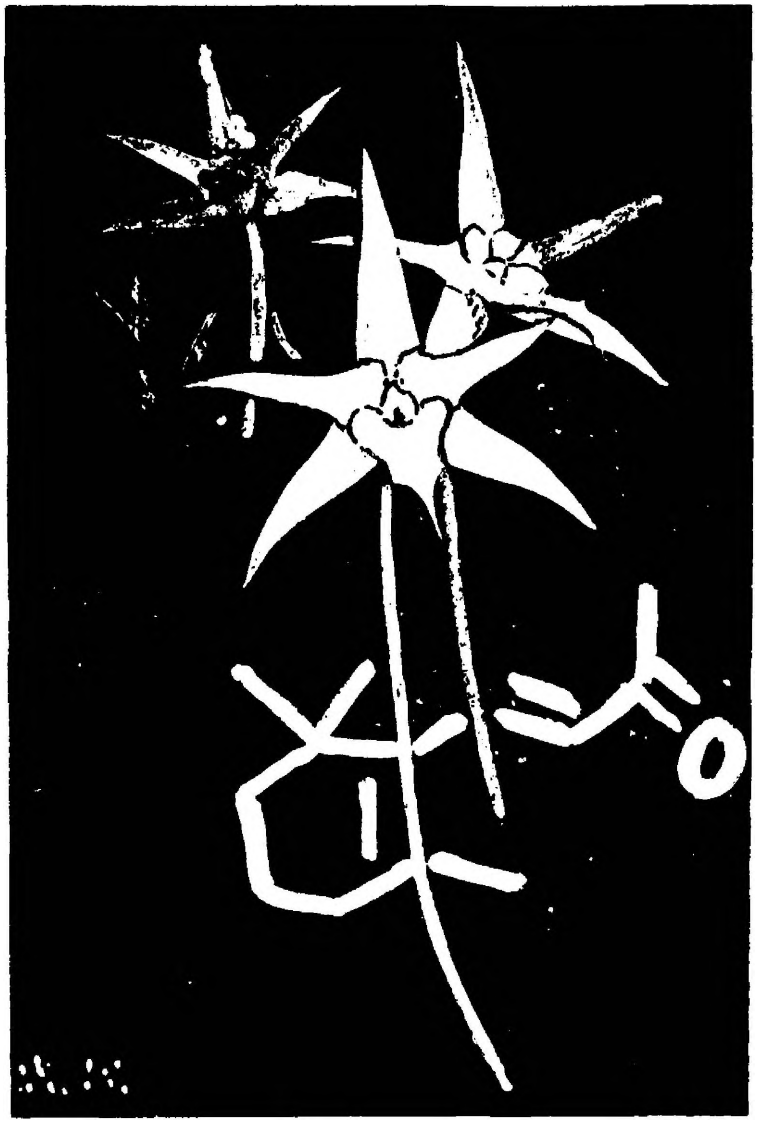
24th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS



FINAL PROGRAMME
ABSTRACTS
LIST OF PARTICIPANTS

JULY 21-24, 1993

TECHNISCHE UNIVERSITÄT BERLIN

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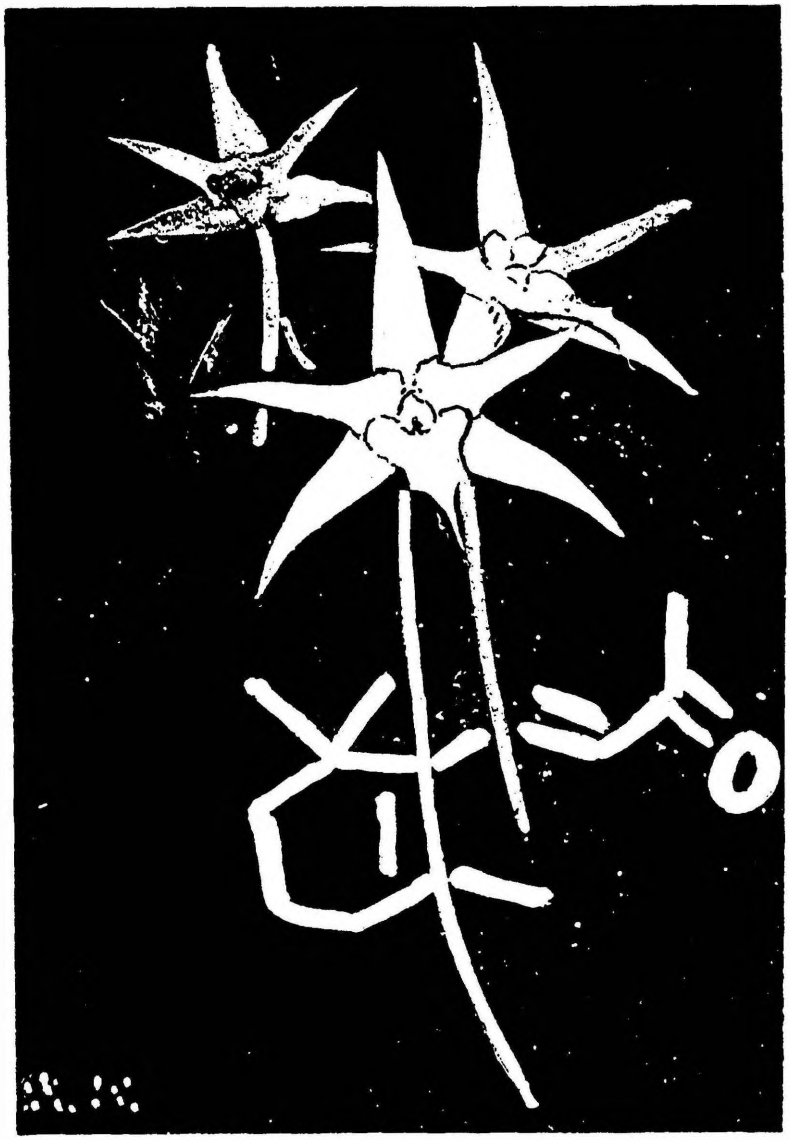

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TECHNISCHE UNIVERSITÄT BERLIN

24th International Symposium on Essential Oils
July 21-24, **1993**, Berlin, Germany

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We are very grateful to all these sponsors whose generous financial support has been so valuable for the organization of the 24th ISEO.

The organizers

7-th int. Symp. ... France 5/Sept → 7 Sept

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24th International Symposium on Essential Oils
July 21-24, 1993, Berlin, Germany

PROGRAM 21.07.93

14.00-19.15 Registration at the ISEO office, Franz-Fischer-Bau (**TC**) of the Technische Universität, Straße des 17. Juni 124, entrance hall.

19.30-22.30 Get-together-party at the "Lichthof" in the main building (**H**) of the Technische Universität.

Welcome address by the President of the Technische Universität, Professor Dr. **Dieter Schumann**

Reception sponsored by **W. Spitzner**, Arzneimittelfabrik GmbH, Ettlingen.

22.07.93	**23.07.93**
09.00-10.15 Lectures	09.00-10.35 Lectures
10.35-11.00 Coffee/Tea	10.35-11.10 Coffee/Tea
11.00-12.00 Lectures	11.10-12.30 Lectures
12.00-14.00 Lunch	12.30-14.00 Lunch
14.00-15.25 Lectures	14.00-15.20 Lectures
15.25-15.50 Coffee/Tea	15.20-15.50 Coffee/Tea
15.50-16.50 Lectures	15.50-17.10 Lectures
17.00-19.00 **Poster session**	17.10-17.30 Closing remarks

23.07.93

19.00	Departure of the busses from Franz-Fischer-Bau (**TC**) to the small island of Lindwerder
20.00	ISEO Dinner as an Old Berlin Garden Party
23.30	Return by bus to the city centre (**ZOO** station)

24.07.93

9.00	Departure of the bus from the Franz-Fischer-Bau (**TC**) for the botanical excursion **"Märkische Schweiz"** (lunch is optional).
17.00	Return to **ZOO** station

24th ISEO address:
Prof. Dr. P. Weyerstahl
Inst. Org. Chem. - Sekr. TC 2
TU Berlin
Str. des 17. Juni 135
D-10623 BERLIN
tel 0049-30-314 22355
fax 0049-30-314 23619

22.07.93 **LECTURES**

9.00 - 9.10 Welcome address by the Dean of the Faculty of Pharmacy, Freie Universität Berlin, Prof. Dr. **E. Eich**

Joulain, D., Presiding

9.10 - 9.55 **OHLOFF, G., Geneva, Switzerland:**
The significance of fragrance in the history of civilisation

9.55 - 10.15 **ISOE, S., Osaka, Japan:**
Progress in the synthesis of iridoids and related natural products

10.15 - 10.35 **TATEO, F., Milano, Italy:**
Ketals and and glycosides in flavourings

Bicchi, C., Presiding

11.00 - 11.20 **NÄF, R., Geneva, Switzerland:**
New constituents of Agarwood

11.20 - 11.40 **JOULAIN, D., Grasse, France:**
Is GC/MS a suitable tool for the identification of sesquiterpenes in essential oils?

