FORST., is a perennial shrub or even a small tree („red tea tree") widespread and common all over New Zealand. The plant belongs to the Myrtaceae family such as *Melaleuca alternifolia* CHEEL, the source of the Australian tea tree oil.

Earlier studies have proven the existence of three chemotypes of *L. scoparium*, a high-pinene chemotype, a high-triketone chemotype and a type containing a complex mixture of sesquiterpene hydrocarbons [1]. Our investigations of 16 different commercial oil samples demonstrated that they originated exclusively from the high-triketone chemotype, which predominates on the East Cape. The compositions of all the commercial oils were relatively similar, containing 10 to 20% triketones, mainly leptospermone (mean 11.6%). Within the sesquiterpene hydrocarbon fraction (60 to 70% of the oils) mainly calamenene (mean 12.5%) and δ-cadinene (mean 6,3%) were found in addition to minor amounts of several other hydrocarbons; oxygenated sesquiterpenes amounted to 10 to 18%. Monoterpene hydrocarbons were present in low concentrations (mean 4.5% in total), a reliable criterion to differentiate between Manuka oil and Kanuka oil (*Kunzea ericoides* (A.RICH.)J.THOMPSON; „white tea tree"), which usually contains about 75% α-pinene. Terpinene-4-ol and 1.8-cineole, the most important constituents of the Australian tea tree oil (from *Melaleuca alternifolia*), could be detected only in negligible amounts ($\leq$1% each) demonstrating the remarkable difference between these two competitive tea tree oils.

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SESQUITERPENE HYDROCARBONS OF DISTILLED AND COLD-PRESSED LIME OILS

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The composition of the non-polar sesquiterpene hydrocarbons of distilled and cold-pressed lime oils of different geographic origin was investigated in detail.
GC and GC/MS studies under optimized separation conditions permitted the unambiguous identification of the single representatives of this compound class.
Within the scope of the present work we succeeded in verifying a number of sesquiterpenes which were so far unknown in the lime oils.

α-Santalene, epi-β-santalene, β-sesquiphellandrene, 4(14),7(11)-selinadiene and (E)-α-bisabolene were identified for the first time in distilled lime oils from Mexico and Peru.
γ-Curcumene could be added to the previously described sesquiterpenes of cold-pressed lime oil.

While distilled lime oil, which as a steam distillate of the mixture of juice, peel and oil can be considered as a process flavouring, the gentle production process for the cold-pressed oils — which derive from the same fruit — leads to a completely different type of oil. This is also reflected in the respective sesquiterpene hydrocarbon patterns, qualitative and quantitative differences will be shown and discussed.

The unambiguous identification of 4,7(11)-selinadiene, a sesquiterpene which is present in distilled lime oil in a ratio of 0.2 - 0.3%, required the molecule in pure form. After successful isolation from this matrix, detailed structural analysis via NMR studies was carried out.

THE ESSENTIAL OIL FROM LEAVES AND RIPE BERRIES OF LAURUS AZORICA (SEUB.) FRANCO, GROWN ON MADEIRA

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Laurus azorica (Seub.) Franco is an endemic species of Madeira, the Azores and the Canary Islands, that is found as a dominant tree in the Madeiran laurel wood zone from 200 to 1200m. The tree grows up to a height of 15-20m, with a dense crown formed by dark green glossy leaves, strongly aromatic when crushed. The berries, black when ripe, are the favourite food of *Columba palumbus* ('pombo torcaz'). 'Loireiro', 'loureiro' or 'louro' as *L. azorica* is most commonly known, has become scarce above 1200m due to overgrazing, burning and cutting. Being one of the first colonizers in the zones of recovery and regeneration of the Madeiran natural vegetation, the laurel has importance in several domains [1-4].

The essential oils from fresh leaves and ripe berries of *L. azorica* were isolated by hydrodistillation to estimate the oil yields, and by distillation-extraction to determine the percentage composition of the oils. The oils isolated from the leaves and berries were obtained in a yield of 0.83% and 0.4% (v/w), respectively. Fifty components of the oil from the leaves and fifty-five of that from the ripe berries were identified, amounting to 88% and 82% of the total oils, respectively. The monoterpene fraction was dominated by α-pinene (32%), β-pinene (15%) and 1,8-cineole (13%) in the leaf oil, and by *trans*-β-ocimene (11%) and α-pinene (11%) in the berry oil. The sesquiterpene fraction, that attained only 6% in the leaf oil, reached 22% in the berry oil, *allo*-aromadendrene (6%) and β-elemene (4%) being major components of the latter oil. *allo*-Aromadendrene (1%) was also the main component of the sesquiterpene fraction from the leaf oil. A third fraction, that of phenylpropanoids, was present only in the leaf oil, elemicin (2%) being its major component.

In a previous study of the steam distilled leaf oil of *L. azorica* grown in the Hortus Botanicus of the University of Groningen, Hokwerda *et al.* [5] also found α-pinene, β-pinene and 1,8-cineole as main components, although in different amounts (13%, 7% and 10%, respectively) compared with those found in the present study. *allo*-Aromadendrene was the main component of the sesquiterpene fraction, although the exact amount was not reported (5-10%). Cinnamyl acetate and elemicin (both 1-5%) were the major constituents of the phenylpropanoid fraction. The oil isolated by Hokwerda *et al.* [5] was obtained in a lower yield (0.36%) than that from the present study.

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VOLATILES OF CARROT SEEDS SUPERCRITICAL CO_2 EXTRACT

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Chemical composition of volatile portion of Carrot Seeds *(Daucus carota .L)* isolates obtained by commercial CO_2 extraction was studied. Over 60 components were identified using GC-MS technique. The major odoriferous constituents of the extract were found to be carotol (11%), geranyl acetate(2.6%), methyl eugenol (2.0%), juniper camphor (2.0%), geraniol(1.9%) and asarone (0.8%).

Lippia alba Mill N.E. Br. (Verbenaceae) as source of linalool (29TH ISEO)

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The essential oil from the leaves of *Lippia alba* (Verbenaceae) was analysed by GC (retention indices and GC/MS. Linalool proved to be the major constituent (78,9%), besides 1,8-cineole (6.5%), germacrene D (3.5%), trans-caryophyllene (2.7%) and alpha-terpineol (2.0%). This species is worthy of examination as a rosewood oil substitute.

Linalool is one of the most useful monoterpene alcohols for the perfumery industry, as well as in the large scale route to vitamin E [1]. A natural source for linalool, even though commercially no longer competitive, it is still represented by rosewood from Brazilian *Aniba* genera [2]. Since the major species of rosewood face imminent extinction, an urgent substitution has been in demand for more than thirty years [3]. Among species of Verbenaceae which have been shown to be sources of metabolites closely related to linalool, especially citral and cineole [4], *Lippia alba*, a herbaceous plant growing in the Eastern region of São Paulo state, showed 67-83% of linalool in its oil (1% from leaves), when analysed by GC/MS, IR and ^{1}H-NMR.

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CHEMOTAXONOMIC STUDY OF *PICEA* SPECIES

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The genus *Picea* comprises about 40 species originating from temperate and subtropic areas around the world. Systematically the species are very close to one another and spontaneous crossing is possible, which leads to the occurrence of hybrids. von Schantz and Juvonen [1] studied the essential needle oil from different *Picea* species and showed that the composition, and especially the oxygen containing compounds (borneol and bornyl acetate) can be used as chemotaxonomic markers. In this study we investigated the needle oil of the following *Picea* species collected from Mustila arboretum, Elimäki, south Finland: *P. jezoensis* (Sieb. et Zucc.) Carr., *P. omorika* (Panc.) Purk., *P. omorika „nana", P. koraiensis* Nakal, *P. asperata* Mast., *P. pungens* Engelm., *P. pungens „glauca", P. glehnii* (F. Schmidt) Mast., *P. glauca* var. albertiana (S. Brown) Sarg., *P.* mariana (Mill.) B.S.P., *P. obovata* Ledeb., *P. glauca* (Moench.) Voss., *P. glauca „conica", P. mariorika, P. rubens* Sarg., *P. sitchensis* (Bong.) Carr. and *P. engelmannii* Parry ex Engelmann.

Branches from the above mentioned species were collected in January 1997. The needles were ripped off and submitted to hydrodistillation in a Karlsruher-Stahl apparatus for 2 h. The essential oils were analysed by GC and GC-MS using a polar (NB-351, 25 m x 0.32 mm i.d.) and a chiral (β-cyclodextrin, 25 m x 0.32 mm i.d.) column. α-Pinene and camphene were the main monoterpene hydrocarbons, sometimes limonene was present in high amounts. Usually the (-)-enantiomers of α-pinene and camphene were predominant. Bornyl acetate was the main oxygenated hydrocarbon. The data will be submitted to cluster analysis in order to group the material. Cluster analysis has earlier been shown to be effective in grouping this kind of data [2].

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THE ESSENTIAL OIL COMPOSITION OF' TWO RARE OILS, *RAVENSARA AROMATICA* AND *THYMUS MASTICHINA*, USED IN AROMATHERAPY

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Ravensara aromatica J.F.Gmel (Lauraceae) is a 18-20 m high, evergreen tree growing on the island of Madagascar. The leaves contain an essential oil smelling of camphor and eucalyptus oil [1]. It is used by the locals as an universal remedy for physical and mental disorders. The leaves are used for the preparation of ointments and cough mixtures and extracts of the bark and leaves are used for indigestion. An aromatherapist would use ravensara oil for the treatment of asthma, influenza, inflammations in the respiratory tract and Virus infections, such as *herpes simplex* and *herpes zoster.* A sample of *Ravensara aromatica* oil (Crearome Ab, Gamleby, Sweden) analysed by GC-MS showed 1,8-cineole as main component (75%), followed by α-terpineol (16%).

Thymus mastichina L. (lamiaceae) the Spanish „forest marjoram" *(Mejorana silvestre* or *Tomillo blanco)* or as it is vernaculararly called *„Almoraduz",* grows wild in the low sections of the provinces Sevilla, Huelva, Granada and Almeria. The plant flowers from end of April to June and has a camphoraceous odour. The oil is reported to contain ~72% cineole, 7-8% α-pinene, linalool and a phenol [2]. In addition α-terpineol is reported in an oil from Portugal [3]. *T. mastichina* oil obtained from Crearome Ab (Gamleby, Sweden) analysed by GC-MS had 1,8-cineole (48%) as main component, followed by linalool (14%). Camphor, α-terpineol and thymol were also detected together with oxygenated monoterpenes and sesquiterpene hydrocarbons.

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CRYPTOCARYA ODORATA FROM NEW CALEDONIA: COMPOSITION OF THE LEAF, BARK AND HEARTWOOD ESSENTIAL OILS

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Cryptocarya odorata Panch. et Séb. (Lauraceae) is a tree growing up to 10 m in height in New Caledonia. The leaves, bark and heartwood were collected and submitted to hydrodistillation using the Clevenger apparatus. Essential oils were thus obtained in 0.09%, 0.12 % and 0.046% yields respectively, and submitted to GC-MS analysis. While the bark and heartwood essential oils mainly contain various proportions 6-alkyl- and 6-alcenyl-5,6-dihydro-2H-pyran-2-ones (the so-called «C10, C12, C14 and C16 massoya-lactones») along with minor proportions of sesquiterpenes, the leaf essential oil is made of sesquiterpenoids exclusively.

A number of minor or even trace components which had been found previously in Massoya bark (*C. massoy* Kosterm.) essential oil from Irian Jaya [1], were also found in *C. odorata* bark oil.

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COMPARISON OF TEA TREE OIL AND MARJORAM OIL

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The principle components of tea tree oil (essential oil of *Melaleuca altemifolia)* like terpinen-4-ol, α- and γ-terpinene, p-cymene, α-thujene, α- and β-pinene, sabinene, myrcene, α-terpineol, β-phellandrene, limonene and linalool are also present in considerable amounts in the essential oil of majoram *(Majorana hortensis).* Tea tree oil of high quality applied for therapeutical purposes should contain more than 30 % of terpinen-4-ol and less than 15 % of 1,8-cineole. Marjoram oil used for flavouring should contain much less terpinen-4-ol, but as much as possible of the isomeric alcohol (Z)-sabinene hydrate and its ethyl ester, respectively. (Z)-sabinene hydrate isomerises easily to terpinen-4-ol in acid conditions, subsequent products can also be the terpinenes, which are typical for both essential oils. The composition of marjoram oil can be controlled by variation of reaction time and pH value. This way an oil is obtained, which is similar to tea tree oil (Figure 1) but without its main negative compound 1 ,8-cineole.

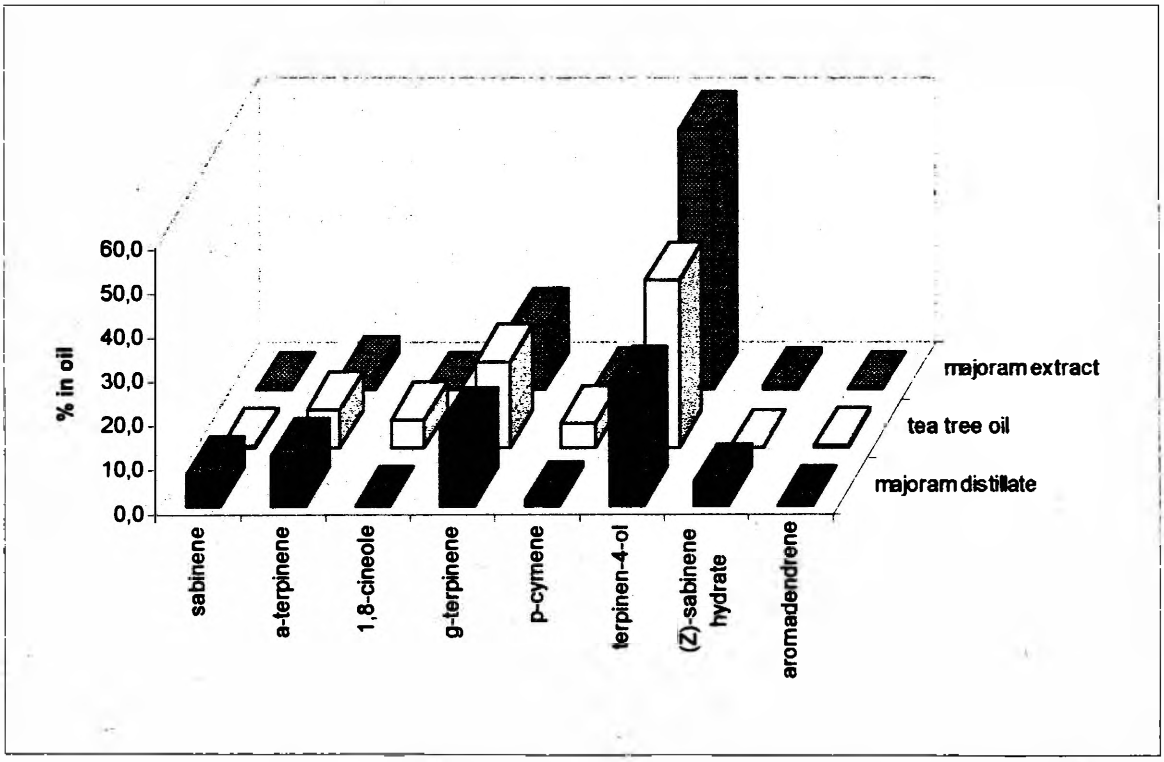

Figure 1. Principle components of a marjoram isooctane extract (air-dried leaves were homogenised in 0,1N HCl and extracted after 6 hours), a commercial tea tree oil and an essential oil of marjoram (the leaves were homogenised in a pH 3 buffer and distilled after 3 hours).

CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM *DAUCUS CAROTA* GROWING IN POLAND

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Both wild carrot and cultivated ones, *Daucus carota L. ssp. carota* and *Daucus carota L. ssp sativus*, respectively, are very popular in Poland.

The aim of the presented study was to compare the chemical composition and the yield of carrot seed essential oils derived from 9 varieties of cultivated carrot and wild carrot collected from natural stations. The oils were analysed by GC and GC/MS, and their yields were estimated by hydrodistillation. In the 9 cultivares of carrot seed, the yield of essential oil varies from 0.6 to 1.5%, whereas there is 3.2% in the wild carrot seed. The main constituents of seed carrot oil from *Daucus carota L. ssp sativus* are: α-pinene (3.7 - 28.4%), sabinene (1.8 - 23.9%), β-pinene (2.8 - 4.7%) and carotol (20.9 - 48.0%). The essential oil from *Daucus carota L. ssp. carota* mainly contains: α-pinene (39.7%), sabinene (53.1%) and β-pinene (2.3%).

The chemical composition of essential oils obtained by steam distillation of carrot seed extracts was also studied. Carrot seeds were extracted, using Soxhlet apparatus, with methanol, ethanol, chloroform and ethyl acetate. The results, in comparison with the direct hydrodistillation method will be given.

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dissolved in BGB-25 (25% phenyl-, 75% methylpolysiloxane)
Temperature Range: 30°C to 250°C/270°C

BGB-173 *50% 2,3-diacetyl-6-tert-butyldimethylsilylated-α-cyclodextrin*
dissolved in BGB-1701 (14% cyanopropylphenyl-, 86% methylpolysiloxane)
Temperature Range: 20°C to 220°C

BGB-174 *50% 2,3-diacetyl-6-tert-butyldimethylsilylated-ß-cyclodextrin*
dissolved in BGB-1701 (14% cyanopropylphenyl-, 86% methylpolysiloxane)
Temperature Range: 20°C to 220°C

BGB-175 *50% 2,3-diacetyl-6-tert-butyldimethylsilylated-γ-cyclodextrin*
dissolved in BGB-1701 (14% cyanopropylphenyl-, 86% methylpolysiloxane)
Temperature Range: 20°C to 220°C

BGB-176 *50% 2,3-dimethyl-6-tert-butyldimethylsilylated-ß-cyclodextrin*
dissolved in BGB-5 (5% phenyl-, 95% methylpolysiloxane)
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ESSENTIAL OIL OF WILD GROWING MENTHA PULEGIUM L., LAMIACEAE FROM YUGOSLAVIA

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According to the Flora of Serbia [1], Mentha pulegium L. (= Pulegium vulgare Mill.) is an aromatic plant species, widely distributed over many natural localities. In Yugoslavia, it is well known under numerous common names [2] and often used in traditional medicine instead of cultivated Mentha piperita L., with the same therapeutic purposes. The chemical composition of its essential oil has not been clarified yet, so it became a subject of our interest. The quantity of essential oil in dried aerial parts of this plant species was determined by steam distillation of the herb, according to the Proceeding I of the Yugoslav Pharmacopoeia [3], when we gained 0.18 to 0.22 %. Analysis of the obtained essential oil was performed using GC and GC/MS; component identification was conducted by comparing the experimental retention indices with those of standard substancies added to the mixture. The identification was also confirmed by GC/MS. The principal component was identified as p–menthone (30.90 %). The other important components were pulegone (14.10 %) and neomenthol (13.81 %), along with caryophyllene–trans–oxyde (9.01 %), isomenthone (5.53 %), neomenthyl–acetate (4.34 %) and piperitone (3,36 %). In analyzed essential oil, limonene, 1,8–cineol, octane–3–ol and camphor occured in quantities of about 1 % (1.00, 1.34, 1.76 and 1,03 % respectively). Knowing that pulegone as well as menthone could be easily converted into menthol, that these two components make a total amount of about 45 % in our essential oil and the fact that Mentha pulegium is widely distributed in nature, we can make a conclusion that the essential oil of this plant species could become a plentiful and inexpensive resource for semisynthetic industrial production of menthol.

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COMPOSITION OF THE ESSENTIAL OIL OF NEPETA RTANJENSIS DIKLIĆ ET MILOJEVIĆ, LAMIACEAE FROM SERBIA

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Found and established in 1974, this plant species endemic to Serbia is spreaded over only a few natural localities on the southern slopes of mountain Rtanj [1,2,3]. This plant species has been a subject of some morphologic, anatomic and chemical researches [4,5]. The principal compound of its essential oil was identified as nepetalactone (64 %), but the chemistry of the others remained unrevealed yet.

The essential oil was obtained by steam distillation of the aerial parts of the plant. Analysis of the essential oil was performed using GC and GC/MS. Component identification was conducted by comparing the experimental retention indices with those of standard substancies added to the mixture. The identification was also confirmed by GC/MS. The analyzed essential oil contained over 80 components, with 19 identified. The principal one was nepetalactone (cis, trans) – 86.40 %. Nepetalactone (cis, cis) has also been identified, but in significantly lower amount (0.87 %). The essential oil of Nepeta nuda and the other species of this genera except N. rtanjensis, can be characterized by the germacrene–D contents (0.2–23 %); in our essential oil, this structure was absent. Among the other components, 7 of them was present in both essential oils: α– and β–pinene, 1,8–cineole, linalool, α–copaene, β–bourbonene, β–caryophyllene and α–terpineol, along with nepetalactones previously mentioned. The essential oil of N. rtanjensis is also characterized by presence of eugenol and isoeugenol (0.63 and 0.3 %, respectively), two phenolic structures which could not be found in essential oils of N. nuda and the other species of this genera.

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CONTRIBUTION TO THE CHEMICAL STUDY OF THE ESSENTIAL OILS OF THE *CYPERACEAE*

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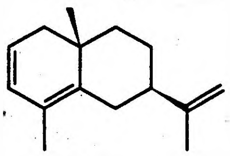
The *Cyperaceae* constitute a familly of grass-like plants, which usually flourish in marshy or aquatic habitats. The genus *Cyperus* forms an important part of this familly, since *Cyperus* contains many useful species. Their use against migraine, abdominal pains or as fumigants and to flavour food has been reported [1, 2].

The investigation of the essential oil of *C. alopecuroides*, *C. papyrus* and *C. rotundus* showed a great similarity in their chemical composition. Cyperene [3] is the major hydrocarbon constituent of the three oils. Cyperotundone [4] has been identified as the main oxygenated component in the oil of *C. papyrus* and *C. rotundus*, while α-cyperone [5] is the major oxygenated constituent of *C. alopecuroides*, although cyperotundone is also present. However some differences appear in the distribution of newly isolated compounds. The eudesmane sesquiterpenoids (1)[6], (2)[6], (3) and (4) were identified only in *C. alopecuroides* while the compound (5) was isolated only from *C. rotundus* and *C. papyrus*. The new compounds were characterised by spectroscopic analysis and their structure confirmed by chemical transformations.



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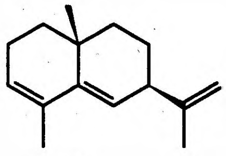

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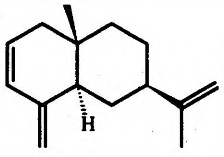

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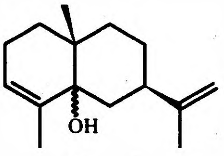

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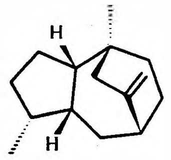

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FLAVOR COMPONENTS OF JAPANESE TRADITIONAL FOOD, VEGETABLE *(CYPTOTAENIA JAPONICA* HASSK „MITSUBA") FOR JAPANESE FOOD.

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Abstract *Cyptolaenia japolzica* HASSK („Mituba" in Japanese) is one of the Japanese traditional vegetable for topping of Miso soup and Japanese Stile steamed egg. There are three kinds (Nemituba (1), Kirimituba (2) and Itomituba (3)) of Mituba. The composition of the volatile oil from these three kinds of *Cyptotaenia japonica* HASSK have been investigated by capillary GC, GC-MS. 1 and 2 were similar components, but 3 was different The oil contained more than 40 volatile components of which mainly components were terpenoids. The main terpenoid of 1 and 2 were α-selinene (39.63% and 38.54%, respectively) and 3 was β-cubebene (24.77%).

COMPOSITION AND CHEMICAL POLYMORPHISM OF THE ESSENTIAL OIL FROM *PIPER LANCEAEFOLIUM*

Marisa Mundina[1], Roser Vila[1], Felix Tomi[2], Xavier Tomas[3], Jose F. Ciccio[4], Tomas Adzet[1], Joseph Casanova[2], Salvador Cañigueral[1]
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Continuing our work on the chemical composition of the essential oils from Latinoamerican *Piper* sp. (1,2) we report here the volatile constituents of the essential oil from leaves and spikes of *Piper lanceaefolium* H.B.K. from Costa Rica. Essential oils obtained by hydrodistillation from leaves and spikes of *Piper lanceaefolium* H.B.K. from Costa Rica were analysed by GC-FID, GC-MS and ^{13}C-NMR. Main constituents found in the oil from leaves were sesquiterpene hydrocarbons, especially ß-caryophyllene and germacrene D, and phenylpropanoids, being elemicin and parsley apiol the major ones. The volatile oil from spikes showed monoterpene hydrocarbons namely ß-pinene, and the same phenylpropanoids as in the oil from leaves as the major constituents. Results obtained in the analysis by GC-FID and GC-MS of the essential oils from individual plants of different geographic origin were submitted to chemometric Cluster Analysis and Principal Component Analysis, showing the presence of three different types of oils (i) parsley apiol/elemicin, (ii) elemicin/ parsley apiol/ dill apiol, and (iii) parsley apiol/ dill apiol.

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CHEMICAL COMPOSITION OF ESSENTIAL OILS OF *HYPTIS SPECIGERA* AND *HYPTIS SUAVEOLENS* FROM CAMEROON

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Many species of the genus Hyptis (Lamiaceae) are used for culinary and medicinal purposes, and numerous reports on the composition of essential oils of *Hyptis suaveolens* from many countries, have been published. Recently L.V. Hac analysed the essential oil of *H. suaveolens* from Vietnam [1] , a few reports existed on the *H. specigera* [2]. To the best of our knowledge, the essential oils from these two cameroonian species have not been investigated until now. The objectives of this study were to analyse the oil from different parts of the plant material by GC and GC/MS using conditions reported by J. Jirovetz et al [3], and to correlate the chemical composition to the odor impression.

The best sources of the essential oil from plant material are leaves for *H. suaveolens* and flowers for *H. specigera*. Yields of essential oil obtained by hydrodistillation of these materials are: *H. suaveolens* (0.12% from fresh leaves, 0.3% from dried leaves), *H. specigera* (0.3% from fresh flowers and 0.9% from dried flowers).

The olfactory evaluations of the different oils from the plants are: The essential oil of the leaves of *H. suaveolens* is described as green and terpineol-like (direction of *Pinus sylvestris*) respectively the oil from *H. specigera* as pinene-, cypress-tree- and spruce-like.

More than sixty five compounds have been identified in *H. suaveolens* oil from fresh leaves, the major components belong to the monoterpene group (56%) represented by sabinene (23.6%), limonene (5.3%) and terpinolene (5.9%). The oil from the fresh flowers of *H. specigera* contains less components (fifty two compounds), with the monoterpenes as the main group (96%) represented by sabinene (20.3%), α-thujene (11.5%) limonene (7.6%), terpinen-4-ol (7.3%). These two species contain a higher percentage of sabinene (>20%), but differ also by their composition, especially as to α-thujene which content is much higher in *H. specigera*.

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ANALYSES OF THE ESSENTIAL OIL OF THE LEAVES OF THE MEDICINAL PLANT *COLEUS ZEYLANICUS* (BENTH.) *CRAMER* FROM INDIA

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The composition of the essential oil of the leaves of *Coleus zeylanicus* was analysed by GC-FID, GC-MS and olfactoric evaluation. In this essential oil used in traditional *(Ayurvedic)* Indian medicine [1-6] more than 80 compounds were detected. Totally 64 compounds could be identified with monoterpenes (especially geraniol and nerol derivatives), hexane- and octane-derivatives as main constituents. The effects of these volatile, responsible for the pleasant odour of this sample as well as particularly for the reported therapeutic properties, will be discussed.

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1,8-CINEOLE RICH ESSENTIAL OILS FROM MOZAMBIQUE: *EUCALYPTUS TERETICORNIS AND E. CINEREA*

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The water distilled essential oil from the leaves of *Eucalyptus tereticomis* and *E. cinerea,* from Mozambique were obtained using a Clevenger-type apparatus, in a yield of 3.34% and 7.8% (v/w), dry weight basis, respectively.

The oils were analysed by means of GC and GC/MS systems with DB-Wax GC column. Forty and thirty five components were identified in the *Eucalyptus tereticomis* and *Eucalyptus cinerea* oils, representing 87,7% and 94.2% of the total components detected, respectively.

The major constituents of *Eucalyptus tereticomis* were 1,8-cineole (68.2%), limonene (9.1%), p-cymene (2%) and α-bisabolol (2%), while those of *Eucalyptus cinerea* oil were 1,8-cineole (69.3%), α-terpinyl-acetate (10.9%), α-terpineol(5.7%), limonene(4.6%) and α-pinene (3.7%).

Key words index

Eucalyptus tereticomis, Eucalyptus cinerea, Myrtaceae, essential oil composition, 1,8-cineole, α-terpinyl-acetate

CAPILLARY GAS CHROMATOGRAPHIC ANALYSIS OF CAMOMILE (*CHAMOMILLA RECUTITA* /RAUSCH./) ESSENTIAL OIL AND "FLORASOL" EXTRACT

E. Hethelyi[1], M. Palfi-Ledniczky[2]. P. .F Wilde[3], I. Zambo[4], [1]University of Horticulture and Food Industry, Dept. of Medicinal Plant Production, H-1114 Budapest, Villányi u. 29/31,[2]AROMAX Ltd, H-1211 Budapest, Szallitok u. 4, [3]The ",Ol-factory" 91 Front Street, Sowerby Thirsk UK-Y07 1JP, [4]Pharmafontane Ltd. H-1106 Budapest, Csillagvirag u. 8.

Both herbal tea and essential oil made of the flowers of blue camomile (*Chamomilla recutita)* have been used in traditional medicine internally for digestive disorders because of their antiphlogistic, vulnerary properties and general sedative action and externally for their healing effect when there is aching or pains generally and because of their analgesic, antiphlogistic, cicatrisant and antiseptic properties. Some of the components (e.g. α-bisabolol, chamazulene, bisabolol-oxide I and II) are known to contribute to these properties.