11.40 - 12.00 **KÖNIG, W. A., Hamburg, Germany:**
Characterization of essential oils:
Enantiomeric ratio of terpenoid hydrocarbons

Isoe, S., Presiding

14.00 - 14.45 **ASAKAWA, Y., Tokushima, Japan:**
Essential oils of hepaticae-structures and synthesis of mossy odorous and pungent terpenoids and aromatic compounds

14.45 - 15.05 **BICCHI, C., Torino, Italy:**
Further acquisition in the analysis of the components of *Iris pallida* rhizomes

15.05 - 15.25 **PETRI, G., Budapest, Hungary:**
Chervil a wildgrowing aromatic plant in Hungary

Kubeczka, K.-H., Presiding

15.50 - 16.10 **GORA, J., Lodz, Poland:**
The possibilities of essential oils utilization for the limitation of some insects population or their repellency

16.10 - 16.30 **ZHELJAZKOV, V., Plovdiv, Bulgaria:**
Studies on the effect of heavy metals upon the growth, productivity and quality of lavender (*Lavandula vera* D.C.) production

16.30 - 16.50 **HENGLEIN, M., München, Germany:**
The fragrance circle: a phenomenological approach for the application of fragrance materials

17.00 - 19.00 **POSTER SESSION**

23.07.93 **LECTURES**

Schilcher, H., Presiding

9.00 - 9.45 **DELLA LOGGIA, R., Trieste, Italy:**
Antiinflammatory activity of essential oils
and its experimental evaluation

9.45 - 10.15 **KOBAL, G., Erlangen, Germany:**
Olfactory event-related potentials: A physiological technique
to record brain activity induced by odors

10.15 - 10.35 **BUCHBAUER, G., Vienna, Austria:**
New results in aromatherapy research

11.10 - 11.30 **ILMBERGER, J., München, Germany:**
Effects of essential oils on human attentional processes

11.30 - 11.50 **RIECHELMANN, H., Mainz, Germany:**
Clinical application of volatile oils in pneumology
and otolaryngology today

11.50 - 12.10 **MERK, H., Köln, Germany:**
Dermatopharmacological properties of eugenol
and eugenol containing oils

12.10 - 12.30 **KRALL, B., Eschwege, Germany:**
Efficacy and tolerance of *Menthae arvensis* aetheroleum
Report on an open randomized study

Boelens, M. H., Presiding

14.00 - 14.20 **FRANZ, C., Vienna, Austria:**
Recent developments in the production of essential oil crops

14.20 - 14.40 **MOSANDL, A., Frankfurt/M., Germany:**
Origin assessment of essential oils - Scope and limitations

14.40 - 15.00 **BOUWMEESTER, H. J., Wageningen, The Netherlands:**
Volatilization of essential oil from caraway

15.00 - 15.20 **BASER, K. H. C., Eskisehir, Turkey:**
Non-Labiatae essential oils of Turkey

Stahl-Biskup, E., Presiding

15.50 - 16.10 **BOELENS, M. H., Huizen, The Netherlands:**
Chemical characterization of oils from some *Cymbopogon* species

16.30 - 16.50 **TSVETKOV, R., Kazanlak, Bulgaria:**
Bulgarian oleaginous rose and rose oil.

16.10 - 16.30 **TÜMEN, G. Balikesir, Turkey:**
Compositon of the essential oil of Taurus Cedar wood oil

16.50 - 17.10 **KNOBLOCH, K., Erlangen, Germany:**
The aroma-garden in Erlangen

PROGRESS IN THE SYNTHESIS OF IRIDOIDS AND RELATED NATURAL PRODUCTS

Sachihiko Isoe

Institute of Organic Chemistry, Osaka City University

Sumiyoshi-ku, Osaka 558, Japan

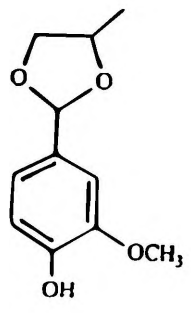
A number of terpenoid dialdehydes have been isolated from plants, animals, insects, and microorganisms.
Nearly all kinds of dialdehydes are biologically active and some of them have been found to exhibit remarkable antimicrobial and antitumor activity and to inactivate phospholipase A_2 and aldose reductase.
Our research projects are to develop the useful synthetic method generally applicable to the synthesis of terpenoid dialdehydes and to elucidate the structure-activity relationship.
In this paper we deal with the synthesis of polyfunctional iridoid (loganin, penstemide, didrovaltrate, plumericin, allamandicin, garjasmin, gardenoside, asperuloside) secoiridoids (secologanin, sweroside, kingiside, morronoside, gentiopicroside, sarracenin) and diterpenoids related to iridoid (petiodial and udoteatrial).

KETALS AND GLYCOSIDES IN FLAVOURINGS.

F.TATEO*, A.ORLANDI*, A.FERRILLO*, C.CAPODANNO*, L.PANZA**, G.RUSSO**.

* Dipartimento di Scienze e Tecnologie Alimentari, Università degli Studi di Milano, 2 Via Celoria, 20133 Milano, Italia.
** Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, 21 Via Venezian, 20133 Milano, Italia.

The problem of the spontaneous formation of cyclic ketals during the storage of flavourings and the production of some foods in presence of 1,2-propanediol was first analyzed by F.Tateo et al. during the 22nd International Symposium on Essential Oils(Saint Vincent - 1991).
The same author published in 1991 and 1992 other papers om this topic and proposed New ketals and Glycosides for the Formulation of Flavours and Fragrances at the 12th International Congress on Flavours and Essential Oils (Vienna - 1992).
The paper currently presented reports the kinetic analysis of the formation of ketals derived from vanillin by reacting with 1,2-propanediol (I) and the assessment of spontaneous formation of the same ketal during industrial cake production.
Furthermore, it reports the analytical characterization of the ketal synthetized from benzaldehyde with 1,2- propanediol (II) and the release kinetics of acetaldehyde from a precursor glycoside such as vinyl-beta-D-glucopyranoside (III).
The results allow to draw conclusions on the possible amounts of vinyl-beta-D-glucopyranoside which may spontaneously form and on the possible use of the other two synthesis products analyzed in this paper.



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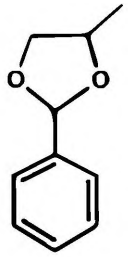

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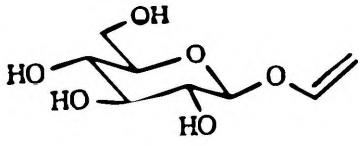
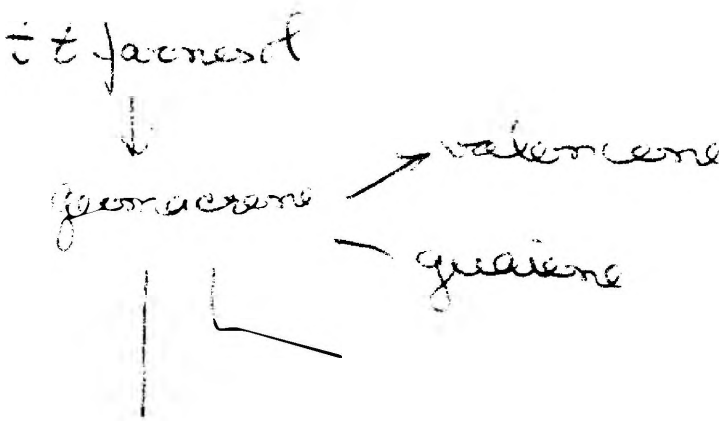


III

NEW CONSTITUENTS of AGARWOOD

Regula Näf and Alain Velluz
Research Laboratories, FIRMENICH SA
Postbox 239, CH-1211 Geneva 8

Agarwood oil is the essential oil from the diseased wood of *Aquilaria agallocha* Roxb., a softwood found in the evergreen forests of northeastern India. The oil, obtained by steam distillation, has a characteristic balsamic odour with a sandalwood-ambergris-like tonality and is widely used as oriental incense.
In our contribution, the GC/MS profile of the volatile parts of this oil is presented. Apart from two aromatic compounds, it is a complex mixture of sesquiterpenoids, based principally on the skeletons of agarofuran, vetivene, eudesmane, eremophilane or valencane. We isolated about twenty previously unknown constituents; their structures were elucidated and will be discussed in detail.



IS GC/MS A SUITABLE METHOD FOR THE ANALYSIS OF ESSENTIAL OILS ?

Daniel Joulain, Research Laboratories
ROBERTET S.A., B.P. 100,
F-06333 Grasse, France

Today, gas chromatography/mass spectrometry (GC/MS) is one of the most widely used methods for the rapid analysis of volatile compounds in natural mixtures, including essential oils. In most cases, this very convenient method can indeed give good results, provided a number of conditions are met and certain precautions are taken. It possesses, however, severe limitations when new or unsufficiently described compounds are involved. Known substances displaying chromatographic and mass spectral data of poor specificity can generate ambiguities that may not be easily detected.

The author will discuss examples of sesquiterpenes and other constituents from well-documented essential oils: tea tree leaf (*Melaleuca alternifolia* Cheel), clove bud (*Eugenia caryophyllus* Spreng.), massoy bark (*Cryptocarya massoya* Kosterm.). Studies from lesser known essential oils will be also presented, with a special attention to case of the *Neocallitropsis pancheri* (Carr.) heartwood oil.

Some recommandations for the appropriate use of GC/MS will be proposed.

CHARACTERIZATION OF ESSENTIAL OILS: THE ENANTIOMERIC RATIO OF TERPENOID HYDROCARBONS

Wilfried A. König

Institut für Organische Chemie, Universität Hamburg

Monoterpene hydrocarbons constitute a considerable proportion of plant volatiles and contribute greatly to the overall character of an essential oil. Many of these compounds are chiral and may be present in two enantiomers, some of which with distinct differences in odour. The enantiomeric ratio of monoterpene hydrocarbons may also be associated with the provenience or different production processes of an essential oil. It is therefore of basic interest to investigate the enantiomeric ratio of these volatiles. We have recently proposed a gas chromatographic system where samples are simultaneously injected onto two capillary columns with two different cyclodextrin derivatives [1]. Each pair of enantiomers is resolved on at least one of the two columns and quantitative measurements are obtained with the signals from two flame ionization detectors and a two-channel integrator. With this system a specific pattern of the monoterpene hydrocarbon fraction of an essential oil is obtained which can be used for its characterization and reliable identification. The structural complexity of sesquiterpene hydrocarbons and the lack of racemic reference compounds has so far been a serious obstacle to extend the described technique to this equally important group of essential oil constituents. We have now started to synthesize racemic sesquiterpene hydrocarbon standards and shown that in the case of α-curcumene and β-bisabolene both enantiomers are present in the essential oils of related plant families. For stereochemical assignments preparative enantiomer separation with short packed gc columns, coated with cyclodextrin derivatives, was applied [2].