An extraction method offers new components. This new extraction technique Is solvent extraction with "Florasol" the hydrofluorocarbon, HFC 134a (1,1,1,2-tetra-fluoro-ethane). The HFC134a extract (prepared in Great Britain) and essential oil of the flowers (*Chamomillae anthodium*) obtained by steam distillation have been analysed with a Shimadzu GC-14B capillary gas chromatograph. The content of the main components have been compared. The main components are listed in order of increasing retention times are:

Component	EO (%)	Extract (%)
Bo-II*	7,0	2,3
Alfa-B**	9,5	2,8
Chamazulene	2,3	
BO-I***	54,5	31,0
cis-Spiro-ether	18,0	23,5
Herniarine		1,9
Others	8,7	38,5

There are two obvious findings, one: chamazulene is formed when the essential oil is distilled (heat and water are needed), second: herniarine, a particular UV-B filter compound is present in the HFC 134a extract.

ESSENTIAL OILS FROM TWIGS AND BERRIES OF DIFFERENT POPULATIONS OF *JUNIPERUS BREVIFOLIA*

J. A. da Silva[1], *L. G. Pedro*[1], *P. A. G. Santos*[1], *A. C. Figueiredo*[1], *J. G. Barroso*[1], *R. P. Tenreiro*[1], *C. A. Ribeiro*[2], *S. G. Deans*[3], *A. Looman*[4], *J. J. C. Scheffer*[4], [1]*Departamento de Biologia Vegetal, Faculdade de Ciências de Lisboa, C2, Campo Grande, 1780 Lisboa, Portugal;* [2]*Departamento de Biologia, Universidade dos Açores, Rua da Mãe de Deus 58, 9502 Ponta Delgada, Portugal;* [3]*Department of Food Science & Technology, Scottish Agricultural College, Auchincruive, Ayr KA6 5HW, Scotland, U. K.;* [4]*Division of Pharmacognosy, LACDR, Leiden University, Gorlaeus Laboratories, PO Box 9502, 2300 RA Leiden, The Netherlands.*

Juniperus brevifolia (Seub.) Antoine (= *J. oxycedrus* L. var. *brevifolia*), locally known as „cedro-das-ilhas“, „cedro-do-mato“, „cedro-da-terra“ or „zimbro“, is an endemic species of the Azorean archipelago that occurs on all the islands, except S. Maria and Graciosa [1, 2]. Due to the high quality wood of this species, it has been continuously cut and old stands of some size are now rarely seen in the archipelago [2]. As part of a screening programme of the aromatic flora of Macaronesia, the present study deals with the analysis of the essential oils from *J. brevifolia* collected at five Azorean islands.

The composition of the essential oils isolated from twigs and from unripe berries of several populations of *J. brevifolia* was analysed by GC and GC-MS. All oil samples were dominated by their monoterpene fraction (84-94%), limonene (41-79%) and α-pinene (6-40%) being the main oil components. The sesquiterpene fraction occurred in a concentration ranging from 1% to 10% and diterpenes amounted up to 6%. Cluster analysis of the essential oils revealed two distinct groups, one from the twigs and the other from the berries. The enantiomeric composition of α-pinene and limonene showed a clear homogeneity between all the oil samples.

Acknowledgments: This study was granted by the Fundação para a Ciência e a Tecnologia (FCT), Lisboa, under research contract no PBIC/C/BIA/2070/95. We are grateful to FCT for scholarships to J. A. da Silva and P. A. G. Santos.

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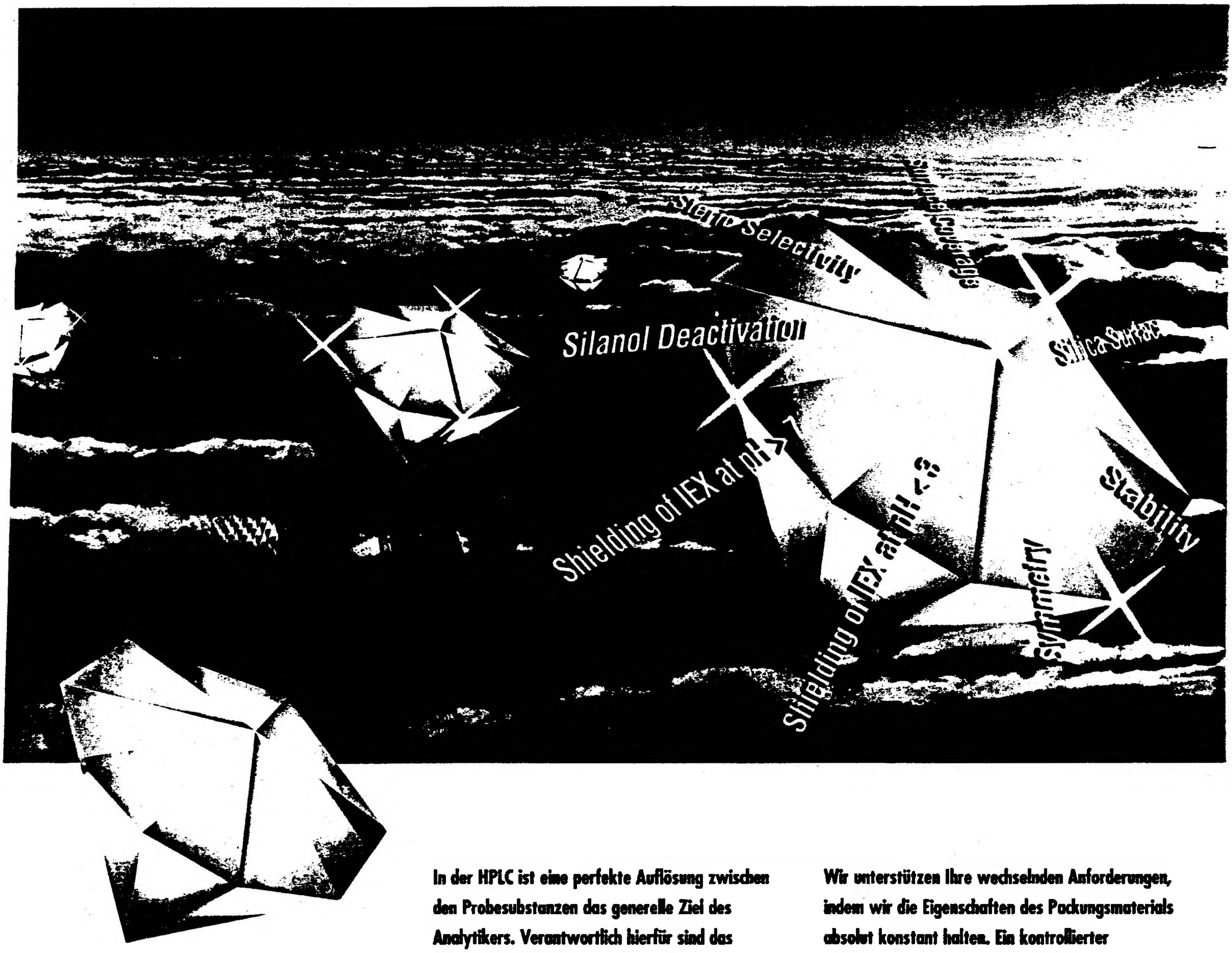

Doxycyclin gehört zu einer Gruppe von antibakteriellen Substanzen, den Tetracyclinen. Die USP 1990 schreibt eine chromatographische Methode zur Bestimmung vor. Die Trennung wird auf einer C18-Säule mit N,N-dimethyl-octylamin als Peakverbesserer durchgeführt.
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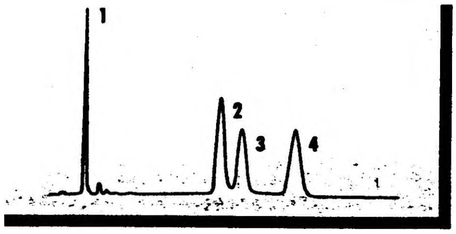

In der HPLC ist eine perfekte Auflösung zwischen den Probesubstanzen das generelle Ziel des Analytikers. Verantwortlich hierfür sind das System, die Zusammensetzung der mobilen Phase, die Reproduzierbarkeit der stationären Phase sowie die Qualität und Haltbarkeit der Packung. Die Reproduzierbarkeit der letzten beiden Parameter ist eine Grundvoraussetzung für Ihre erfolgreiche und sichere Analytik, die mit Purospher® RP-18 endcapped gewährleistet ist.

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CHARACTERIZATION OF SOME SPECIES OF (*TAGETES SPP.*)

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Marigold is an important source of numerous secondary metabolites such as carotenoids (1), flavonoids, thiophens and essential oils having biological activity.

The oils are known to possess insecticidal activity

The content and composition of the oil is strictly related to the species and greatly influenced by the environmental conditions.In our study, seven species of marigold were grown in Po Valley near Bologna to evaluate their bioagronomic characteristics. The tested species resulted very different for the morphological characters and for the lenght of the vegetative cycle. This latter, in a few cases, was too long and not suitable for our mild climate (2). The harvested fresh material (flowers and leaves) was separately steam distilled in a Clevenger-type apparatus to evaluate the essential oil yields. The oils were characterized byGC/MS and showed significant differences in the quali-quantitative composition confirming an important infraspecific variability (3).

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STEREOCHEMICAL ANALYSIS OF DITERPENES BY ENANTIOSELECTIVE CAPILLARY GAS CHROMATOGRAPHY

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A number of enantiomers of diterpene hydrocarbons ($C_{20}H_{32}$) was isolated as reference compounds from plants and the enantiomeric separation of several of these diterpene hydrocarbons was demonstrated by enantioselective gas chromatography with cyclodextrin derivatives as chiral stationary phases .

As a rule from higher plants, like *Pinus* species, diterpene hydrocarbons can be obtained as enantiomers with the *usual* configuration. In exceptional cases, e.g. *Araucaria araucana*, also a higher plant, only diterpene hydrocarbons with the *unusual* configuration [1] were obtained.

A quite different situation is found in *Araucaria heterophylla*, where the cooccurence of the enantiomers with the *usual* and the *unusual* configuration is observed. As a remarkable result, both enantiomers of the diterpene hydrocarbon sclarene could be detected in *A. heterophylla* in a ratio of (+) : (-) = 5.2 : 1. Diterpenes have never before been described as enantiomeric mixtures in individual plants.

Diterpene hydrocarbons with the *unusual* configuration are predominantly found in liverworts.

In order to check the limits of enantioselective gas chromatography with cyclodextrin derivatives as stationary phases for the resolution of diterpenes and to see if enantiomeric separation is applicable to more polar compounds, both enantiomers of sclareol were separated. The (-)-enantiomer is a constituent of *Salvia sclarea* and is industrially used for the preparation of Ambrox®, an important fragrance compound. (+)-Sclareol is a constituent of *Conyza trihecatactis* (Asteraceae) [2]. Both enantiomers of sclareol were dehydrated with $POCl_3$ in pyridine at 0°C and again the enantiomers of the hydrocarbon products could be resolved without any difficulty on cyclodextrin phases.

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[2] R. Torrenegra, J. Robles, R. Waibel, M. Löwel, H. Achenbach, Phytochemistry **35** (1994) 195

DETERMINATION OF VOLATILE COMPONENTS OF ESSENTIAL OIL OBTAINED FROM *LAURUS NOBILIS* L. COLLECTED IN SOME PROVENANCES OF AGEAN REGION

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Laurus nobilis L. is a Mediterranean plant which grows wild in coastal provenances of Turkey and it is also widely used for industrial purposes. Two major parts of *Laurus nobilis* L. are used in industry: one of them is laurel leaf, other part is laurel berry. Laurel berry is except of this study, but main subject of our study is to determine the essential oil content and composition of volatile components in laurel leaf. This study consists of four main steps. These steps are as follows:

1. Collection of laurel leaf samples from coastal provenances (Karaburun , Manisa Gümüldür , Izmir-Kemalpaşa, Izmir-Bornova, Marmaris, Mordağı)
2. Drying of samples
3. Obtaining of volatile oil samples by water distillation (3 hours)
4. Qualitative and quantitative identification of major volatile constituents of volatile oil (GC and GC/MS)

(1) J. Pino, P. Borges and E. Roncal, Die Nahrung, 37 (1993), 592-595
(2) D. A. Pearson, E. N. Frankel, R. Aesbach and J. B. German, 45 (1997), 578-582

COMPOSITION OF THE ESSENTIAL OILS FROM TWIGS AND BERRIES OF *JUNIPERUS OXYCEDRUS* GROWN IN PORTUGAL

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Continuing our investigations on the genus *Juniperus* from Portugal we now report on the composition of twigs and berries essential oils of *Juniperus oxycedrus* (43 populations and 174 individual plants) grown at several locations of Portugal.

The essential oils were isolated by hydrodistillation and submitted to qualitative and quantitative analysis by GC on two different polarities columns and by GC-MS. The identity of the oil components was assigned by comparison of their retention indexes and MS, with those of authentic samples. Major compounds identity was confirmed by 13C-RMN.

More than 40 components were identified amounting up to 98 % of the total oils. Either for twigs and berries oils, monoterpene hydrocarbons were the main components (65-92%). In the oils from twigs, α-pinene was the major constituent attaining 85%. Oils from berries, besides the α-pinene (41-56%), contain important percentages of Germacrene D (1-19%).

On the contrary of other portuguese *Juniperus* species, oils from populations and individual plants of *J. oxycedrus* shown a homogeneous composition, attesting that no infra-subspecific variability occurs at this *taxon.*

ESSENTIAL OIL COMPOSITION OF *CURCUMA LONGA* AND *ZINGIBER OFFICINALE* WILD RHIZOMES FROM S.TOMÉ AND PRÍNCIPE

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The rain forest of the Republic of S. Tomé and Príncipe is of immense value to man since it is a source of medicines, health related products and foods. Little information was available about the essential oils from plants of this country. For this reason, we have undertaken a study on the aromatic plants of S. Tomé and Príncipe.

Continuing our study on the essential oils of the medicinal aromatic plants from S. Tomé and Príncipe, we now present the chemical composition of the essential oils of *Curcuma longa* L. and *Zingiber officinale* Rosc., plants whose rhizomes are widely used in these islands. *Curcuma* rhizomes are used by the traditional healers for the treatment of jaundice and rheumatism, and also as a spice and food colorant. The rhizomes of *Zingiber* are very popular because besides their use as a food spice, they are highly valued against gastro-intestinal disorders, rheumatism, flue and are also reputed as aphrodisiac.

The essential oils of these plants were obtained by hydrodistillation of the rhizomes. They were analysed by GC and GC-MS, using two fused silica capillary columns of different stationary phases (carbowax and methylsilicone), and by ^{13}C-NMR.

The major compounds in the volatile oil of *Curcuma longa* are α-turmerone (23.9%), ar-turmerone (12.8%), curlone (11.5%), α-phellandrene (15.5%) and 1,8-cineole (10.2%). The essential oil of *Zingiber officinale* is characterized by the five well-known sesquiterpenes α-zingiberene (15.1%), β-sesquiphellandrene (7.3%), β-bisabolene (5.7%), E,E-α-farnesene (4.6%) and α-curcumene (3.4%), and also by a high content of geranial (14.4%) and neral (9.1%).