The extension of enantiomeric ratio determinations to the sesquiterpene fraction will allow a more detailed characterization and the investigation of biogenetic questions in this important group of essential oil constituents.

[1] W. A. König, A. Krüger, D. Icheln, T. Runge, J. High Res. Chromatogr. **15** (1992) 184.

[2] I. Hardt, A. Rieck, W. A. König, J. Chromatogr., in press.

FURTHER ACQUISITION IN THE ANALYSIS OF THE COMPONENTS OF IRIS PALLIDA RHIZOMES

Carlo Bicchi, Monica Fresia and Patrizia Rubiolo

Dipartimento di Scienza e Tecnologia del Farmaco
via P.Giuria 9 - 10125 Torino (Italy)

Iris rhizome absolute is widely and successfully used in the perfume industry, thanks to their attractive smell of violets. This characteristic is due to irones (cis- and trans- α -irone and cis- γ -irone), a group of terpenoids which are formed by oxidative degradation of triterpenoids characteristic of the Iris genera, the iridals, in particular the bicyclic iridals iriflorental and iripallidal.

This communication will reports the most recent results obtained in the analysis of the constituents of Iris pallida rhizomes.

In particular a supercritical fluid extraction (SFE) method for the simultaneous extraction of irones and iridals from the rhizomes will be described. The results obtained with SFE will be compared to the classical methods in terms of extraction times and yield.

The results of the analysis through HPLC coupled to MS via a particle beam interface in different ionization modes (EI, PCI and NCI) will also be described. The identification of the consituents of the iridal ester fraction in the total extract will be discussed in detail.

CHERVIL A WILDGROWING AROMATIC PLANT IN HUNGARY

Gizella Petri,[1] Éva Lemberkovics [1], L.Lelik[2], Gy. Vitányi[2]

1 Semmelweis Medical University Institute for Pharmacognosy, Budapest
2. University of Horticulture and Foodindustry, Budapest

Chervil (Anthriscus cerefolium L., Apiaceae) is an aromatic plant native in Europe. It contains essential oil, flavonoids and bitter substances. In the folk medicine it is used as a diuretic and digestive having metabolism stimulating effect and it is also used as a chronic skin aliments. The fresh leaves are salat, soup or spice-products.

In our work we studied the quantitative and qualitative formation of essential oil composition of chervil growing wild in our country.

The samples of investigations were collected from different geographical places of Hungary (Budapest, the Plain and Transdanubia). The plants were gathered during the vegetation is Spring and also in Autumn. We studied the oil-composition dependent on development stages of plant jung state, before flowering, after flowering and investigated also the essential oils obtained from fresh herb and dried one.

Obtaining of essential oil was carried out by watersteam distillation and also with organic solvent. For the determination of the total oil content we used the method of the Seventh Hungarian Pharmacopoea. The qualitative evaluation of each oilcomponents was carried out by gas chromatographic (GC) and capillaric GC-MS techniques.

We established that the main part ($\sim$95 %) of the essential oil is formed from 3 characteristic components, such as:

undecane ($C_{11}H_{24}$) paraffine hydrocarbon, methylcaviol (esdragol) and the structure isomer compound of eugenol methyl ether. (=1 allyl-2,4 dimethoxy benzene). We established that the quantitative distribution of the methylchavicol and the eugenol methyl ether isomer compounds is influenced significantly by the vegetation period: flowering or only leafy stadium of plant. The place and the season of gathering did not influence essentially the composition of essential oil.

THE POSSIBILITIES OF ESSENTIAL OILS UTILIZATION FOR THE LIMITATION OF SOME INSECTS POPULATION OR THEIR REPELLENCY

J. Gora, S. Lakota*, A. Kurowska, D. Kalemba, A. Raszka* and J. Kula

Institute of General Food Chemistry, Technical University of Lodz, 90-924 Lodz, Poland

*Institute of Organic Industry, 43-200 Pszczyna

The results of biological studies of the essential oils influence on some environmentally onerous insects have been presented. Over 20 essential oils were tasted against mosquito (*Aedes aegypti L.*), house-fly (*Musca domestica L.*) and fruit-fly (*Drosophila melanogaster Meig.*).

Highly repellent effect on mosquito was observed in the case of the essential oils from juniper (*Juniperus communis L.*), valerian (Valeriana officinalis L.), garden thyme (*Thymus vulgaris L.*), herb of goldenrod (*Solidago graminifolia L.*), pine (*Pinus sylvestris L.*), coriander (*Coriandrum sativum L.*), larch (*Larix decidua Mill.*), douglas fir (*Pseudotsuga menziesri Franco*), tansy (*Tanacetum vulgare L.*) and fir (*Abies alba Mill.*). Moreover, a strong insecticidal influence on mosquito larvae was revealed for the following essential oils: douglas fir, pine, larch, American Arborvitae (*Thuja plicata Donn.*), fir, orange, and sesquiturpentine and its fractions. These oils, at concentrations 0.012 - 0.05%, killed 100% mosquito larvae after 3 hours. It was found, by biotests, that the essential oils from thyme, valerian, sweet flag (*Acorus calamus L.*), curcuma (*Curcuma longa L.*) and caraway (*Carum carvi L.*) are lethally active against house-fly. In a similar bio-experiment with fruit-fly, the essential oils from douglas fir, silver fir, motherwot (*Artemisia vulgaris L.*), American Arborvitae, juniper and a fraction of the sesquiturpentine appeared to be insecticidal.

The results obtained are indicative of large possibilities of the essential oils use in pro-ecological preparations for the fight against environmentally onerous insects.

STUDIES ON THE EFFECT OF HEAVY METALS UPON THE GROWTH, PRODUCTIVITY AND QUALITY OF LAVENDER (LAVANDULA VERA D.C.) PRODUCTION

Valcho Zheljazkov, Department of Plant Growing, University of Agriculture, Plovdiv, Bulgaria

S u m m a r y

In the period 1989-1992, we made field experiments to study the effect of the aerosol and soil heavy metal pollution upon the growth, development and quality of Lavandula vera D.C. production. We used as experimental material cvs Hemus and Druzhba grown on two different plots - the first in the region of the Non-Ferrous Metals Combine near Plovdiv at aerosol and soil heavy metal pollution, and the second in the experimental field of the Plant Growing Department at the Agricultural University in Plovdiv. Industrial plantings were used as a second control, too.

The following indices were estimated: initiation of the different phenological stages, height and branching of plants, yield of fresh herbage, essential oil content in the fresh herbage, yield of essential oil, chemical (GC analysis) essential oil composition, germinating capacity of seeds, heavy metal content in the essential oil, the plants and distillation wastes.

The data from the investigation has shown that the rate of the aerosol and soil pollution in the region of the Non-Ferrous Metals Combine near Plovdiv does not affect significantly the productivity of the tested Lavandula vera D.C. cultivars. The results obtained could be used for determination of crops' structure both in the mentioned and other regions with a similar rate of industrial pollution.

THE FRAGRANCE CIRCLE
A PHENOMENOLOGICAL APPROACH FOR THE APPLICATION OF FRAGRANCE MATERIALS

Martin Henglein,
Westenriederstraße 24, D - 8000 München 2

Starting with a short overview on some of the basic questions concerning the methodology of Aromatherapy and Aromatology, the speaker will present his more recent work, a new phenomenological approach towards interdisciplinary fragrance application. Over the last few years he has developed the Fragrance Circle, which helps to integrate Data from various fields of perception. Smell Types, based on chemical constituents and their unique expressions in plant metabolisms can be related to morphological and chronobiological aspects. As emotions and smell reactions are closely linked through the limbic system, human reactions towards certain types of smell can be interpreted. A series of Smell - tests help to determine problem - zones and emotional affinities. Some examples of applications in different fields of treatment will be given.
Closing up with a summary of the present situation, especially stressing the importance of ecological thinking - intergrating plant life , human life and healthcare and environmental conditions - , the speaker hopes to encourage future interdisciplinary research and application.

Olfactory Event-Related Potentials: A Physiological Technique to Record Brain Activity Induced by Odors.

Gerd Kobal

Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Universität Erlangen-Nürnberg, Krankenhausstr. 9, 8520 Erlangen FRG

The first chemosensory event-related potentials (CSERP) have been obtained by Finkenzeller (1966) and Allison and Goff (1967). Latencies of the earliest peaks were in the range of 300-400 msec. However, Smith et al. (1971) contested the olfactory nature of these responses, on the grounds that they were not obtainable in patients who had lost trigeminal sensitivity. In 1988 Kobal and Hummel were able to demonstrate that there were indeed olfactory responses to vanillin in man. In patients whose filae olfactoriae had been ruptured, e.g. after cranial-cerebral trauma stimulation with vanillin evoked no potentials whatsoever. On the other hand, non-odorous carbon dioxide was always perceived and elicited chemo-somatosensory event-related potentials (CSSERP) mediated by the trigeminal nerve. On the grounds of these findings it can be assumed that responses to vanillin are indeed olfactory event-related potentials (OERP). Also, topographical mapping on the skull has permitted the differentiation between these two kinds of CSERPs. To date, only late nearfield olfactory event-related potentials have been recorded in man. Already here, experimenters have found themselves confronted with considerable difficulties, since it is of paramount importance to employ suitable, specially devised olfactometers. They must be able to repeatedly present identical stimuli of steep onsets (rise time < 50 msec) without simultaneously exciting other, non-chemosensory modalities. Rapid habituation, a characteristic feature of olfaction in which it differs from all other sensory modalities, precludes high repetition rate which is necessary to improve the signal-to-noise ratio. Besides intensifying the fundamental research, which includes the finding of a definition of cortical generators, the ascertaining of interactions with the trigeminal system, etc., clinical studies are indispensable, in order to determine whether CSERPs will attain the same importance and quality of being useful, a position, auditory and visual event-related potentials have securely kept now for several years.