THE ESSENTIAL OIL OF *ACORUS CALAMUS* L.

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Sweet flag, Acorus calamus L., is a perennial plant that grows more or less abundantly throughout the northern hemisphere, inhabiting pond edges, marshes, swamps, and the banks of rivers and streams. The rhizome (rootstock) of this medicinal plant contains a pleasantly aromatic oil and its preparation is used in appetizers, bitters, spasmolytics and antiulcer preparations and antihistaminergic activity was presented too [1].

The essential oil was obtained by hydrodistillation from rhizomes, which were collected in the Zatin locality, on the East-Slovakian lowland. GC/MS analyses were carried out on a Hewlett-Packard 5890/5970 equipment. 57 compounds are representing 70.8 % of the essential oil could be identified. The results of qualitative-quantitative essential oil composition are showed that this medicinal plant from the East Slovak localities has very interesting properties. Sufficient content of components (α-; - β asarones: 25.3 %, cis-; trans- asaronaldehydes: 6 %, shybutones: 16 %, isoshybunone: 5.5 %, calamenene: 2 %, cadinols: 2.9 % and further sesqviterpenes: 4 %) can enable to use this Slovak Sweet flag population for a medicinal utilization.

[1] K. P. Odenthal, 22nd ISEO, St. Vincent, Italy (1991), p. 57

THE VOLATILE LEAVE OILS OF *JUNIPERUS EXCELSA* COMPARED WITH *J. EXCELSA SSP. POLYCARPOS*

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Results of many studies showed (e.g. Adams 1992) that *J. excelsa* have more seeds per cone than *J. excelsa ssp. polycarpos* (4-6 Vs. 2-4, respectively).

However *J. excelsa* is monoecious, but *J. excelsa ssp. polycarpos* is dioecious and both of them appears in Iran. Essential oils from leaves of *J. excelsa* (monoecious with 2-4 seeds per cone) collected in spring, summer and autumn 1995 in mountains of Iran was analysed by GC-MS.

The volatile leaves oils of male and female trees (*J. excelsa ssp. polycarpos*) and male-female trees (*J. excelsa*) were similar in spring and autumn (samples was dried under laboratory conditions), But content of these were varied in summer. Then sampling season is significant in classification. In addition, character of seed number per cone related with climate as seed number per cone in raining year were increased to 5-8 in both of *J. excelsa* and *J. excelsa ssp. polycarpos*. We must classify Iranian Juniper (*J. excelsa* and *J. excelsa ssp polycarpos*) as *J. excelsa*.

COMPOSITION OF ESSENTIAL OILS OF *JUNIPERUS EXCELSA* LEAVES DURING DIFFERENT TIMES OF YEAR

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Leaves essential oil from *Juniperus excelsa* collected in spring, summer and autumn in 1995 from Iranian mountains and analysed by GC-MS. The total oil (yield 1 %) consisted of almost 20 constituents.

Seasonal investigation of the essential oils showed mainly constituents variations among quantitative changes. Content of some constituents decreased significantly in summer (e.g. α-pinene was 67.5%, 11.6% and 55.1% in spring, summer and autumn, respectively). But content of another constituents increased (e.g. verbenone was 2.5%, 6.1% and 3.3% in spring, summer and autumn, respectively). However content of some constituents did not show any change (e.g. β-caryophyllene was 1.8%) in three seasons.

STUDY OF ESSENTIAL OIL FROM JUNIPERUS EXCELSA "BERRIES" (CONES) IN NATURAL HABITATS (IRAN)

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GC-MS analysis of the essential oil from the cones of *Juniperus excelsa* in natural habitats was carried out. The total oil (yield 2.5 %) consisted of almost 22 constituents. Seasonal investigation of essential oils showed mainly quantitative and qualitative variations. The total oil was minimum (0.8 %) in spring increase after growth season. Maximum total oil was seen in autumn and winter.

The constituents variation of the essential oil was interesting. For example α-pinene content was 6.2% ,75.8 % and 57.4 % in spring, summer and autumn, respectively. Results indicated high content of *trans*-verbenol (24.8%), verbenone (9.3%), elemol (6.3%) in spring, but content of these was trace in summer and autumn. However, other constituents (e.g. myrcene , limonene) were trace in spring.

CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF TWO SALVIA SPECIES FROM IRAN, *SALVIA VIRGATA* Jacq. AND *SALVIA SYRIACA* L.

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Fifty-eight species of die genus *Salvia* (Lamiaceae) are to be found in Iran, seventeen of them are endemic. Due to the usage of this genus or their essential oils in the food, drug and perfumery industries [1,2], we are investigating the oils of *Salvia* species which grow wild or are cultivated in Iran. The chemical composition of the essential oils of *Salvia virgata* Jacq. and *Salvia syriaca* L. growing wild in Iran were examined by GC and GC-MS. Fifteen components were characterised for *S. virgata* with β-caryophyllene (46.6%), germacrene B (13.9%), β-caryophyllene epoxide (13.2%), spathulenol (6.4%) and germacrene D (5.7%) as the major constituents. Twenty-two components were identified for *S. syriaca* with germacrene B (34.8%), germacrene D (29.2%), α-ylangene (3.6%) and spathulenol (3.4%) as the major constituents.

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ESSENTIAL OIL GC/MS ANALYSES OF TWO BULGARIAN THYME CULTIVARS

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Bulgarian thyme cultivars with essential oil fragrances different from the commonly accepted reminiscent of thymol thyme odour [1] was bred in the Institute of Botany.

Cv. "Slava" was selected from *Thymus vulgaris* L. (Czech origin) [2]. Twenty-four essential oil compounds were identified by GC/MS and the main are geraniol (24.17%), geranyl acetate (20.94%), germacrone (10.57%), geranial (7.30%), β-caryophyllene (5.62%), neral (4.09%). The expert's assessment of the fragrance was "delicate, fresh, with citral notes".

Cv. "Pagane" was selected from *Thymus marshallianus* Willd. (Czech origin) [3]. Nineteen compounds were identified and significant participation of geraniol (66.04%) and geranyl acetate (11.93%) determined the frangrance as "fresh, delicate, strong, sweet, new geraniol-type essential oil".

High essential oil content of both varieties (Slava - 0.46%, Pagane - 0.50%) and high yield of aboveground mass (up to 1100 kg/dka, 1300 kg/dka, respectively) combined with possibilities for twice harvesting a year ensure high yield of essential oil (7 kg/dka and 8.2 kg/dka, respectively).

Desirable characteristics of these varieties enhance the traditional use of thyme essential oil in phytotherapy and pharmacy and favour their application in perfumery and cosmetics.

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[2] Y. Yankulov, T. Stoeva and R. Taleva, Zemedelie (Sofia), 5 (1990), 43-44.
[3] Y. Yankulov, T. Stoeva and R. Taleva, Zemedelie (Sofia), 8 (1990), 24-25.

COMPOSITON OF THE ESSENTIAL OILS OF THYMUS *PECTINATUS VAR. PECTINATUS* FROM TURKEY DURING DIFFERENT STATES OF DEVELOPMENT

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The genus *Thymus* (Lamiaceae) is represented by 39 species and all together 64 taxa in Turkey, 23 taxa being endemic. This paper describes oil composition from herbal parts of *Thymus pectinatus* var. *pectinatus,* endemic in Turkey, collected three months before flowering, one month before flowering and at flowering stage. The water distilled oils were analysed by GC-MS. Best oil yield (1.8%) was obtained from flowering plants. Maximum thymol content (62%) was observed in the oil from plant material collected one month before flowering.

QUALITATIVE-QUANTITATIVE CHARACTERISTICS OF CHAMOMILE ESSENTIAL OILS FROM PRODUCTION CONDITIONS OF AGRICULTURAL COOPERATIVE FARM “ROZKVET” IN NOVA LUBOVNA, SLOVAKIA

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German Chamomile, *Chamomilla recutita* (L.)Rauschert, belongs to the most popular medicinal plants in the Slovak phyto-medicine and aromatheraphy.

Slovakia is one of the European countries in which particular attention has been devoted to research of chamomile in all its aspects, including the breeding of this medicinal plant [1]. Based on the study of chamomile's pharmacodynamic properties, the sesquiterpenes: /-/- α-bisabolol and chamazulene are considered to be the most valuable constituents. Gradually, within the years 1972 - 1992 the chamomile varieties ‘ BONA ’, ‘ALFA ’, ‘GORAL ’and ‘LUTEA ’ have been bred at the Agricultural Cooperative Farm “ROZKVET” in Nova Lubovna, Slovakia. The chamomile essential oil of these very good-quality varieties (Table) is in the production programme of this company.

Table : Essential oil qualitative-quantitative characteristics of the Slovak chamomile varieties from the large-scale distillation (the production season: 1996/1997).

Slovak chamomile varieties	key component contents (%) into chamomile essential oil			
	Bo	Cha	BoA	BoB
variety ‘ BONA ’, diploid	30 ± 2	10 ± 2	1 ± 1	1 ± 1
variety ‘ ALFA ’, diploid	32 ± 2	15 ± 2	1 ± 1	0.1 ± 1
variety ‘ GORAL ’, tetraploid	25 ± 2	10 ± 2	5 ± 2	5 ± 2
variety ‘ LUTEA ’, tetrapoid	35 ± 2	13 ± 2	0.1 ± 1	0.1 ± 1

Bo: /-/-α-bisabolol, Cha: chamazulene, BoA: /-/-α-bisabololoxide A, BoB: /-/-α-bisabololoxide B

The raw-material for essential oil distillation is cultivated by the large-scale chamomile production. The essential oils are destilled in the large-scale distillation apparatus specifically designed for aromatic and medicinal plants.

[1] I. Salamon, J of Herbs, Spices, and Medicinal Plants. **1(1/2)** (1992) 37

SESQUITERPENE CONSTITUENTS OF THE LIVERWORTS *CALYPOGEIA MUELLERIANA* AND *BAZZANIA TRILOBATA*

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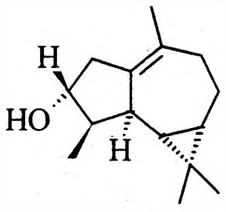
The lipophilic constituents of the liverworts *Calypogeia muelleriana* and *Bazzania trilobata* were investigated. In addition to many known compounds three new sesquiterpenes could be isolated and identified: two sesquiterpene alcohols with aromadendrane skeleton, (+)-3-hydroxyledene (**1**) and (+)-4,5-dehydroviridiflorol (**2**), from *Calypogeia muelleriana* [1] and a new sesquiterpene hydrocarbon with cadinane skeleton, (-)-cis-1(6),4-cadinadiene (**3**), from *Bazzania trilobata*. The components of the essential oil were separated by preparative GC and HPLC. The structure elucidation was carried out mainly by NMR spectroscopic techniques: ^{1}H-, ^{13}C-, $^{1}H^{1}H$-COSY-, HMQC-, HMBC- and NOESY experiments. For verification of structural assignments, chemical conversions were carried out. The absolute configuration was determined by a chemical correlation. The products of the correlation reaction were compared with reference compounds by enantioselective GC using cylodextrin phases.



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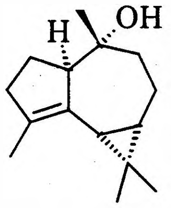

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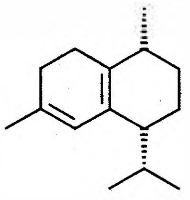

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SEASONAL VARIATION OF SOME ESSENTIAL OILS COMPONENTS OF *THYMUS ALBICANS* HOFFMANNS & LINK

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Thymus albicans Hoffmanns & Link is a straight shrub with nearly 80 cm high, similar to the *T. mastichina*, growing on sandy soils of Iberian Peninsula Southwest. In Portugal, this plant can only be found at Algarve and even so in very diminute quantities.

In this work the chemical composition of the essential oils of *T. albicans* at different development stages were evaluated. For this, the plant material was collected in April, June and July at Gambelas, Algarve.

Five batches of plant material were collected: flowers and leaves during full flowering (June and July) and only leaves during the flowering beginning (April).

The oils were isolated from fresh plant material by hydrodistillation, for 4 hours, using a Clevenger-type apparatus.

The analyses showed that the major component was 1,8-cineole whose percentages ranged from 57.7 % to 65.8 %, either in leaves or flowers.

From April to July, the concentrations of sabinene (2.2-1.6), 1,8-cineole (65.1-57.7), *trans*-ocimene (0.5-0.1), linalool (1.1-0.3) and camphor (2.7-1.0) decreased, whereas the percentages of borneol (2.5-8.6), α-terpineol (3.7-4.4), bornyl acetate (0.2-0.5), globulol (0.8-1.4) and intermedeol (0.2-0.6) increased, in leaf samples.

From June to July, the concentrations of camphene (3.2-2.4), *trans*-ocimene (0.3-0.1), linalool (2.6-1.7), camphor (2.1-0.5) and α-terpineol (5.4-3.7) decreased, while the percentages of α-terpinene (0.3-0.5), *p*-cymene (0.3-0.5), 1,8-cineole (58.2-65.8) and γ-terpinene (0.5-0.9) increased, in flower samples.

In June, the flowers were richer in α-pinene, camphene, sabinene, β-pinene, myrcene, linalool, borneol and α-terpineol, and less rich in *p*-cymene, 1,8-cineole and camphor than the respective leaves. In July, the flowers were richer in α-pinene, 1-8-cineole and linalool and less rich in sabinene, camphor, borneol and α-terpineol than the respective leaves.

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CHARACTERIZATION OF FLAVOR COMPONENTS IN WINE WITH SOLID PHASE MICROEXTRACTION (SPME), GC AND GC/MS

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Characterization of Volatile in wines provides important information on the origin and method of preparation. One class of volatiles, terpene alcohols, is critical in assuring the proper taste and aroma of wines, particularly Muscat and Cabernet Sauvignon.

A simple, time consuming sample preparation method is presented in this work using SPME.

A recent study with SPME involved determination of flavor volatiles in a fruit beverage (1).

The terpene alcohols in the wine samples were identified by comparison with the NIST 92 library in the saturn software. For 4 typical terpene alcohols the minimum detection levels are determined with a polyacrylate fiber in a 12 % ethanol-water mix.

Conclusion: Without any sample preparation, other than pipetting the wine into the Autosampler vials, SPME was found to be very effective for determing trace alcohols in wine at ppb levels (2). Both PDMS (Polydimethylsiloxane) and Polyacrylate SPME fibers were useful. The linear response upon spiking with analyte indicated that in wines with up to 20 % ethanol, the fibers were not saturated.

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MULTIDIMENSIONAL GAS CHROMATOGRAPHY, ONLINE COUPLED WITH ISOTOPE RATIO MASS SPECTROMETRY (MDGC-IRMS): A NEW COUPLING TECHNIQUE FOR ANALYTICAL AUTHENTICATION OF GENUINE FLAVOUR COMPONENTS

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In recent years, enantioselectivity and isotope discrimination during biosynthesis have been recognised as important principles of authenticity evaluation.

Evaluation of these principles requires efficient clean-up methods to determine characteristic components in complex mixtures.