New Results in Aromatherapy Research

G. Buchbauer[1], L. Jirovetz[1], M. Czejka[1], Ch. Nasel[2] and H. Dietrich[3]

[1] Institute of Pharmaceutical Chemistry, University of Vienna, A-1090 Vienna, Austria
[2] Department of Radiology, University of Vienna, Division: Neuroradiology, A-1090 Vienna, Austria
[3] Central Laboratory Animal Facilities, University of Innsbruck, A-6020 Innsbruck, Austria

In continuation of our work with animals inhaling fragrance compounds or essential oils with ascribed sedative properties (1,2), as well as with humans (EEG-, CBF- and vigilance experiments) (3), volatiles with assumed stimulating effects were investigated.

In total 17 essential oils or pure fragrances were tested in animal experiments some of which show an increase in the motility of the tested animals in a significant way. Additional investigation of their stimulating effects after a sedation of the mice by an i.p.-application of diazepam showed a clear influence on motility observed after inhalation of jasmine oil, fenchone, patchouli oil, ylang-ylang oil and basil oil. In blood samples of the test animals fragrances could be detected and identified in an range of 0.2 to 4 ppb using GC-FID and GC/FTIR/MS. Benzaldehyde as well as carvone as representative samples of fragrance compounds were also able to pass the blood-brain-barriere and could therefore be identified in brain samples (left and right part as well as cortical and other areas of the mouse brain in a low ppb level). Mice experiments with chiral fragrance compounds showed no significant difference in motility between the two enantiomeres.

Finally, studies on the cerebral blood flow using 1,8-cineol as activating accepted fragrance compound in Xe-CT-experiments are presented. The CBF in an anosmic person after fragrance inhalation is discussed.

(1) G.Buchbauer, L.Jirovetz, H.Dietrich, Ch.Plank, E.Karamat: *Z.Naturforsch.* **46c,** 1067 (1991)

(2)G.Buchbauer, L.Jirovetz, W.Jäger, Ch.Plank, H.Dietrich: *J.Pharm.Sci.***82**,.... (1993)

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EFFECTS OF ESSENTIAL OILS ON HUMAN ATTENTIONAL PROCESSES

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Based on earlier findings that inhalation of the fragrance of essential oils changed the amount of exploratory behavior in mice we conducted a study to investigate the effects of essential oils on human attentional processes. Human attention is not a unitary concept; several distinct areas have to be discriminated. One basic concept is that of alertness which can be defined as the general speed of information processing. Alertness is measured in conventional reaction time tasks. Another important aspect of attention is vigilance, meaning the ability to sustain attention to a given task over longer periods of time, especially when the task shows a low temporal density of stimuli, i.e. relevant stimuli occur infrequently.
Three groups of subjects, a control group and two experimental groups, were tested on choice reaction time (alertness) and vigilance tasks wearing surgical masks. A test session was divided into two parts consisting of the same set of tasks. In the first part of a session in both groups water was applied to the surgical masks; in the second part, in the control group again water was applied, but the experimental groups were exposed to either lavender or jasmin. For each subject difference scores were calculated between the results of the two parts of the session, and those scores were then averaged within groups.
Results clearly indicate excitatory effects of jasmin and sedative effects of lavender on the results of the vigilance task. No effects could be shown in the choice reaction task measuring alertness. Essential oils in general may have differential effects within different domains of human attentional processing.

Abstract International Symposium On Essential Oils, Berlin, 1993.

Clinical applications of volatile oils in pneumology and otolaryngology today

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Viscoelastic properties of airway mucus and mucociliary transport are altered in acute and chronic inflammation of airway mucosa. In various studies on acute and chronic bronchitis, the effectiveness of topically and systemically administered volatile oils has been proven. Airway secretory glands are stimulated, mucus surface tension is reduced, mucociliary transport and symptoms of acute and chronic tracheobronchitis improve. However, the effects of volatile oils on ciliary activity of isolated human respiratory cells are still controversial. Preliminary results regarding this problem are reported. A yet unpublished study demonstrates the benefit of orally administered volatile oils also in acute and chronic sinusitis. In addition, nasal ointments containing volatile oils have been found to increase the sensation of nasal airflow, acting by stimulation of nasal sensory nerve endings.

DERMATOPHARMACOLOGICAL PROPERTIES OF EUGENOL AND EUGENOL CONTAINING OILS

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In wasp venom sentizitation, the antiinflammatory effect of clove oil was investigated and a significant reduction of this inflammatory reaction was found. Further investigations of the antiinflammatory effect of eugenol as main component of clove oil showed an dose-dependent induction of NADP(H): quinone reductase. This result suggests that the inflammatory activity is mediated among other factors by influencing oxygen metabolism.

EFFICACY AND TOLERANCE OF MENTHAE ARVENSIS AETHEROLEUM
Report on an open randomized study

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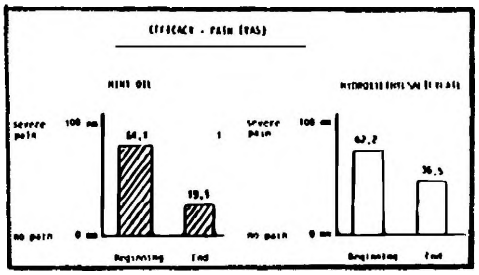
Introduction
The study was planned to evaluate efficacy and tolerance of JHP Gel (mint oil 30 %) in comparison with standard medication Phlogont Gel (hydroxyethylsalicylate 10 %) in patients suffering from acute and subacute complaints of the periarticular system, e.g. distortions, tendopathies.

Design, patients, medication, duration
Open, randomized study in 100 patients suffering from acute (n=49) and subacute (n= 51) complaints. Age: 36,6 years. Stratification: 5 groups dependent on diagnosis. 50 patients were treated with mint oil, 50 patients were treated with hydroxyethylsalicylate. Dependent on severity of complaints duration of treatment extended 10 to 20 days.

Methods
Intensity of pain (main target) was evaluated by VAS (visual analog scale), ranging from 0 mm (no pain) to 100 mm (severe pain). Additional tenderness on pressure, spontaneous pain, pain on movement and tolerance were examined.

Results
Before treatment 64,1 % of the mint oil-group and 62,2 % of the hydroxysalicylate-group were suffering from severe pain. At the end of the study 19,1 % of the patients treated with mint oil and 36,5 % of the patients, treated with hydroxyethylsalicylate were suffering from severe pain. The difference was statistically significant.



78 % of the patients treated with mint oil and 34 % of the patients treated with hydroxyethylsalicylate estimated the therapy to be very good or good. In 78 % mint oil therapy was estimated to be very good or good by the physician, whereas only in 50 % therapy with hydroxyethylsalicylate was estimated to be very good or good by the physician.
Best efficacy was seen in patients suffering from acute complaints treated with mint oil. 6 from 50 patients stopped treatment with hydroxyethylsalicylate because of inefficiency. No patient out of the mint oil-group stopped treatment.
In the mint oil-group 1 side effect (smell of mint oil in the nose) was reported, whereas 10 side effects (3 times erythema, 7 times itching) were reported from patients treated with hydroxyethylsalicylate.

Conclusion
Mint oil-therapy proves to be safe and effective in treating patients suffering from subacute and especially acute complaints of the periarticular system.

RECENT DEVELOPMENTS IN THE PRODUCTION OF ESSENTIAL OIL CROPS

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During the recent years the production of essential oil crops has changed mainly due to the following reasons:

1. The economical and political changes in Eastern Europe, which led first to an excessive supply (by selling the stock), followed by a slight rise of prices and especially of the costs, and subsequent by a decreasing acreage.

2. The structural alteration of the European agriculture and the changing consumers desires; the high technical standard and the introduction of quality assurance systems resulted in new production centers e.g. in France, Spain, Italy and Germany.

3. The efforts of UNIDO and several governmental and non governmental organizations to include essential oil crops in rural (industrial) development programmes. Even if the number of failures is rather high by neglecting e.g. the market rules, success stories are also known from several countries and new essential oil crops (e.g. Vietnam, Tansania, Brazil; Tagetes lucida, Clausena anisata).

It is evident, that the production and its possible increase depends mainly on the internal rather than internation market and demands, and successful introductions are often based on domestication of local plant species. Besides, mainly innovative and qualitatively high value products find broad acceptance. Quality, however, depends on remarkable investments beginning with breeding of high value strains or cultivars not feasible due to an unfavourable cost benefit ratio. The judgement of the German Federal Supreme Court in favour of the patent "tetraploid camomile Manzana" punctuates that investments also in this field may be remunerative. This should stimulate breeding and quality production of essential oil crops.

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Origin Assessment of Essential Oils - Scope and Limitations

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Both phenomena, **enantioselectivity** as well as **stable isotope discrimination** during biosynthesis may serve as endogenic parameters in the authenticity control of natural flavours and essential oils, if suitable methods and comprehensive data from authentical sources are available.

Enantioselective capillary gaschromatography (enantio-cGC) and comparative stable isotope ratio mass spectrometry (IRMS), on-line coupled with cGC have proved to be valuable tools in the origin assessment of essential oil compounds. Promising developments in both fields of research will strongly accelerate insights into the biochemical pathways of natural flavour and essential oil compounds.

Volatilization of essential oil from caraway

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Interest in essential oils recently increases because they may be used as natural biocides. This interest is mainly based on data in the literature on biological effects of the plants from which the oils are extracted, on certain organisms. For a compound to act as a repellant or attractant it is a prerequisite that the organism to be affected becomes aware of the compound.
The biological necessity for excretion by plants conflicts with the human interest in high contents of the compounds to be harvested. Although there is evidence that volatilization of monoterpenes from Pinus spp. and flowers occurs, as yet no research was done on losses from essential oil crops.
For caraway it has been hypothesized that during the last stages of seed ripening losses of essential oil through volatilization take place. Caraway essential oil consists for over 95% of the monoterpenes carvone and limonene. In a greenhouse experiment we showed that exposure of plants with seeds to wind, produced by ventilators, reduced essential oil content by 80%.
To enable measurement of release of carvone and limonene, umbels with seeds in different stages of development were enclosed in glass flasks. The umbels were not detached from the plants, so release could be measured repeatedly on the same umbels. The headspace was sampled by pumping off air at 150 ml/min through a glass column containing the volatile-trapping agent Tenax. Incoming air was purified through a second Tenax column. At the completion of a run, volatiles were eluted from the column with hexane and measured using GLC. Release of carvone and limonene was demonstrated, there were large variations between individual plants, release was greater from older fruits, and in general more limonene than carvone was detected. During 24 h up to 30 ng/fruit limonene and 20 ng/fruit carvone were released from young fruits, and up to 40 ng/fruit limonene and 30 ng/fruit carvone from ripening fruits.
The large variations between plants may indicate differences in damage, caused by handling of the umbels. Such damage, however, undoubtedly also occurs in the field by wind. Moreover, wind may increase volatilization as it likely decreases the concentration of the monoterpenes in the fruit's boundary layer.
Results of further measurements of carvone and limonene volatilization from umbels of caraway, in the laboratory as well as in the field, are presented and discussed and an estimate of the losses in monoterpene yield caused by volatilization is made.

Non-*Labiatae* Essential Oils of Turkey

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The rich and diverse Flora of Turkey has a considerable percentage of aromatic plant species. More than 1/3 of the ca. 9000 flowering plant taxa known the occur in Turkey are believed to be aromatic plants.

A review of the essential oil composition of the Anatolian *Labiatae* was previously communicated (1).

This paper covers the non-*labiatae* essential oils of Turkey studied previously or at present. The families subjected to this review includes *Anacardiaceae, Araceae, Caprifoliaceae, Compositae, Cupressaceae, Cyperaceae, Gramineae, Hamamelidaceae, Lauraceae, Moraceae, Myrtaceae, Pinaceae, Rosaceae, Rutaceae, Oleaceae, Umbelliferae, Valerianaceae, Verbenaceae.* Present information includes many unpublished results.

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CHEMICAL CHARACTERIZATION OF OILS FROM SOME CYMBOPOGON SPECIES

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The plant-family of the grasses, the *Gramineae* is one of the most extensive plant-families in the world. This family consists of more than 500 genera with over 5000 species. A sub-family is the tropical family of *Poaceae* to which belong a group of aromatic grasses of the genus *Cymbopogon*, which comprises about 40 species. Some of the *Cymbopogon* species are important for the production of essential oils.

The most important oils isolated from *Cymbopogon* species are:
- citronella oils from *Cymbopogon nardus* (L.) Rendle and from *Cymbopogon winterianus* Jowitt;
- lemongrass oil from *Cymbopogon citratus* (DC.) Stapf, or *Cymbopogon flexuosus* Stapf;
- palmarosa oil from *Cymbopogon martinii* (Roxb.) Stapf, var. *motia* or *Cymbopogon martinii* (Roxb.) Wats, var. *martinii*;
- gingergrass oil from *Cymbopogon martinii Stapf*, var. *sofia*.

Citronella, lemongrass and palmarosa oils are chemically mainly characterized by the occurrence of aliphatic monoterpene derivatives, such as geraniol, citral, citronellol and citronellal.
Gingergrass oil, however, is featured by the fact that is contains about 90% monoterpenoids with p-menthane skeleton, such as: limonene, p-menthatrienes and p-menthadienols.
The chemical composition of the oils will be treated in more detail.

Essential oils isolated from some other *Cympobogon* species are of scientific interest.

BULGARIAN OLEAGINOUS ROSE AND ROSE OIL

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1. The origin of the Bulgarian oleaginous rose and favourable conditions for the rose production in Bulgaria. Unique characteristic of the climatic and soil conditions of the "Valley of The Roses", favourable to the accumulation of essential oil into the flowers of Bulgarian oil bearing rose.
2. The methods of preservation of the genotype of the Bulgarian rose - named Kazanlak rose.
3. Agrobiological bases of this field in Bulgarian agriculture. Technology of creation and cultivation of the rose plantation in contemporary conditions of private and cooperative rose production.
4. The origin, physical-chemical properties and perfumery qualities of the Bulgarian rose oil.
5. Installations and technology for the production of the Bulgarian rose oil. Combined system of florentine flasks ensure total extraction of essential oil without excess accumulation of stearopteus which lowers the rose oil quality.
6. Secondary processing of the rose oil form all producing installations:
   - Reasons for the introduction of this typical practice of the Bulgarian production of Rose oil.
   - Unique characteristics of the secondary processing in comparison with the practice of other rose oil producing countries.
   - Secondary processing - essence and importance for the stability of the Bulgarian rose oil quality.
7. The contribution of the "Bulgarska Rosa-Sevtopolis" Ltd., Kazanlak, to the rose production in Bulgaria.

Composition of the Essential Oil of Taurus Cedar Wood Oil (*Cedrus libani*)

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Cedrus libani A. Rich (*Pinaceae*) grows mainly on Taurus Mountain in Turkey and in Lebanon. Its timber is highly esteemed for its durability, strength and fragrance.

Cedrus libani is also known as Lebanon Cedar or Taurus Cedar. The other *Cedrus* species known are *Cedrus atlantica* Manetti (Atlas Cedar), *Cedrus brevifolia* Henry (Cyprus Cedar) and *Cedrus deodora* Loud. (Himalayan Cedar). These are "true Cedars" Fragrant timbers of some *Larix, Juniperus, Cupressus* and *Thuja* species are also known as Cedar wood.

This paper reports on the composition of the water and steam distilled essential oils from the chipped wood of *C. libani* Capillary GC and GC/MS analyses were carried out and the results were compared with those of *C. atlantica* and *C. deodora*. Main components in the *C. libani* oils were trans-α-atlantone, himachalol, α-β and γ-himachalens.

THE AROMA-GARDEN IN ERLANGEN

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The idea of an "Aroma-Garden", where one could assemble aromatic plants of the moderate climate zones, was realized more than 12 years ago at the University of Erlangen-Nuremberg in Franconia in the northern part of Bavaria. Our garden at Erlangen was worldwide the first garden of this kind; it still remains to be the largest Aroma-Garden.

An area of 9000 m^2 in a beautiful and protected landscape, in the midst of the City of Erlangen, close to the Schwabach Creak, was prepared to serve as a park with sunny or shady and dry or wet places. About 100 different aromatic plants were assembled, each of them covering a bed of several square meters. This way the visitor will find plant material sufficient to become familiar with the respective plant of his special interest. Plant material for analytical purposes is always available in non-limiting amounts.

The beautiful garden always has changed its appearance during the past vegetation periods; and each year, the different seasons attribute to a distinct sight of different sceneries.

Most of the aromatic plants in the garden produce essential oils. Among the several plant families, three of them appear to be predominant. Especially members of the Apiaceae, the Asteraceae and the Lamiaceae are present in larger amounts. Aromatic plants from other continents and from foreign human cultures have been gathered in the garden.

Aromatic plants and their essential oils have been used at all times and in all human cultures to serve as a medicine, as a spice or a cosmetic means, respectively.

We have studied intensively several plants from our garden. We did observe rather pronounced seasonal dependences in the production of essential oils among different species. We did analyze a number a essential oils and, important enough, did separate their main constituents.

The Aroma-Garden at Erlangen is open to the public from March to October and may be visited all day from the morning to the evening hours.

POSTER

1

COMPOSITION OF THE ESSENTIAL OIL OF *ARTEMISIA ARGENTEA* L'HÉR.

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The genus *Artemisia*, probably the largest and the most widely distributed genus of the Asteraceae, comprises about 400 species. Most of these species are characterized by the presence of strong aromatic odours mainly based on mono- and sesquiterpenes which are accumulated both in glandular hairs and schizogenous secretory ducts.[1]

A. argentea L'Hér. is an endemic species of Madeira, Desertas and Porto Santo islands that grows wild as small shrubs in the dry soils of the coast.[2] This species is also cultivated in homegardens, and an infusion of the leaves and inflorescences is locally used as an emmenagogue, a tonic, a stomachic and a vermifuge.[2,3]

The essential oil of *A. argentea* was isolated by hydrodistillation and distillation-extraction from leaves collected during the vegetative phase of the plant. The oil possessed a bluish-green colour and a strong odour, and was obtained in a yield of 0.2 % (v/w). The oil was a mixture of about 50 components, 30 of which were identified, representing 90 % of the total oil. The monoterpene fraction was the major one (69 %), containing mainly hydrocarbons (47 %). α-Phellandrene was the most abundant component of the oil (26 %), while pinanone (9 %) and pinocarvone (7 %) were the main components of the oxygen-containing monoterpenes. The sesquiterpene fraction (20 %) consisted mainly of oxygen-containing components; β-eudesmol and β-caryophyllene were the major constituents of this fraction (8 % and 6 %, respectively). In addition, chamazulene was found to be present in traces.