On the basis of the relationship between stable isotopes ($^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{15}N/^{14}N$ and $^{18}O/^{16}O$) many facts have accrued about the genuineness of natural compounds. Online combustion of gas effluents from capillary gas chromatographic columns have made great strides in direct determination of $^{13}C/^{12}C$-ratios of individual components. Last years, in the origin specific analysis of flavours and fragrances an integral evaluation of isotopic data was established and characteristic fingerprints of different essential oils were deduced. But unfortunately capillary gas chromatographic separation in GC-IRMS has not always been sufficient to determine characteristic compounds from complex matrices. Multidimensional gas chromatography, equipped with a preseparation column, online coupled with a main column, fulfils highest performance in direct sample clean-up.

This poster presents the direct coupling of the MDGC-System via an interface with the isotope ratio mass spectrometer as a powerful method of origin specific analysis [1,2]. This technique achieves utmost accuracy and precision of IRMS-measurements. Scope and limitations of this sophisticated coupling technique are discussed.

The chromatographic separation with the MDGC-system enables to the isotopic analysis of many components in citrus oils. Characteristic fingerprint curves were found for genuine oils. Integral authenticity profiles including isotopic data, enantiomeric distributions as well as quantification of compounds analyzed are proved to be a reliable indicator of genuineness.

[1] D. Juchelka, T. Beck, U. Hener, F. Dettmar and A. Mosandl, J. High Resol Chromatogr, **21** (1998) 145-151.
[2] D. Juchelka, F. Podebrad and A. Mosandl, GIT-Fachz Lab, **4** (1998) 363-367.

CHARACTERISATION OF ESSENTIAL OILS PRODUCED BY STEAM DISTILLATION CLOSE TO THE FIELD

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Essential oils of thyme, parsley, sage and basil for example were produced using a steam distillation plant close to the field.

The steam distillation process was developed by the Fraunhofer-Institute for Environmental, Safety, and Energy Technology UMSICHT (Oberhausen, Germany) in close co-operation with an agricultural company founded by farmers and is described elsewhere.

The maximum oil content of the herbs was determined using hydrodistillation according to DAB. Yields of hydrodistillation and steam distillation were compared.

The essential oils generated by the steam distillation process close to the field were characterised according to standard procedures and quality data sheets were set up.

The separation and identification of the individual constituents was performed using non-chiral and chiral GC methods.

In addition the essentials oils originating from the steam distillation process were compared to different oils from several suppliers available on the market.

On-going research covers the field of Supercritical Fluid Extraction (SFE) using pure carbon dioxide and Solid Phase Micro Extraction (SPME).

SPIROSESQUITERPENES IN THE ESSENTIAL OIL OF *LEPECHINIA* SPECIES (LAMIACEAE) FROM VENEZUELA

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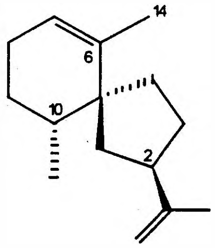
In the course of our investigations of essential oil plants from the Venezuelan Andes, *Lepechinia bullata* (BRIQ.) EPL. and *Lepechinia salviaefolia* (KUNTH) EPL. (Lamiaceae) were studied.

Especially two sesquiterpene hydrocarbons attracted our attention because of their unknown mass spectra. Isolation of the two compounds was achieved by a combination of column chromatography over silica gel and preparative GLC.

The structural elucidation was accomplished by means of spectroscopic methods, namely EI-Mass, ^{13}C-NMR, ^{1}H-NMR and 2D NMR experiments.

The compound which amounts to about 45 % in the essential oil of *L. bullata* and to about 20 % in *L. salviaefolia* turned out to be premnaspirodiene (**1**) a hydrocarbon with a spirovetivane skeleton. It was isolated in the early eighties from *Premna latifolia* ROXB. [1] and *Premna integrifolia* Linn. (Verbenaceae) [2] but has never turned up in other essential oils. Its exact configuration could now be confirmed by NOESY experiments.



1

The second compound of our interest occurs up to about 10 % in the essential oil of *L. bullata* and seems to be as well a spiro[4.5]decane but with a different skeleton. This compound is still under investigation. A complete structure elucidation will be presented.

[1] Rao, Ch.B., Raju, G.V.S., Krishna, P.G. (1982) Indian J. Chem. 21B: 267-268.
[2] Rao, Ch.B., Krishna, P.G., Suseela, K. (1985) Indian J. Chem. 24B: 403-407.

THE ESSENTIAL OIL AND GLYCOSIDICALLY BOUND VOLATILE COMPOUNDS OF *JUNIPERUS COMMUNIS* (L.)

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The content and composition of the essential oil and glycosidically bound volatile compounds of *Juniperus communis* were studied in this paper. These investigations were performed with dried needles. The isolation of the essential oil was performed by hydrodistillation in modified Clevenger type apparatus with simultaneous extraction of glycosides. The yield of essential oil was W = 0.18%. The essential oil was fractionated on microcolumn of silica gel with solvents of different polarity. One fraction of terpene hydrocarbons and four fractions of oxygenated terpene compounds were obtained.

After glycoside isolation from aqueous extract, the final purification was performed by column chromatography [1]. The glycosides were also isolated from plant material by cold ethyl acetate extraction [2]. After enzymatic hydrolysis of glycosides, the obtained aglycones, as well as fraction of the essential oil were analysed by gas chromatography-mass spectrometry using two columns with different polarity.

Sixty-three compounds were identified in essential oil fractions. On contrary, only twenty-eight compounds were identified in the essential oil without fractionation. The main components of essential oil were: α-pinene, sabinene, terpinene-4-ol, widrene, β-phelandrene, γ-terpinene, β-terpinene, α-terpinene and other compounds in smaller amounts.

The content of aglycones was 10.7 mg/kg of plant material and 68.0 mg/kg, depending on the method of isolation. Sixteen aglycones were identified by means of water extraction, respectively twentytwo by means of ethyl acetate extraction. Aglycones obtained by the two methods of isolation were: 3-hexene-1-ol, 1-octen-3-ol, methyl salycilate, benzyl alcohol, α-methylbenzyl alcohol, methyl-3-hydroxybenzoat, 2-phenylethanol, 2-phenylpropanol, 3-phenyl-2-propenal, p-cymen-8-ol, o-methoxybenzyl alcohol.

[1] J. Mastelić and D. Kuštrak, Acta Pharm., **47** (1997) 133-138.
[2] M. Miloš, J. Mastelić and A. Radonić, Croat. Chem. Acta, **71** (1998) 139-145.

ESSENTIAL OIL COMPOSITION OF *MICROMERIA JULIANA* (L.) BENTHAM EX REICHENEB.

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Micromeria juliana (L.) Bentham ex Reicheneb. is growing wild in Dalmatia, southern Croatia and only in some Mediterraniean regions. The content of essential oil in this plant is low and composition is very complex. The essential oil was isolated from green parts of the plant by simultaneous hydrodistillation-extraction in a modified Likens-Nickerson type apparatus for three hours. The yield of essential oil for dried plant material prior to flowering was W = 0.07% , respectively W = 0.11% for plant material during flowering.

The essential oil was fractionated on microcolumn of silica gel with solvents of different polarity. One fraction of terpene hydrocarbons and four fractions of oxygenated terpene compounds were obtained. All fractions, as well as essential oil were analyzed by gas chromatography-mass spectrometry (GC-MS) on two columns with different polarity of stationary phases [1]. The chromatograms of essential oils are very complex and contain hundred or more separated peaks. The more complete analysis was obtained from GC-MS analysis of the fractions. Monoterpene, sesquiterpene and nonterpene compounds were identified in the essential oil. The major components were: caryophyllene, linalool, α-pinene, β-pinene, α-gurjunene, δ-+γ-cadinene, borneol, Ar-curcumene, limonene and smaller percentage of other components. 3-octanol, 1-octen-3-ol, phenylacetaldehyd, p-methylacetophenone, methyl salycilate, β-damascenone and octanoic acid were identified as nonterpene compounds.

The contents of linalool, caryophyllene, α-gurjunene, α-humulene and cadinene were several times increased and these of borneol, β-cubebene, Ar-curcumene, β-bisabolene, manool and limonene were decreased several times during the flowering of the plant.

[1] J. Mastelić, M. Miloš, D. Kuštrak and A. Radonić, Croatica Chem. Acta, **71** (1998) 147-154.

DILL ETHER AND ITS *CIS*-STEREOISOMERS: SYNTHESIS AND ENANTIOSELECTIVE ANALYSIS

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Key Words:

Dill ether, stereoselective synthesis, dill ether stereoisomers, enantioselective GC

Dill (*Anethum graveolens L.*) is known as a remedial herb and spice all over the world. The composition of the essential oil differs in various plant parts and changes during the ripening of the dill.

Dill ether (3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzo[*b*]furan) is the most important constituent in dill oil from an organoleptic standpoint.

Given that the sterically unfavorable *trans*-ring connection is not present, four stereoisomers are possible. The stereochemistry of dill ether has been clarified, showing the *3S,3aS,7aR*-configuration.

So far, no data about simultaneous enantioselective analysis of the stereoisomers have been reported. Thus, a new stereoselective synthesis and the enantioselective analysis of dillether and its *cis*-isomers are presented.

The simultaneous analysis of all four stereoisomers was achieved by enantioselective capillary gas chromatography (enantio-GC) using octakis(2,3-di-O-butyryl-6-O-*tert*-butyldimethylsiyl)-γ-cyclodextrin (di-butyryl-TBDMS-γ) in OV 1701-vi as the chiral stationary phase.

The elucidation of the relative and absolute configurations of the stereoisomers was deduced from H,H-COSY in connection with ^{1}H-NMR/NOE measurements.

The enantioselective analysis of an authentic dillweed oil unequivocally confirms dill ether as the only natural stereoisomer to be found in dill.

By use of the enantioselective gas chromatography/olfactometry (enantio-GC/O) the odor characteristics of the four stereoisomers were evaluated. Only dill ether is proved as the unique character impact compound of dill, while its stereoisomers show different, but unspecific odor characteristics. [1]

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THE ESSENTIAL OIL OF *PETASITES HYBRIDUS*

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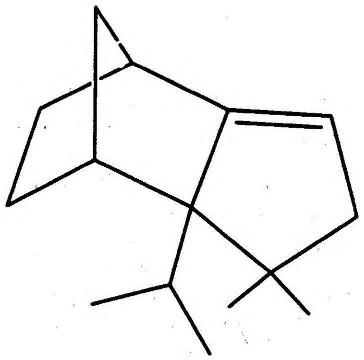
The essential oil of *Petasites hybridus* (*Asteraceae*) has been used for the treatment of several diseases in traditional medicine. Previous articles on the secondary metabolites of this plant reported the identification of several sesquiterpenoic compounds [1, 2]. During our preliminary analysis of the essential oil using GC-MS, we found that many compounds in the hydrocarbon fraction have not been identified. In this communication we present a new investigation of the hydrocarbon fraction. This fraction contains many commonly found compounds like eremophilene, α-humulene, β-bisabolene etc. In addition, we identified many rare sesquiterpenes e.g. modhephene, silphinene, α- and β-isocomene etc.

Moreover, a new sesquiterpene hydrocarbon, petasitene **1**, was isolated. The structure of this compound was determined by GC-MS, 1D- and 2D-NMR techniques.



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[1] Debrunner, B., Neuenschwander, M., *Chimia* **1994,** 48 (12), 564-569
[2] Brunke, E.-J., DRAGOCO Report **1993**, 3, 102-121

FRAGMENTATION MECHANISM OF ROSE OXIDE IN ELECTRON IMPACT MASS SPECTROMETRY

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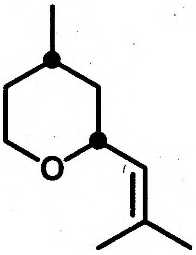
The so called rose oxide (*cis-/trans*-2-(2-methyl-1-propenyl)-4-methyltetrahydropyran **1**,**2**) is a cyclic monoterpenoid ether. (-)-*cis*-rose oxide was isolated in 1959 from Bulgarian rose oil by Seidel and Stoll [1]. Although rose oxide is a minor compound in rose and geranium oils it contributes substantially to the unique bloomy-green top notes of these essential oils. Rose oxide is indispensable in creating of artificial rose and geranium oils and therefore much work has been done in its synthesis, and in studies of its constitutional and conformational isomerism. It is rather surprising that no information is available about the fragmentation of rose oxide in mass spectrometry on the basis of labelling experiments. The fragmentation mechanism of *cis-/-trans*-rose oxide in mass spectrometry is therefore investigated using specifically deuterium labelled isotopomers. The main fragmentation pathway is not initiated by the loss of the 4-methyl group as previously assumed. The labelling experiments are in agreement with a fragmentation mechanism which includes a hydrogen rearrangement of H-2



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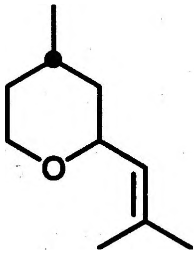

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[2] M. Wüst, S. Fuchs, A. Rexroth, T. Beck and A. Mosandl, Eur Mass Spectrom, submitted

STRUCTURE ELUCIDATION OF *CIS*- AND *TRANS*-ROSE OXIDE KETONE AND ITS ENANTIOSELECTIVE ANALYSIS IN GERANIUM OILS

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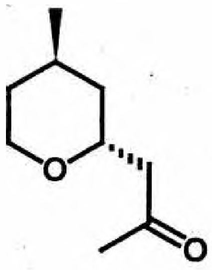
cis- and *trans*-Rose oxide ketones (**1-4**) are prepared enantioselectively using (*R*)-3-methylpentanedioic acid monomethyl ester as a chiral building block. All four stereoisomers of rose oxide ketone can be analyzed simultaneously by enantioselective GC using heptakis-(2,3-di-O-acetyl-6-O-*tert*.-butyldimethylsilyl)-ß-cyclodextrin in OV-1701-vi as the chiral stationary phase. The enantiomeric distribution of *cis*- and trans-*rose* oxide ketone in geranium oils of different origin was investigated and found to correlate with the enantiomeric ratios of *cis*- and *trans*-rose oxide. A pathway for the biogenesis of rose oxide ketone in *Pelargonium* species is proposed including rose oxide as the precursor of rose oxide ketone [1].