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2

COMPOSITION OF THE ESSENTIAL OIL FROM INFLORESCENCES OF *NEPETA TUBEROSA* L. SSP. *TUBEROSA*

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Nepeta is one of the most extensive genera of the Lamiaceae with almost 250 species occurring in Europe, Asia and few areas of Africa.[1] Twelve species are described for the Iberian Peninsula and the Baleares Islands.[1,2] The essential oils isolated from some species of this genus have also been studied,[3] and some of their constituents possessed biological activities.[4] Nepetalactone, the main constituent of the essential oil of *N. cataria* (catnip), as well as its isomers have shown a feline attractive effect.[5] Other studies have shown that these compounds could also act either as insect repellants[6,7] or attractants.[6]

In this work we report on the analysis of the essential oil from the inflorescences of *N. tuberosa* ssp. *tuberosa*. This species grows wild in Portugal and is one of the best represented species in the South West of the Iberian Peninsula.

The essential oil isolated by hydrodistillation and distillation-extraction showed a yellow colour and was obtained in an yield of 0.5% (v/w). The essential oil analysed by GC and GC-MS was a mixture of 13 components, 11 of which were identified, representing 98.9% of the total oil. The oil consisted mainly of monoterpenes (89%), 5,9-dehydronepetalactone (69%) and geranyl acetate (17%) being the main components. The sesquiterpene fraction amounted to (8%) and was dominated by β-caryophyllene (5%).

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3

COMPOSITION OF THE ESSENTIAL OIL OF *MICROMERIA VARIA* BENTH. SSP. *THYMOIDES* (SOL. EX LOWE) PÉREZ VAR. *THYMOIDES*

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Micromeria varia ssp. *thymoides* (=*Satureja thymoides*) is a common endemic species of Madeira, Desertas and Porto Santo islands.[1] This species is locally known as hyssop and its reported life zone reaches up to 1000 m height in the rocky scarps of the islands.[1]

An infusion of the leaves and of the flowers is traditionally used as an antispasmodic, a stimulant and an expectorant.[1,2]

The essential oil of *M. varia* was isolated by hydrodistillation and distillation-extraction, from leaves collected during the vegetative phase and at the beginning of the flowering phase, respectively. The oils were obtained in the same yield (0.3 %, v/w), but their main components were different, although both oils were dominated by the monoterpene fraction.

Thirty-six components were identified of the oil isolated from the leaves collected during the vegetative phase of the plant, amounting to 96 % of the total oil. This oil consisted mainly of monoterpenes (69 %); the hydrocarbons and the oxygen-containing compounds attaining 39 % and 31% of this fraction, respectively. α-Pinene (20 %) and geranial (16 %) were the major constituents of the monoterpene fraction. The sesquiterpene fraction (27 %) was dominated by *trans*-nerolidol (15 %) and β-caryophyllene (5 %).

Of the oil isolated from the leaves collected at the beginning of the flowering phase it was also possible to identify thirty-six components, representing 98 % of the total oil. In this oil, the monoterpene fraction was again the main one (86 %), however, a major difference was found between the amounts of the monoterpene hydrocarbons (6 %) and the oxygen-containing monoterpenes (80 %). Neral (30 %) and geranial (45 %) were the main constituents of the monoterpene fraction. The sesquiterpene fraction (12 %) was dominated by β-caryophyllene (6 %), whereas *trans*-nerolidol, one of the major components of the leaf oil during the vegetative phase was only found in traces.

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4

THE ESSENTIAL OILS OF TWO ENDEMIC MADEIRA ISLAND *ARGYRANTHEMUM* SPECIES : *A. PINNATIFIDUM* (L. FIL.) LOWE SSP. *PINNATIFIDUM* AND *A. HAEMOTOMMA* (LOWE) LOWE

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Six endemic *Argyranthemum* species are recorded in the Flora of the Madeira archipelago.[1] *A. pinnatifidum* (L. fil.) Lowe ssp. *pinnatifidum* (= *Chrysanthemum pinnatifidum* L. fil.; *Pyrethrum pinnatifidum* (L. fil.) Choisy; *A. pinnatifidum* (L. fil.) ssp. *pinnatifidum* Humphr. p.p.) is a common species that grows mostly in laurel woods.[1] In contrast, *A. haemotomma* (Lowe) Lowe (= *Chrysanthemum haemotomma* Lowe; *Chrysanthemum barreti* Costa) is one of the rare species, that grows as a shrub in the northern rocky scarp coasts of Madeira and Desertas islands (from 50 m to 400 m height).[1]

The essential oils of both species were isolated by hydrodistillation and distillation - extraction from leaves collected at the beginning of the flowering phase. The essential oil of *A. pinnatifidum* was obtained in an higher yield (0.1 %, v/w) than that of *A. haemotomma* (< 0.05 %, v/w). Twenty - three components of the oil of *A. pinnatifidum* were identified and twenty - one of that of *A. haemotomma*, amounting to 97 % and 96 % of the total oils, respectively.

Both oils were dominated by the monoterpene fraction (82 % for *A. pinnatifidum* and 72 % for *A. haemotomma*), β - myrcene being its main component (62 % for *A. pinnatifidum* and 50 % for *A. haemotomma*). Bornyl acetate was the major constituent of the oxygen - containing monoterpenes of the two oils, although in very different amounts (11 % for *A. pinnatifidum* and 3 % for *A. haemotomma*). The sesquiterpene fractions were dominated by hydrocarbons (11 % for *A. pinnatifidum* and 17 % for *A. haemotomma*), germacrene - D being the main constituent in both cases (10 % for both oils). The oxygen - containing sesquiterpenes amounted to 0.7 % in *A. pinnatifidum* oil and were only found in trace amounts in *A. haemotomma* oil. Another component found in both oils was *trans* - 2 - hexenal, amounting to 3 % in *A. pinnatifidum* and to 7 % in *A. haemotomma*.

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5

HYPERICUM HUMIFUSUM L. ESSENTIAL OIL FROM PORTUGAL. COMPARATIVE CHEMICAL COMPOSITION STUDY WITH *HYPERICUM PERFORATUM* L.

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In Portugal there are numerous *Hypericum* species with traditional utilization in home medical treatment. *Hypericum* L. is an herbaceous plant of the family of *Guttiferae* growing spontaneously in humid soils. This work is carried out to study the chemical composition of the essential oil of *Hypericum humifusum* L., comparing it with *Hypericum perforatum* L., which has been previously studied. The essential oil of *H. humifusum* was obtained in a Clevenger type apparatus, from fresh plant material collected in different vegetative stages and its chemical composition was studied by GC and GC/MS.

The main components were identified (α pinene 60%, β pinene 5%, undecane 2,5%, germacrene D 6%, β caryophyllene 2%) and compared with the main components of *H. perforatum* (methyl-2 octane 26%, nonane 3,4%, α pinene 36%, β caryophyllene 5,5%).

6

The essential oils from Spanish *Elaeoselinum* species (Umbelliferae)

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Elaeoselinum genus (Umbelliferae) is represented in Europe by four species and two subspecies, all of them found in the Iberian Peninsula.[1]

The essential oils from the fruits of *Elaeoselinum asclepium* (L.) Bertol subsp. *asclepium* and subsp. *millefolium* (Boiss.) García Martín & Silvestre, two plants that are morphologically quite similar, reveal a different composition. The GC/MS analysis of the essential oil from the subspecies *asclepium*, yielded 68 substances from which 48 were structurally identified (T_R and MS) representing 99.1% of the oil. Accordingly, the essential oil analysis of the subsp. *millefolium* showed 51 substances from which 38 (99,3%) were identified. Although in both cases α- and ß-pinene are present in a high proportion (76% and 94%, respectively) other less abundant or trace components can be used as markers to distinguish both subspecies, i.e. the sabinene content of subsp. *millefolium* (0.3%) is very small as compared with the content of subsp. *asclepium* (15%) and there are also minor components which are present in only one of the subspecies.

The essential oils of *E. gummiferum* (Desf.) Tutin (= *Margotia gummifera* (Desf.) Lange) and *E. foetidum* (L.) Boiss, do also contain a high proportion of α- and ß-pinene (>55%). In contrast, *E. tenuifolium* (Lag.) Lange, shows a quite different composition. In this plant the pinenes are minor components (3%) while the more abundant are myrcene (48%), sabinene (24%) and limonene (7%). This difference in the essential oil composition, and also in other secondary metabolites,[2] supports the proposed exclusion of this plant from the *Elaeoselinum* genus based in taxonomic and cariological characters. The genus and name now accepted is *Distichoselinum tenuifolium* (Lag.) García Martín & Silvestre.[3]

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7

VOLATILE COMPONENTS OF GENERA *COREOPSIS* AND *DICHROCEPHALA* (ASTERACEAE) FROM CAMEROON

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The purpose of the present study is to compare the chemical features of Asteraceae collected in Cameroon.

The esential oils, obtained by hydrodistillation of leaves of *Coreopsis grandifolia* and *Coreopsis barteri* (yields: 0.1%), of leaves and flowers of *Dichrocephala integrifolia* (yield: 0.06%), were analyzed by gas chromatography and GC/MS.

The volatile extracts obtained from the two *Coreopsis* species present very different chemical compositions: Germacrene D is the major constituent of the first one (42%) accompanied by a few amount of phenylheptatriyne (5%) and precocene I (0.5%), when *C. barteri* essential oil consists mainly of monoterpene hydrocarbons (with limonene, 50% and α- phellandrene, 13%).

The volatile extract of *D. integrifolia* is more complex; it contains a majority of sesquiterpenes (70%) among which germacrene D (34%) is predominant.

8

The Essential Oils of Turkish Eucalypts

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Eucalyptus sp. were introduced to the Southern coastal line of Turkey in late 19 th Century. Since then they have naturalized and The Ministry of Forestry has encouraged the establishment of state and private eucalyptetums.

This paper reports on the essential oil composition of the leaves of eight *Eucalyptus* species grown in a major experimental eucalyptetum. These are *E. citriodora*, *E. gamphocephala*, *E. occidentalis*, *E. globulus* subsp. *bicostata*, *E. globulus* subsp. *globulus*, *E. globulus* subsp. *maideni*, *E. grandis* and *E. camaldulensis*. The water and/or steam distilled oils were subjected to capillary GC and GC/MS analyses.

9

Composition of the Essential Oils of *Melissa officinalis* Subspecies of Turkey

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The genus *Melissa* (*Labiatae*) is represented in Turkey by a single species comprising three subspecies, namely, *Melissa officinalis* subsp. *officinalis*; subsp. *altissima* and subsp. *inodora*.

The water distilled essential oils of the three subspecies collected from various locations were analysed by capillary GC and GC/MS. The results are listed in tabulated form and their significance is discussed.

10

Essential Oils of the Five Subspecies of *Sideritis libanotica* Occurring in Turkey

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Sideritis (Labiatae) is represented in Turkey by 40 species comprising 46 taxa, of which 35 taxa are endemic. Most of these spesies are used as herbal tea in folk medicine.

We report on the results of our work on the water distilled essential oils of the five subspecies of *Sideritis libanotica* occurring in Turkey. These are: subsp. *violascens* (endemic), subsp. *microchlamys* , subsp. *kurdica*, subsp. *libanotica* and subsp. *linearis* (endemic). The oils were subjected to capillary GC and GC/MS analysis. To the best of our knowledge this is the first report on the essential oil analysis of *Sideritis libanotica*.

11

CHEMICAL COMPOSITION OF THE ESSENTIAL OILS FROM RHIZOME, STEM AND LEAF OF ALPINIA SPECIOSA GROWN IN EGYPT

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Essential oils prepared by hydrodistillation of the rhizomes, stem and leaf of Alpina speciosa (=Alpina zerumbet, Zingiberaceae) were analyzed by GC-MS. Components were identified by their Kovats indices and their MS which were compared with published material or with the data of reference compounds. Quantitative determination was carried out based on peak area integration.

The oils of the three organs of Alpinia speciosa grown in Egypt are fairly rich in monoterpenoids. Nearly equal proportions of oxygenated and unoxygenated compounds were found in them. Mono- and sesquiterpene hydrocarbons constitute the largest proportion of each of the three oils. About 25% of alcohols were found in each of the three oils; terpinen-4-ol being the main alcohol. Appreciable amounts of 1,8-cineole (12-14%) are present in the oils of the three organs.

Detailed discussion of the identification of components of the essential oils prepared from rhizome, stem and leaf of Alpinia speciosa grown in Egypt and comparison with oils prepared from Alpinia species growing abroad will be presented.

12

ESSENTIAL OILS OF SOME PERSPECTIVE PLANTS FOR MOLDOVA

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The chemical composition and yields of a range of essential oils prepared from perspective plants cultivated or which are going to be introduced in culture in Moldova have been investigated: *Dracocephalum moldavica* L., *Nepeta cataria var. citriodora* L., *Hyssopus officinalis* L., *Hyssopus meretacens* L., *Hyssopus montana* L., *Artemisia annua* L., *Tagetes signata Bartl., Origanum vulgare* L.; *Origanum heracleoticum* L., *Lophanthus annisatus Benth., Satureia montana* L., *Calamintha nepetoides* L., and *Calamintha nepeta ssp. glandulosa.*

13

ESSENTIAL OIL OF DIFFERENT FORMS OF ARTEMISIA BALCHANORUM KRASCH

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Artemisia balchanorum cultivated in the climatic conditions of Moldova proved to be a strong polymorphic species with respect to the morphological signs as well as to the productivity of the essential oil and its chemical composition.

The essential oil of 96 forms of *Artemisia balchanorum* has been investigated. The yield of essential oil varies from 0.50 to 3.85%, that of natural population being about 0.80%.

The following 12 compounds (in the order of their decreasing content) linalool, citral, geraniol and its acetate, α- and ß-thujone, 1,8-cineole, limonene, α-terpineol, p-cymene, nerol and linalyl acetate predominate in the essential oils of different forms of *Artemisia balchanorum*.

14

ESSENTIAL OIL FROM THE FOLIAGE AND BRANCHES OF DOUGLAS FIR PLANTATIONS GROWN IN BULGARIA

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The percentage of essential oil in needles and branches of Pseudotsuga menziesii (Mirb.) Franco grown in plantations from 15 up to 40 years old, was found, as well as the composition and dynamics of this same oil in the different seasons.

The terpene hydrocarbons and oxygen-containing compounds of the essential oil obtained in different objects, were found, too.

The extracted oil amounted from 0.31 up to 0.37 % of the green foliage and thin branches, and it varied, having a spring minimum and a winter maximum.

The main components were: α -pinene, ß-pinene, camphene, sabinene and bornylacetate, and they varied in amounts, depending on the Douglas fir provenances.

15

COMPARATIVE STUDY OF THE CONTENT AND COMPOSITION OF THE ESSENTIAL OILS FROM THREE CALAMINTHA SPECIES

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Calamintha sylvatica Bromf. is widely distributed in Croatia, both in the coastal region and the continental area of the country.

The area of Calamintha nepeta /L./ Savi subsp. glandulosa /Req./ P.W. Ball is restricted to the karst of the Mediterranean zone.

Specimens of these two species were collected during blossoming in Istria.

The third Calamintha species, marked as "Calamintha sp." is an undetermined bastard which has been grown for over thirty years in the Botanical garden of the Faculty of Pharmacy and Biochemistry in Zagreb.

GC analysis of the hydrodistilled oils of the Calamintha species examined, revealed their characteristic composition and the following predominant components in each species:
Calamintha sylvatica /total oil content 0.6 %/: pulegone > piperitenone oxide > humulene > carvone

Calamintha nepeta subsp. glandulosa /total oil content 1.1 %/: piperitenone oxide > pulegone > limonene+cineole > carvone

Calamintha sp. /total oil content 0.6 %/: menthol > menthone > neomenthol > carvone > limonene+cineole > pulegone > menthylacetate

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MONOTERPENES IN FOUR *Solidago* SPECIES

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The essential oils from four *Solidago* (goldenrods) species growing in Poland - *S. virgaurea* L., *S. canadensis* L., *S.gigantea* Ait. and *S. graminifolia* (L.) Salisb. were obtained by hydrodistillation and investigated by the GC-MS method. The herb of *S. graminifolia* was found to contain the highest amount -1,77% and of *S. virgaurea* the lowest amount- 0,30% of oil.

The oils were obtained from herbs, flowers, leaves and stems. There were some variation in the yield of oils but there were no significant differences in their composition in the individual parts of the same species. In general the leaves contained the most of oil.

Monoterpenes and germacrene D - a sesquiterpene characteristic of this genus - were identified and their content of herb oils was established. All the oils contained germacrene D: *S. virgaurea* - 16,0%, *S. canadensis* - 24,6%, *S. gigantea* - 30,0% and *S. graminifolia* - 3,0%. Other main components were : *S. virgaurea* - sabinene - 11,2%, β-pinene 10,7%, *S.canadensis* - α-pinene - 16,3%, limonene - 9,30%, *S. graminifolia* - sabinene - 18,2%, β-pinene - 9,3%.

In our studies we established that considerable differences occur in the yield and composition of essential oils among the four species of goldenrod.

In addition we compared yield and composition of the oils of *S. canadensis* from seven different origin in Poland. The yield of oil varied from 0,69% to 1,44%. The main constituents were the same but their contents were different, e.g. α-pinene -13,0-29,9%, limonene - 6,8 - 20,8%, germacrene D - 13,7-41,3%.

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COMPARISON OF ESSENTIAL OILS COMPOSITION OF BETONICA AND STACHYS -DOMESTIC PLANTS IN POLAND

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Abstract

The essential oils obtained from dry herbs of 8 species of Betonica and Stachys genera were investigated.

The essential oils were obtained as a result of destillation with water vapour using Deryng's apparatus. Their content in raw material was estimating simultaneously (0.2 - 0.6%).

Main components of essential oils were distributed by use of thin layer chromatography on Merck plates in ethyl acetate - benzene system (1:5).

Qualitative and quantitative analysis of essential oils was carried out using gas chromatography with mass detector Hewlett - Packard 5890/5970. As a result of chromatographic distribution 65 fractions were obtained. It was assumed that there are the following main essential oils components: cadinens (fractions no 9,11,14,16), phthalate (no 44), phytol (no 50) and unidentified ones (no 18,41,45,46).

The relationship among above species was presented using graphic dendrite method. This dendrite reflects chemotaxonomic similarities of described species and confirm the system presented in Flora Europea.

Next biosystematic studies of these species are in progress.

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The Essential Oil of Balsamita major Desf. from St.-Petersburg region

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The essential oil of leaves and inflorescence of Balsamita major Desf.(Syn. Pyrethrum majus (Desf.) Tzvel.; Tanacetum balsamita L.; Pyrethrum tanacetum D.C.; Chrysantemum majus Aschers ; Asteraceae=Compositae), that cultivated in the Bota nical Gardens of St.-Petersburg, was produced by water distillation. The essential oil was obtained in 0.13 % from leaves and 0.27 % from inflorescence.

The quantitative amounts of the identified components of the leaf oil are as follows: limonene and 1,8-cineole (1.0 %), linalool (11.0), tujone (1.3), E-alloocimene and camfora (1.2), 1-terpineole and pinocarveole (0.6), Z-alloocimene and pinocamfeole (0.7), linalool oxide (1.1), terpinene-4-ol and borneole (0.7), myrtenal (0.8), α-terpineol and myrtenol (3.4), pulegone (71.0), piperitone (0.5), bornile acetate (1.5), α-terpenilacetate (1.9), and longifolene (0.5).