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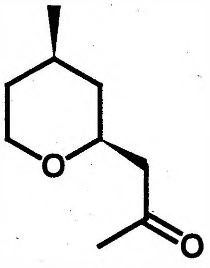
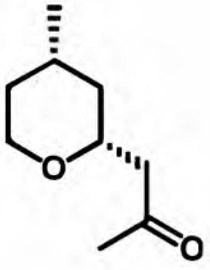
(2*R*,4*R*)



2

(2*S*,4*R*)



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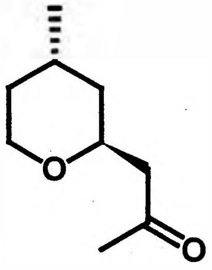
(2*R*,4*S*)



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(2*S*,4*S*)

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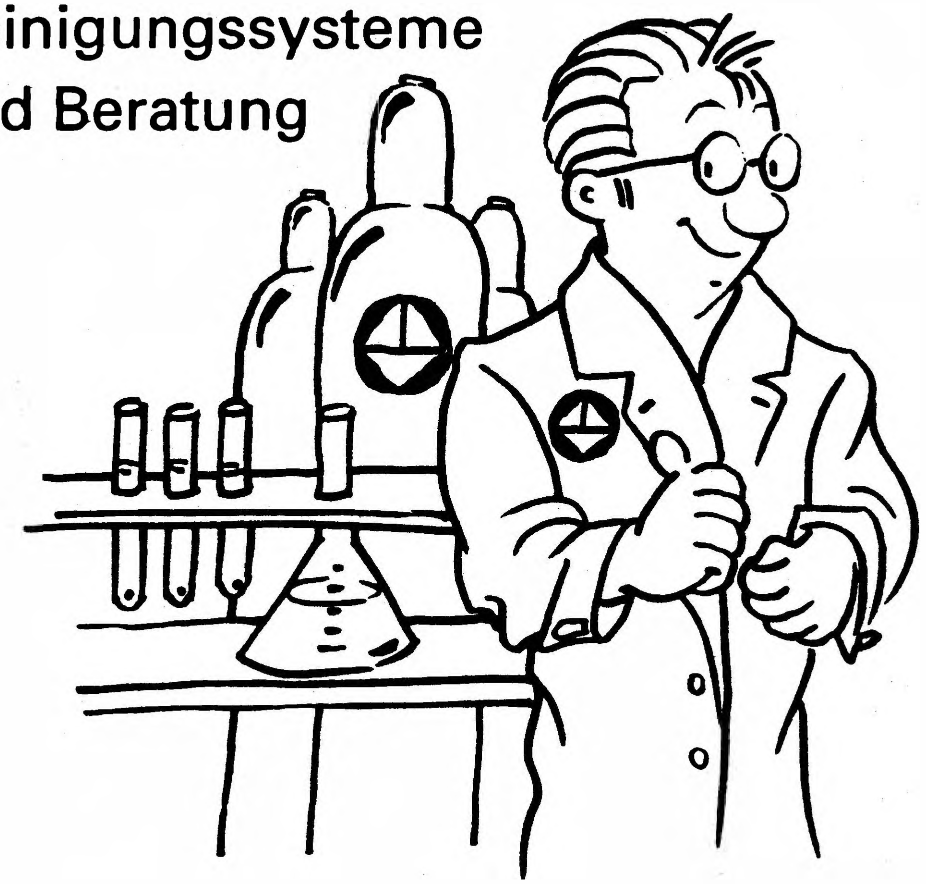

NETWORK ON THE VALORIZATION OF PLANT MATERIALS (AFRICA)

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Canada through the International Development Research Center (IDRC) has financially supported R & D projects related to the valorization of plants with the objective of finding applications of unexploited raw material and of improving currently used inefficient methods of production which would eventually lead to creating employment and to generating income for underprivileged people. About 15 projects are currently in progress and involve twelve african countries.

The products presently under study are essential oils (thyme, verbena, mints, Ocimums, basil, artemisia,...), alguae, cassava, vegetable olis (karity butter, Neem oil,...), as well as specific chemical products: *l*-camphor, *d*-borneol, indigo, tabersonin, carrageenan,...). The countries involved include Morocco, Malawi, Guinea, Senegal, Togo, Benin, Ghana, Burkina Faso, Nigeria, Cameroun, Rwanda, la Tanzania and Zimbabwe.

In addition to specific objectives and the means used to incite synergism between the projects, the research groups and their projects will be presented. Several marketable samples will also be available.

SEASONAL VARIATION AND PROCEDURES INFLUENCING THE YIELD AND QUALITY OF THE ESSENTIAL OIL OBTAINED FROM *JUNIPERUS OXYCEDRUS* SSP. *OXYCEDRUS* BERRIES

Maria Jose' Vieira[1] *and* *Stavros Th. Katsiotis*[2]
[1] *International Centre for Advanced Mediterranean Agronomic Studies MAICH, POB 85, Chania, Crete, Greece,* [2] *Department of Pharmaceutics, School of Pharmacy, Aristotle University of Thessaloniki, 54006, P.O.Box 1589, Thessaloniki, Greece*

ABSTRACT

The effects of the seasonal variation, the ripeness stage, the plant material comminution and the isolation techniques (Hydrodistillation and SDE - simultaneous distillation extraction - of different durations) were evaluated on the essential oil yield and composition obtained from the wild growing *juniperus oxycedrus* ssp. *Oxycedrus* berries, collected from Kallergi (Crete, Greece). The essential oil composition was determined by GC and GC/MS analysis. More than eighty components were detected and thirty-three of them, representing approximately 96.50% of the total oil, were identified. The main constituents were α-pinene (24.45-38.6%), β-myrcene (33.23-40.59%), limonene (2.04-3.25%) and γ-cadinene (15.73-21.20%). The collection period and the different winning procedures proved to have highly significant effects on the essential oil yield (0.07-2.26-%). Regarding the essential oil composition, quantitative variations were recorded for both different collection periods and extraction procedures as well, in the consideration of the main components α-pinene, β-myrcene, limonene and γ-cadinene. The hydrodistillation of 1 hour was sufficient to obtain the highest yield of α-pinene, β-myrcene and limonene under different stages of ripeness and collection period from the comminuted berries; however 3 hours of SDE were necessary to obtain the highest yield of γ-cadinene from the comminuted mature berries harvested in july.

THE LARGE-SCALE DISTILATION OF PINENE ESSENTIAL OIL ON THE AGRICULTURAL COOPERATIVE FARM “ROZKVET” IN NOVA LUBOVNA, SLOVAKIA

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The Agricultural Cooperative Farm “Rozkvet” in Nova Lubovna began to be interested about medicinal, aromatic and spice plant production in 1980. The large-scale distilation of essential oils was started in 1983. At present good cultivation methods, breeding, harvesting and processing, produce plants with the high quality essential oils [1]. These agricultural farm is produced about 20 kinds of essential oils and the main production is the large-scale distilation of pinene essential oils (Table).

Table : The qualitative and qualitative characteristics of key component contents into pinene essential oils.

plant species	production capacity per year (kg)	key component contents (%) into pinene essetial oils	
		α – pinene	bornyl acetate
Scots Pine *Pinus sylvestris* L.	1,800	25 ± 2	6 ± 2
Fir Needle *Abies alba* Mill.	1,000	25 ± 2	1 ± 2
Juniper *Juniperus communis* L.	150	35 ± 2	4 ± 1

Analysis of the essential oils was carried out using a Vega Series Carloerba Gas Chromatograph, connected to a Spectrophysics SP 4270 integrator. The following operating conditions were used: column : DB5, 30 m x 0.32 mm i.d., film thickness: 0.25 μm, carrier gas : nitrogen, adjusted to a flox of 1 ml/min, injection and FID-detector temperatures: 220 ^{0}C respectively 250 ^{0}C. Components were identified by their G.C. retention times, and the resulting values were comparable to those of literature. Oil component standards for comparisom were supplied by Extrasynthese Ltd..

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ENCAPSULATION OF *THYMUS MASTICHINA* L. ESSENTIAL OIL IN STARCH RICE

Moldão-Martins, M. [(1)] *Nolasco, M.A.*[(1)]; *Salgueiro, L.*[(2)]; *Cavaleiro, C.*[(2)] *Esquível, M.M*[(3)] *Bernardo-Gil M.G*[(3)] *and Beirão da Costa, M.L.*[(1)]
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[(3)] *Dep. Chemical Engineering, Instituto Superior Técnico, 1096 LISBOA Codex*

Thymus mastichina L. is an aromatic herb usually used as a food ingredient and medicinal herb at the Mediterranean countries. The unavailability of the fresh plant along the year justifies the interest in the production and preservation of the essential oil. On the other hand the dried plant looses the majority of the volatile compounds.

In the present work the composition of the *Thymus mastichina* essential oil collected in the North of Portugal (Trás-os Montes region) was studied. Aiming to protect less-stable essential oil components against oxidative and photochemical degradation, encapsulation in rice starch spheres produced by spray-dried, were used [1]. Suspensions of rice starch using different bonding agents (low methoxyl pectin (LMP), carboxymethylcelulose (CMC) and gelatin) at different levels were spray dried.

The stability of the encapsulated essential oil in the different matrix were evaluated by Head Space-GC.

The yield of essential oil obtained by steam distillation was 2,2% (w/w). The main compounds are 1,8-cineol (about 74%), followed by b- pinene (about 4%) and a-terpineol (about 3%).

Rice starch spheres produced using 0,2%(w/w) of LMP are quite uniform in size and are a good retention medium for essential oil compounds. A similar behavior was observed when gelatin is the bonding agent at 0,25%(w/w). In what concerns CMC bonding agent at a similar level it is quite apparent that a much higher number of spheres were produced.

[1]. J. Zhao and R. L. Whistler, Food Technology, July (1994): 104-105.

Cape Chamomile
Rebirth of *Eriocephalus punctulatus* Essential Oil

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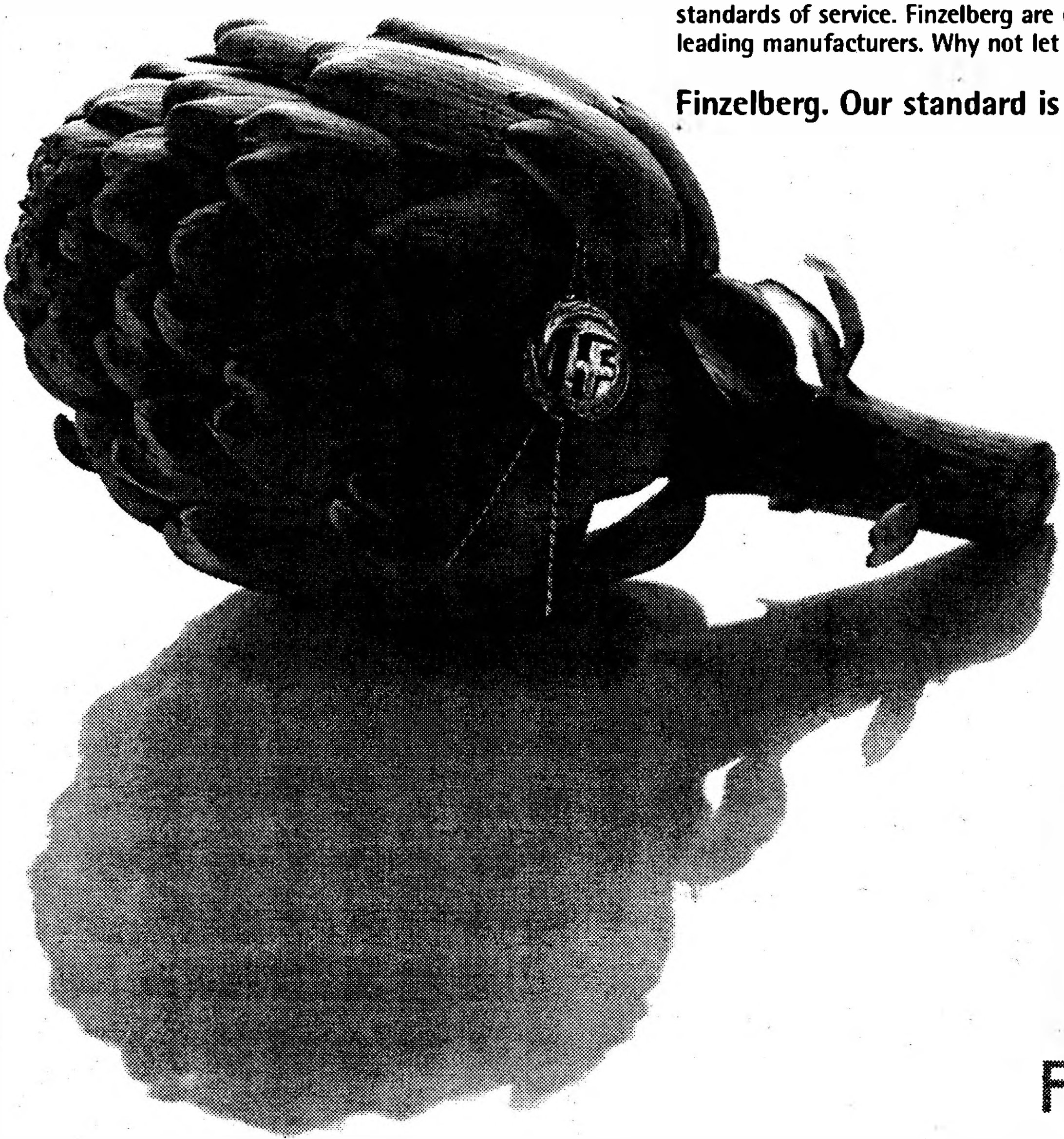
E. punctulatus was first introduced to the European Essential oil industry in the 1970s. Following an enthusiastic response, pilot production was commenced using wild crafted material. During this period two monographs were published [1,2]. Unfortunately it was found that the wild plants did not recover after severe defoliation and the project was abandoned.

Following the development of sustainable harvesting systems and the selection of elite genotypes the crop is now being cultivated in the Western Cape region of South Africa.

The poster deals with many of the practical issues relating to the domestication of this potentially important new essential oil crop, and will provide chemical and physical information on Cape Chamomile oil.

[1] D. Roard, M. Derbesy, H. Peter and M. Remy, Int Flavours Food Addit, **8** (1977)
[2] D. Roard, M. Derbesy, H. Peter and M. Remy Perfum Flavor, **3** (1978)



Influence of phytohormones on growth and essential oil of regenerated plants of *Mentha spicata* L. (Lamiaceae) (29th ISEO)

G. Binder, A. A. Abou-Mandour, F.-C. Czygan, Julius von Sachs Institut für Biowissenschaften, Lehrstuhl für Pharmazeutische Biologie, Julius von Sachs Platz 2, 97082 Würzburg, Germany.

Explants of *Mentha spicata* L. were excised from intact plants. They have been cultured under *in-vitro* conditions on Murashige and Skoog (MS) medium, supplemented with various growth regulators. Callus cultures, obtained from leaves, were regenerated to intact plants.

Growth and morphology were significantly affected by selected phytohormones.

There is no root development in the medium under the influence of (a)-1-Naphtylacetic acid (NAA), but enhanced root formation on the mediums surface. Growth of stem and root is reduced by Abscissicacid (ABA), whereas stolone formation is enhanced. Under the influence of 6-Benzylaminopurine (BAP) regenerates develop to plants with enhanced shoot formation and suppressed shoot, while the root formation is reduced to minimum. Similar effects of the phytohormones as described above are known from the Lamiaceae *Melissa officinalis* L. [1].

By addition of NAA, monoterpenehydrocarbons rise in content, where the content of sesquiterpenes is reduced to about 20-30% of control. All the monoterpene hydrocarbons and some oxygenated ones extremely rise in content by addition of BAP, e. g. pulegone, a trace component in control plants (0,05%), rise up to 1,05% in plants on MS [10 mg*l^{-1}] BAP. The total amount of essential oil is reduced from about 12 mg*g dry weight^{-1} in plants of control to 4 mg*g dry weight^{-1}for regenerates on MS [10 mg*l^{-1}] BAP. Even with ABA some changes in essential oil composition can be noticed.

The addition of phytohormones to the nutrition medium has distinct effects on growth, essential oil content and composition of regenerated plants from *Mentha spicata* L.

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NEW STRAINS OF MINT AS SOURCE OF LINALOOL

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Species of mint are probably the most cultivated and investigated essential oil bearing plants but are well known as sources of menthol and carvon. As it was reported earlier [1], interspecific hybridisation in genus Mentha allows to combine valuable qualities of crossing species and get a number of hybrids with different components of the oil. Our research [2] revealed new strains of mint having linalool as major component. There were distinct differences in fragrance presumably due to the proportions of about 20 minor terpen constituents that make 5–12 per cent of the oil. Essential oils analysis revealed oil components such as α-pinene, β-pinene, limonene, 1,4-cineol, 1,8-cineol, p-cimole, oktanol-3, linalyl acetat, citronelyl acetat, citranelol, α-terpineol, geraniol, geranyl acetat, nerol etc. Strains with bergamot-like odour are out of interest for its high amount of linalool – up to 93 per cent. In our region there is no natural sources of linalool besides coriander. Comparative studies of strains of mint and coriander revealed advantage of mint thanks to high yield of oil, great amount of linalool, absence of borneol, decil aldehyde and camphor in it and good agrotechnical characteristics. The efficiency of isolation of linalool and oxidation it to citral was found much higher than that from coriander (see below).

ID	Amount of oil, %	Linalool, %	Citral, %	Purity of citral, %
Strains of mint: Mi-55, Mi–17, La 15	1.5–2.5	87–93	24–33	90–96
Coriander	0.9–1.2	65–70	Up to 22	Up to 88

The cultivation of mint with linalool is shown to have advantages compare with that of coriander.

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CYTOEMBRYOLOGICAL STUDY ON SALVIA PRATENSIS L. (LAMIACEAE)

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Salvia pratensis is distributed on dried, grassy places and meadows all over Bulgaria. From the air dried herb it has been obtained 1,15% essential oil containing phenolen and cholin. This plant is used in the folk medicine against cough and stomach-ache.

The cultivation of this plant on the experimental field of the Institute of Botany suggested also a cytoembryological investigation. This study concurring the running of the embryological processes in the male generative organs in the flowers.

The structure of the anthers is four layered and comprises an epidermis, an endothecium, an ephemeral middle layer and a tapetum of a secretory type. The running of the meosis is accompanied by great cytomorphological changes in the anther wall layers.

The comparative early degeneration of the tapetum, accompanied in some anthers by a degenerations also of the other layers except the epidermis, is one of the principal causes for the great percentage of the pollen grains degeneration and the impossibility for the further development of the male gametophyte in them.

Hardy-Weinberg Equilibrium of Bisaboloid Chemotypes of Tetraploid Chamomile (*Chamomilla recutita* (L.) Rauschert)

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Various tetraploid highbreed-cultivars of german chamomile differ in their content of essential oil constituents. A population of 198 plants of the bisabolol-containing cultivar 'Manzana' was crossbreeded with 2 plants of the bisabololoxid-containing cultivar BK-2, thus simulating practical conditions in seed production, where accidental crossbreeding could easily happen. The feature „bisabololoxid-containg" is passed on dominantly by the gene O/o. In order to reach a state near the Hardy-Weinberg equilibrium five following generations of this crossbreeding were produced under strict isolation from other cross-pollinating sources. In each generation 500 to 600 individuals were tested with the procedure TAS/TLC for their content of bisabolol and bisabololoxid. The results of this analysis have been comparised with the expected values deduced from a calculation of the Hardy-Weinberg equilibrium for tetraploid organisms. Within this calculation two parameters were unknown, namely the exact genotype of the two BK-2 individuals and the value α, which describes the type of chromosomal segregation during meiosis.

The models formed by variation of this parameters showed a high correspondence with the observed values in each generation except F4. This deviation was probably due to a mistake in the seed manipulation of F4 after the production of F5, as it had no influence on this following generation. The sum of the χ^2-test values of the generations F1, F2, F3 and F5 was used as a measure for assessment of the different models. Significant correspondence was reached by models where the BK-2 individuals had three to four dominant allels and α varied between 0 and 1/7.

COMPOSITION OF THE ESSENTIAL OIL OF *JUNIPERUS EXELSA* M.B. I TO SPECIES RESISTANCE AGAINST PEST-ATTACK OF *GELECHIA SEN* STGR. (LEPIDOPTERA, GELECHIIDAE)

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The studies on the chemical composition of the essential oil from foliage of *Juniperus exelsa* M.B. at different degree of pest attack of *Gelechia senticetella* Stgr. were carried out in 1993 in "Tissata" reserve. Seventeen components have been identified belonging to carbohydrate and oxygen-containing parts of the essential oil. The content of 12 terpene carbohydrates, 3 terpene acetates and 3 free alcohols have been determined. The main component of the carbohydrate part is α-pinene. The content of α-pinene in healthy trees is 41.97%, while in moderate and hardly damaged trees it is respectively 19.06% and 11.32%. The same trend of decreasing can be found for β-pinene - from 2.43% for healthy trees to 0.85% and 0.46% for moderate and hardly damaged individuals. The quantity of limonene varied in a wide range - from 1.39% to 10.35%. Total content of esters is higher than the content of free alcohols. The content of bornylacetate varied from 19.54% to 42.73% showed a trend for increasing in the seriously damaged trees. The main content of alcohol terpinene-4-ol in the healthy trees is 1.27%, while in moderate and hardly damaged trees it is respectively 5.78% and 6.75%. On the base of α-pinene, β-pinene, limonene, Δ-3-carene and γ-terpinene contents in the essential oil, the index of resistance of *Juniperus exelsa* against the *Gelechia senticetella* attack was computed. For seriously damaged trees this index had value 25.22%, while for moderate damaged and healthy trees it was 30.53% and 46.23% respectively. The resistance indices could be used in pest-resistance determination of *Juniperus exelsa*.

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PHYTOCHEMICAL INVESTIGATION AND GROWTH POTENTIAL OF *PERILLA FRUTESCENS* (L.) IN A NORTHERN ENVIRONMENT

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Perilla (*Perilla frutescens* (L.) Britt.) is a tender, frost susceptible, bushy, annual herb belonging to the family Lamiaceae. It is a traditional Japanese herb with both widespread culinary and medicinal uses [1,2]. It can be divided into six different chemotypes, perillaldehyde, perillyl alcohol, phenylpropanoids, furylketones, perillene and citral, and as some of the components of the oil are considered toxic, e.g, perillaketone, it is important that each chemotype is identified correctly [3,4,5]. Two varieties were grown: *P. frutescens* var. *japonica* (green, smooth-leaved) and *P. frutescens* var. *acuta* (purple, crinkled leaves), obtained from various sources. The green variety was a pure perillaldehyde chemotype (83.5-95.1%) or a mixture of perillaldehyde (39.5-56.4%), linalol (2.4-10.6%), limonene (10.0-31.0%) and geranial (16.4-39.0%). The red variety had a light, fragrant scent. Only β-caryophyllene was identified (2.4 - 10.6%) as all other peaks did not match any available standards. Perillaketone was not found in any of the samples. Yields of volatile oil varied between 0.2 and 0.4%. Both varieties had the same form of glandular trichomes. Seed germination was high (94-98%), and after an initial slow start, the plants grew vigorously. In Scotland, weekly growth measurements were taken and growth curves constructed for all main parameters. Of the two, only *P. frutescens* var. *acuta* flowered, indicating a higher possible oil yield at this growth stage. In Finland, the plants were extensively tested for growth and yield to determine the best agrotechnical system of production. It was found that it was possible to grow this plant in more northerly latitudes.

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VARIABILITY IN ESSENTIAL OILS FROM THE GENUS MENTHA CULTIVATED IN DEPARTMENT OF BRASOV, ROMANIA

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The genus *Mentha*, one of the most studied from "Mother Nature's Chemical Factory", consists of about 25 species, a roughly equal number of primary and secundary hybrids and a large number of intraspecific chemical races or chemotipes [1],[2]. Crossing experiments permited to obtain a wide variety of compositions [3]-[8], valuable source of pure terpenoids, or complex systems-essential oils, important in cosmetic, parfumery, or pharmaceutical and food industry. This paper presents the composition of essential oils from different valuable mint hybrids, with a high essential oil content, rust resistance and a good productivity, derived from those obtained by Konka Nicolaie, during his more than fifty years work in mint breeding, only a little part being published [9]. His work is the first Romanian contribution in obtaining other mint chemotypes than menthol/menthone by crossing experiments. Partial results about the composition of some of these hybrids have been communicated [10],[11]. The hybrids, caryologically caracterised (chromosomes number) [12], belong to trans-sabinene hydrate, dihydrocarvil acetate, linalool, menthol/ menthone, carvone/ dihydrocarvone chemotypes. Intrinsic and extrinsic factors affecting yield and oil composition are discussed. Composition of essential oils obtained by hydrodistillation are compared with those of supercritical CO_2 extracts, obtained in batch mode, the last being richer in oxygenated compounds, resposible for fragrant properties [13]. Trans sabinene hydrate chemotype have been explored using supercritical CO_2 extraction in a cvasi-batch mode [14]. Essential oils and supercritical CO_2 extracts were analysed by GC for Kovats indices and quantitative analysis and GC-MS, for qualitative analysis. One oil was separated and analysed by flash chromatography-GC-MS,. [15]- [17]. Encouraging results of biological effect of essential oils against Escherichia coli(67), Escherichia coli (88), Pseudomonas aeruginosa, Staphylococus aureus-Chapman positif, were obtained[18].

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29th ISEO

29th International Symposium on Essential Oils

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Sunday	**Septemper 6, 1998**	**03.00 pm – 07.00 pm**
Monday	**September 7, 1998**	**08.30 am – 06.00 pm**
Tuesday	**September 8, 1998**	**08.30 am – 06.00 pm**
Wednesday	**Septemper 9, 1998**	**08.30 am – 01.00 pm**

UNIVERSITÀ DEGLI STUDI DI TORINO

DIPARTIMENTO DI SCIENZA E TECNOLOGIA DEL FARMACO
VIA P. GIURIA, 9 - 10125 TORINO

Torino 10/09/98

Dr Prof. Figueiredo,

as I sayed you, in Frankfurt, this is the article on Hypericum species from Portugal, published in "Silva Lusitana".

I hope you may find some useful informations

Sincerely yours

Patrizia Rubiolo

29th ISEO, Sept. 6-9, 1998, Frankfurt/ M.

Chair persons

Monday, 7 Sept. 98

A. Mosandl	10:50 am – 12:00 am
A.C. Figueiredo	
W. Boland	02:00 pm – 03:10 pm
P. Weyerstahl	
G. Buchbauer	03:40 pm – 05:00 pm
K.H.C. Başer	

Tuesday, 8. Sept. 98

K.-H. Kubeczka	09:00 am – 10:10 am
H. Becker	10:40 am – 12:00 am
Y. Asakawa	
W. A. König	02:00 pm – 03:10 pm
A. Baerheim-Svendsen	03:40 pm – 05:00 pm
G. Martin	

Wednesday, 9 Sept. 98

D. Joulain	09:00 am – 10:30 am
C. Bicchi	
E. Stahl-Biskup	10:50 am – 12:10 pm
M. Lis-Balchin	

SESQUITERPENE HYDROCARBONS OF DISTILLED AND COLD-PRESSED LIME OILS

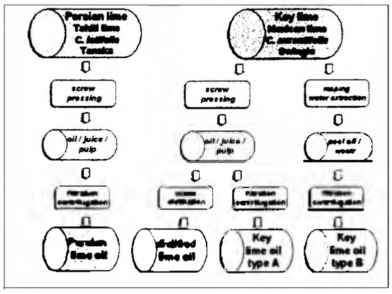
Wolfgang Feger, Herbert Brandauer, Herta Ziegler
ERICH ZIEGLER GMBH , Am Weiher 133, D-91347 Aufsess

1. INTRODUCTION

Lime oils constitute interesting natural matrices which have mainly found application in the soft drink sector.
The two varieties *Citrus aurantifolia* Swingle (Key lime) and *Citrus latifolia* Tanaka (Persian lime) result, depending on the employed production method, in oils with different sensory and analytical properties.
Within the scope of the present work, we focused not only on the investigation of the composition of the sesquiterpene hydrocarbons of the various oil types, but also on elucidating the influence of production processes and analytical methods on this compound class.

2. LIME OILS - FRUIT, ORIGIN AND PRODUCTION METHODS

Persian Lime / Tahiti Lime		Key Lime / Mexican Lime / West Indian Lime
Citrus latifolia Tanaka		*Citrus aurantifolia* Swingle
	Main growing areas:	
USA, Brazil		Mexico, Peru, Egypt
Large seedless fruits		Small seeded fruits



Persian lime
Tahiti lime
C. latifolia
Tanaka

Key lime
Mexican lime
C. aurantifolia
Swingle

screw pressing
screw pressing
rasping water extraction

oil / juice / pulp
oil / juice / pulp
peel oil / water

filtration centrifugation
steam distillation
filtration centrifugation
filtration centrifugation

Persian lime oil
distilled lime oil
Key lime oil type A
Key lime oil type B

3. RESULTS

Fig. 1: GC runs of Key Lime oil Type A; gentle GC conditions
1. run: native oil; 2. run: thermally pretreated oil

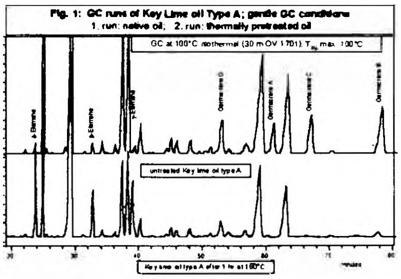
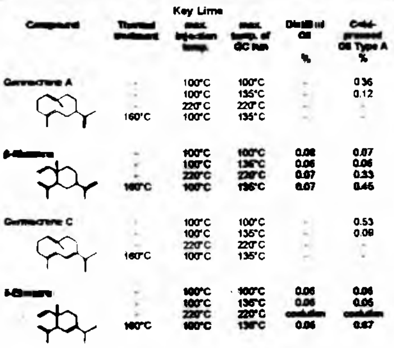


Table 1: Germacrene and elemene quantifications in relationship to GC conditions



Key Lime

Compound	Thermal treatment	max. injection temp.	max. temp. of GC run	Distilled Oil %	Cold-pressed Oil Type A %
Germacrene A	-	100°C	100°C	-	0.36
	-	100°C	135°C	-	0.12
	-	220°C	220°C	-	-
	160°C	100°C	135°C	-	-
β-Elemene	-	100°C	100°C	0.08	0.07
	-	100°C	135°C	0.06	0.06
	-	220°C	220°C	0.07	0.33
	160°C	100°C	135°C	0.07	0.45
Germacrene C	-	100°C	100°C	-	0.53
	-	100°C	135°C	-	0.09
	-	220°C	220°C	-	-
	160°C	100°C	135°C	-	-
δ-Elemene	-	100°C	100°C	0.06	0.06
	-	100°C	135°C	0.06	0.06
	-	220°C	220°C	coelution	coelution
	160°C	100°C	135°C	0.06	0.07

Fig. 2: GC-runs of different lime oils
GC-conditions: column: DB1, 66m; N_2; program: 50°C // 2°/min // 220°C; T_{inj}: 240°C

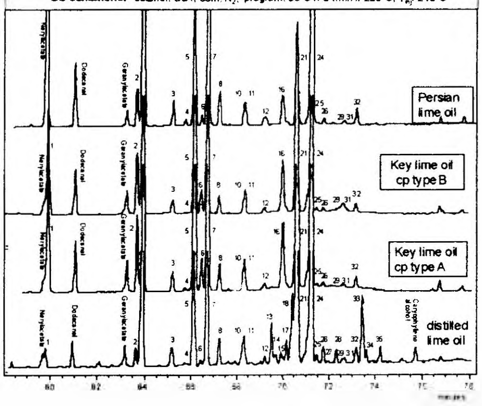
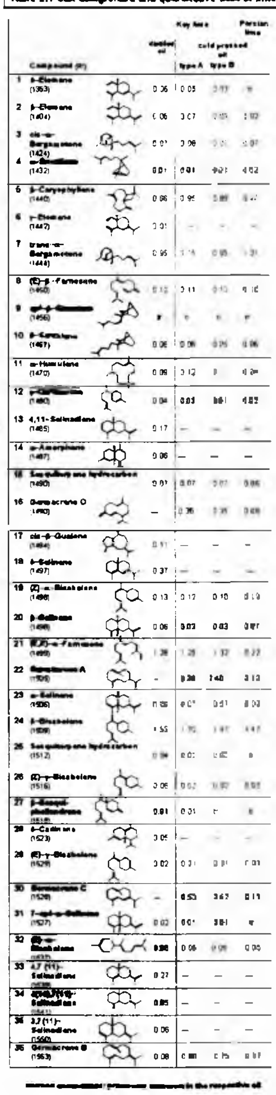


Table 2: Peak assignment and quantitative data of lime oils



Compound (RI)	Key lime distilled oil	Key lime cold pressed oil type A	Key lime cold pressed oil type B	Persian lime
1 δ-Elemene (1353)	0.06	0.05	[illegible]	[illegible]
2 β-Elemene (1404)	0.06	0.07	[illegible]	[illegible]
3 cis-α-Bergamotene (1424)	0.01	0.08	[illegible]	[illegible]
4 [illegible] (1432)	0.01	0.01	[illegible]	[illegible]
5 β-Caryophyllene (1440)	0.66	0.95	[illegible]	[illegible]
6 γ-Elemene (1447)	0.01	—	—	—
7 trans-α-Bergamotene (1444)	0.95	[illegible]	0.95	[illegible]
8 (E)-β-Farnesene (1450)	[illegible]	0.11	[illegible]	[illegible]
9 [illegible] (1456)	tr	tr	tr	tr
10 β-Santalene (1467)	0.06	0.06	[illegible]	[illegible]
11 α-Humulene (1470)	0.09	0.12	[illegible]	[illegible]
12 [illegible] (1480)	0.04	0.03	[illegible]	[illegible]
13 4,11-Selinadiene (1485)	0.17	—	—	—
14 α-Amorphene (1487)	0.06	—	—	—
15 Sesquiterpene hydrocarbon (1490)	0.01	0.07	0.07	0.06
16 Germacrene D (1490)	—	0.36	[illegible]	[illegible]
17 cis-β-Guaiene (1494)	0.11	—	—	—
18 δ-Selinene (1497)	0.37	—	—	—
19 (Z)-α-Bisabolene (1498)	0.13	0.17	0.10	[illegible]
20 β-Selinene (1498)	0.06	0.03	0.03	[illegible]
21 (E,E)-α-Farnesene ([illegible])	1.38	1.28	1.32	0.22
22 Germacrene A ([illegible])	—	0.38	1.40	0.12
23 α-Selinene (1506)	[illegible]	[illegible]	0.51	[illegible]
24 β-Bisabolene ([illegible])	1.55	[illegible]	[illegible]	[illegible]
25 Sesquiterpene hydrocarbon (1512)	[illegible]	0.02	[illegible]	tr
26 (Z)-γ-Bisabolene (1516)	0.06	0.02	[illegible]	[illegible]
27 β-Sesquiphellandrene ([illegible])	0.01	0.01	tr	tr
28 δ-Cadinene (1523)	0.05	—	—	—
29 (E)-γ-Bisabolene ([illegible])	0.02	0.01	[illegible]	[illegible]
30 Germacrene C ([illegible])	—	0.53	[illegible]	[illegible]
31 7-epi-α-Selinene (1527)	0.03	0.01	[illegible]	tr
32 (E)-α-Bisabolene ([illegible])	[illegible]	0.06	[illegible]	[illegible]
33 4,7(11)-Selinadiene ([illegible])	0.27	—	—	—
34 4(14),7(11)-Selinadiene ([illegible])	0.05	—	—	—
35 3,7(11)-Selinadiene (1560)	0.06	—	—	—
36 Germacrene B (1563)	0.08	[illegible]	[illegible]	[illegible]

[illegible] in the respective oil

4. DISCUSSION

The sesquiterpene hydrocarbon fractions of **distilled** and **cold-pressed** lime oils vary, as expected, both qualitatively and quantitatively (see Table1 and 2)

Firstly, the composition of **expressed oils** which, as a result of their gentle production procedure, should be analogous to the patterns natively occurring in plants, will be discussed

For the first time, we succeeded in establishing, both qualitatively and quantitatively, the entire range of the sensitive germacrenes A, B, C and D. This required the usage of a gentle GC- and GC/MS-technique, based on a programmable temperature vaporizer injection system (PTV, Gerstel; 30°C // 2°/sec // 120°C; column temperature: 120°C isothermal); see Figure 1

As a result of thermally induced Cope rearrangements of germacrene A, B and C into β-, γ- and δ-elemene, standard GC conditions partly permit only the detection of the respective rearrangement products. Germacrene A and C, both extremely unstable, were so far unknown in cold-pressed lime oil

Concomitantly, as a result of the gentle GC conditions, the elemenes, quantitatively, shift into the trace component range (0.01 - 0.07%).

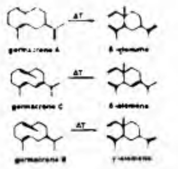
The sesquiterpene patterns of the various expressed lime oils turned out to be qualitatively uniform. Quantitative differences of **(E,E)-α-farnesene**, as well as in the **germacrene range**, permit the **differentiation of the varieties**. Both, (E,E)-α-farnesene and the germacrenes exhibit considerably lower values in Persian lime oil than in the respective oils of the Key lime

The comparison of the sesquiterpenes of **distilled lime oils**, with those of cold-pressed lime oils reveal qualitative differences, which can be partly traced back to the reactivity of the germacrenes present in the source material

Thus, the germacrenes A, C and D are completely lost during the production of distilled lime oil while the content of germacrene B is considerably lower than in the expressed oils

The decrease of the germacrenes can proceed via two reaction types:

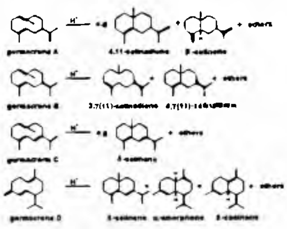
- thermally induced Cope-rearrangement to the elemenes



- acid-catalyzed isomerizations:

  A **series of selinadienes**, (4,11-selinadiene, δ-selinene, β-selinene, 4,7(11)-selinadiene, 4(14),7(11)-selinadiene, 3,7(11)-selinadiene) could be identified as reaction products in distilled lime oil

  Additionally, α-amorphene and δ-cadinene, acid-catalyzed rearrangement products of germacrene D, occur only in distilled lime oils



Generally, distilled lime oils exhibit a considerably more complex sesquiterpene pattern, which can be partly derived from the now visualized germacrenes present in the native material

As germacrene B is well established as one of the characteristic flavour impact compounds of cold-pressed lime oil, it can be assumed that the entire germacrene range is closely associated with the typical character of expressed lime oils. This correlation is of special relevance for the further processing of these matrices, which are usually concentrated before application. In order to retain the unique sensory qualities of cold-pressed lime oils, the employed concentration methods should avoid any thermal stress as otherwise losses of the typical flavour impact of the germacrenes ensue

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Cancellations

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Prof. Dr. Magdalena C. Cantoria, Philippines
Dr. Maria Stoyanova, Bulgaria

Errata

In the Final Programme we recognized some mistakes.

Tuesday, 08th September

16:00	**3 – 5**	K.H.C. Başer, *Eskişehir, Turkey*
16:20	**3 – 6**	A. Ilcim, ***Kahramanmaraş**, Turkey*
16:40	**3 – 7**	S. Nacar, ***Kahramanmaraş**, Turkey*

Wednesday, 09th September

11:10	7 – 2	Z. Fleisher, ***Ardsley, NY**, USA*

We regret these mistakes.

Cancellations

Lecture	**3 – 7**	S. Nacar, Tuesday, 08th September 16:40 The essential oil composition of *Origanum amanum* Post.
Poster	**7 – 3**	C. Daskalova Cytoembryological studies on *Salvia pratensis* L. (Lamiaceae)

The Organizing Committee

THE ESSENTIAL OIL FROM LEAVES AND RIPE BERRIES OF *LAURUS AZORICA* (SEUB.) FRANCO, GROWN ON MADEIRA

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INTRODUCTION

Laurus azorica (Seub.) Franco is an endemic species of Madeira, the Azores and the Canary Islands, that is found as a dominant tree in the Madeiran laurel wood zone from 200 to 1200m. The tree grows up to a height of 15-20m, with a dense crown formed by dark green glossy leaves, strongly aromatic when crushed. The berries, black when ripe, are the favourite food of *Columba palumbus* ('pombo torcaz'). 'Loireiro', 'loureiro' or 'louro' as *L. azorica* is most commonly known, has become scarce above 1200m due to overgrazing, burning and cutting. Being one of the first colonizers in the zones of recovery and regeneration of the Madeiran natural vegetation, the laurel has importance in several domains [1-4].

RESULTS AND DISCUSSION

The oils isolated from the leaves and berries were obtained in a yield of 0.83% and 0.4% (v/w), respectively. Fifty components of the oil from the leaves and fifty-five of that from the ripe berries were identified, amounting to 88% and 82% of the total oils, respectively, Table 1. The monoterpene fraction was dominated by α-pinene (32%), β-pinene (15%) and 1,8-cineole (13%) in the leaf oil, and by *trans*-β-ocimene (11%) and α-pinene (11%) in the berry oil. The sesquiterpene fraction, that attained only 6% in the leaf oil, reached 22% in the berry oil, *allo*-aromadendrene (6%) and β-elemene (4%) being the major components of the latter oil. *allo*-Aromadendrene (1%) was also the main component of the sesquiterpene fraction from the leaf oil. A third fraction, that of phenylpropanoids, was present only in the leaf oil, elemicin (2%) being its major component.

In a previous study of the steam distilled leaf oil of *L. azorica* grown in the Hortus Botanicus of the University of Groningen, Hokwerda *et al.* [6] also found α-pinene, β-pinene and 1,8-cineole as main components, although in different amounts (13%, 7% and 10%, respectively) compared with those found in the present study. *allo*-Aromadendrene was the main component of the sesquiterpene fraction, although the exact amount was not reported (5-10%). Cinnamyl acetate and elemicin (both 1-5%) were the major constituents of the phenylpropanoid fraction. The oil isolated by Hokwerda *et al.* [6] was obtained in a lower yield (0.36%) than that from the present study.

REFERENCES

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[5] da Silva J.A.T., A.C. Figueiredo, J.G. Barroso, L.G. Pedro, M.A. Gomes, S.S. Fontinha and J.J.C. Scheffer, *Flavour Fragr. J.*, **13** (1998) 90-92.

[6] H. Hokwerda, R. Bos, D.H.E. Tattje and Th.M. Malingre, *Planta Medica*, **44** (1982) 116-119.

Table 1. Percentage composition of the essential oils isolated, by distillation-extraction, from the leaves (L) and ripe berries (B) of *Laurus azorica.* [a] Relative to C_9-C_{17} *n*-alkanes on the DB-1 column. t = trace (< 0.05 %).

Compound	L	B
Tricyclene	t	0.7
α-Thujene	0.1	0.4
α-Pinene	**32.4**	**10.6**
Camphene	0.7	0.9
Sabinene	1.2	0.2
β-Pinene	**15.1**	**4.2**
β-Myrcene	0.2	0.7
α-Phellandrene	0.1	0.6
Δ^3-Carene	3.8	4.7
o-Cymene	1.4	1.6
p-Cymene	1.3	0.4
1,8-Cineole	**12.9**	**1.5**
Limonene	0.7	0.6
cis-β-Ocimene	t	4.3
***trans*-β-Ocimene**	t	**11.4**
γ-Terpinene		0.4
trans-Sabinene hydrate	t	
cis-Linalol oxide		0.8
trans-Linalol oxide		0.9
Terpinolene	0.2	
cis-Sabinene hydrate	0.1	
Linalol	0.6	5.0
α-Campholenal	0.2	0.8
trans-Pinocarveol	1.5	1.1
cis-Verbenol	0.1	0.4
trans-Verbenol	0.3	2.6
Pinocarvone	1.6	0.5
δ-Terpineol	0.2	
Terpinen-4-ol	0.7	
p-Cymen-8-ol		0.4
Myrtenal	0.5	0.7
α-Terpineol	0.6	0.2
Verbenone		0.8
Myrtenol	0.6	0.8
trans-Carveol	0.1	0.3
Carvone	0.1	0.2
Linalyl acetate		t
Bornyl acetate	0.4	0.5
Sabinyl acetate	0.3	0.5
Carvacrol	t	
α-Terpenyl acetate	1.6	0.3
α-Cubebene		0.4
α-Copaene	0.3	2.0
Methyl eugenol	0.2	
β-Cubebene		0.8
β-Elemene	t	3.7
β-Caryophyllene	0.4	0.5
trans-Cinnamyl acetate	0.7	
Aromadendrene		0.2
α-Humulene		0.3
allo-Aromadendrene	1.4	6.2
γ-Muurolene		0.5
Germacrene-D		0.3
β-Selinene	1.1	1.0
Valencene	0.9	1.0
α-Selinene	t	0.3
γ-Cadinene	0.1	
δ-Cadinene	0.2	1.7
Elemicine	1.7	
trans-Nerolidol	t	0.8
Spathulenol	0.1	1.1
β-Caryophyllene oxide	1.2	0.9
epi-Cubenol		0.2
T-Cadinol		0.2
Intermedeol		0.2
Total	87.9	82.3
Monoterpene hydrocarbons	57.2	41.7
Oxygen-containing monoterpenes	22.4	18.3
Sesquiterpene hydrocarbons	4.4	18.9
Oxygen-containing sesquiterpenes	1.3	3.4
Others	2.6	
Yield	0.83	0.40

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Composition Of Essential Oil Of Wild Melissa

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The essential oils of 13 wild melissa provenances, seperated by water-steam distillation according to DAB, were analyzed by gaschromatography and mass-spectrometry. Neral and geranial, two components characteristic of melissa, were detected in only four provenances; after drying and storage of the plants, the two components were lacking also in these. In another cut of the melissa plants of June 1998, neral and geranial were lacking in all provenances. It is evident that drying and/or storage caused this loss, but also that these components are not produced any more by plants of a certain age.

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