The components quantitative amounts of the inflorescence oil are as follows: limonene and 1,8-cineole (1.5 %), linalool (8.4), tujone (0.9), E-alloocimene and camfora (1.2), 1-terpineole and pinocarveole (0.6), Z-alloocimene and pinocamfeole (0.8), linalool oxide (0.7), terpinen-4ol (0.4), myrtenal (0.7), α-terpineole and myrtenol (3.1), pulegone (78.0), and piperitone (0.6).

The results of the identification and quantification of the components is based on the GC and GCMS data in combination with its partition coefficients in the hexane/acetonitrile system.

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THE ESSENTIAL OILS OF *LEDUM PALUSTRE* L.: AN ASCARIDOL CHEMOTYPE

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The leaf volatile oil of *Ledum palustre* L. is characterized by a high content of sesquiterpene alcohols palustrol and ledol (1-2). On the contrary, a clearly different pattern has been reported for the oils from *L. palustre* var. *nipponicum* Nakai and var. *yesoense* Nakai. from Japan, which were shown to contain considerable amounts of ascaridol (3). This is also true as regards another Asiatic taxon, viz. *L. hypoleucum* Kom. from Russian Far East (4). Nowadays there is a tendency among taxonomists to include the genus *Ledum* L. (Ericaceae) in *Rhododendron* L. This study describes a *L. palustre* oil with ascaridol as the main constituent.

Leaf samples were collected from southern Finland, the oil was hydrodistilled and analysed by GC and GC-MS. An unknown major component, being thermolabile to some extent in GC conditions, was isolated by TLC. The structure was elucidated by ^{1}H- and ^{13}C-NMR spctroscopy. Palustrol and ledol together represented up to 80% in the known *L. palustre* oil. The volatile oil of the new chemotype contained about 65% ascaridol without the typical sesquiterpene alcohols. The occurrence of ascaridol in *L. palustre* leaf oil was further confirmed by solvent extraction and low temperature GC-MS analysis (130°C). In addition, mixed chemotypes with varying levels of ascaridol (≈10%) were found.

Ascaridol, ^{1}H-NMR: δ(ppm) = 0.99, 1.00 (2xd, 6H, J = 6.92 Hz, 9-H_3 and 10-H_3), 1.38 (s, 3H, 7-H_3), 1.52 (m, 2H, 2-H/3-H), 2.03 (m, 2H, 2-H/3-H), 1.92 (sept., 1H, J = 6.92 Hz, 8-H), 6.41 (d,1H, J = 8.60 Hz, 6-H), 6.50 (d, 1H, J = 8.60 Hz, 5-H). **^{13}C-NMR:** δ(ppm) = 17.17 (q, C-8/C-9), 17.25 (q, C-8/C-9), 21.42 (q, C-7), 25.64 (t, C-2), 29.56 (t, C-3), 32.16 (d, C-8), 74.37 (s, C-1), 79.80 (s, C-4), 133.09 (d, C-5), 136.43 (d, C-6). **GC-MS:** m/z (%) = 168 (M^+,< 1), 121 (100), 43 (89), 93 (77), 136 (68).

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The essential fruit oils from *Smyrnium perfoliatum* L. and *Smyrnium rotundifolium* Miller (Apiaceae)

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In course of our investigations of the essential oils from Apiaceae of the genus Smyrnium the fruit oils of *S. perfoliatum* and *S. rotundifolium* were analyzed. Both species are biennial plants native to South Europe extending to Asia Minor and the Caucasus. Due to its cultivation as a garden plant in former centuries, *S. perfoliatum* also has been naturalized in Western Europe.

Since the two species are regarded to be closely related to each other, we analyzed the respective fruit oils by means of GC-MS to compare their compositions.

The oil of *S. rotundifolium* contains more than 80% oxygenated compounds with 70% furanodiene as native constituent which undergoes thermal Cope-rearrangement during steam distillation and gc-separation. The fraction of hydrocarbons (16%) is composed of 10% monoterpenes with 7% α-pinene and 3% ß-pinene.

In contrast, *S. perfoliatum* is characterized by a high content of hydrocarbons (70%) - above all germacrene-d (46%) - and a smaller proportions of different other sesquiterpenes (together 18%). The oxygenated compounds mainly consist of furanosesquiterpenoids, however, furanodiene - the main constituent of the fruit oil of *S. rotundifolium* - can only be found in traces.

As a result, a clear discrimination of the two species can be performed on basis of the chemical composition of their fruit oils.

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Steam-volatile compounds from the roots of *Geum reptans* L.

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Geum reptans L., belonging to the Rosaceae family, is a perennial plant, which usually grows on tills in the European Alps from 2100 up to 2800 m altitude, but also in the mountainious regions of the Tatra, the South Carpates and the Balkan peninsula.

In course of our investigations of the essential root oils of several Geum species we have found that the essential oil pattern of *Geum reptans* considerably differs from all subalpine Geum species we have investigated so far such as *Geum urbanum, G. rivale, G. fauriei, G. macrophyllum, G. rhodopeum* and *G. bulgaricum*.

In contrast to the essential root oils of the subalpine species which usually consist of eugenol and several compounds mainly belonging to bicyclic oxygenated monoterpenes like cis/trans-myrtanal, myrtenal, cis/trans-myrtanol and myrtenol without any hydrocarbons the essential root oil of *Geum reptans* consists of nearly 7% monoterpene hydrocarbons, more than 30% monocyclic oxygenated monoterpenes with phellandral as main component (5.9%) and approximately 16% of aliphatic acids like dodecanoic, tetradecanoic and hexadecanoic acid (14.3%) which is the compound with the highest amount.

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Composition of the essential oils from different parts of *Ligusticum mutellina* (L.) Crantz

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Ligusticum mutellina (L.) Crantz, belonging to the Apiaceae (Umbelliferae), is a perennial plant, which grows on alpine pastures and meadows in the European Alps from 1500 m up to 2800 m altitude and more rarely in low mountain range areas of Central and South-East Europe.

The roots of *Ligusticum mutellina* are of particular interest because they are used in beverage industry for flavouring a special spirit. The respective essential oil, which has been before subject of a few investigations, consists of approx. 40% hydrocarbons with 11% viridene and 10% myrcene as main constituents, 30% phenylpropanoids - above all myristicine (26.5%) - and 23% phthalides (20.5% ligustilide).

In contrast to the root oil, the essential oils obtained from the herb and the fruits virtually do not contain phthalides. They consist of approximately equal proportions of oxygenated compounds - predominantly phenylpropanoids - and terpene hydrocarbons, however, their qualitative and quantitative patterns differ significantly. Main constituents of the herb oil are myristicin (39%), α-phellandrene (25%), dill-apiole (10%). 3-carene (6%), ß-phellandrene (5.6%), and of the fruit oil myristicin (28%), sabinene (24%), parsley-apiole (13%) and ß-bisabolene (10%). The latter constituent is a characteristic compound of the fruits and was completely absent in the other investigated parts of the plant.

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The Essential Oil in different parts of the head blossoms of *Tripleurospermum inodorum* SCHULTZ-BIP.

Phytochemical investigations of *Tripleurospermum inodorum* SCHULTZ-BIP. (syn. *Matricaria inodora* L.) showed that the title plant belongs to the genus *Tripleurospermum* rather than *Matricaria* based on the compositions of polyacetylenes.[1] Scentless camomile (*Tripleurospermum inodorum* SCHULTZ-BIP.) is occasionally found as falsification in roman camomile (*Chamaemelum nobile* (L.) ALL.). Further, the drug should not be used as substitute for Matricariae flos, DAB 10, as suggested to be due the occurence of polyacetylenes.[2]

The plant material was collected on wasted sewage farms in the south of Berlin and dried carefully at room temperatures. The head blossoms, separated into their components e.g. tubular flowers, lingulate flowers, periclinium leaves with receptacle, semen, were subjected to steam destillation. The essential oil obtained in each part was investigated by GLC and GLC/MS.

The results showed that the amount of essential oil differed in each part. The percentage of essential oil obtained from the complete head flowers was 1,1% whereas that derived from lingulate flowers and periclinium leaves was 0,3% and 1,6%, respectively. Out of 54 substances obvious in the GLC-chromatogram 14 substances could be identified. The composition of the essential oil in each instant differed only in their quantitative amount, not in their qualitative composition. Whereas (2Z,8Z)-deca-2,8-diene-4,6-diynoicacid methyl ester (trivial name: 2-cis, 8-cis-Matricariaester)[3] proved to be the main component in the complete head flowers (80%) investigations in the lingulate flowers showed this component only in 44% of the total essential oil. Further, an hitherto unknown component with a molecular ion M^+174 was present. It is suggested that this substance is a stereoisomer of 2-cis, 8-cis-Matricariaester, the structure elucidation is under investigation.

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CHARACTERIZATION OF SOME FUNGAL VOLATILES OF *GLOEPHYLLUM ODORATUM* GROWN *IN VIVO* AND *IN VITRO*

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The brown-rot fungus *Gloephyllum odoratum* (Wulfen ex Fr.) Imaz. *(Basidiomycetes)* is common on spruce stumps (*Picea*). The fungus produces an odour resembling that of anise or fennel. A number of volatiles depending on the growth conditions have been identified from the cultivated fungus (1, 2, 3). According to our knowledge no data on the composition of natural fungus have been presented. The aim of the present work was to characterize the occurrence of fungal volatiles in natural *G. odoratum* grown in Finland and *in vitro* cultivated fungus.

The natural *G. odoratum* was collected from a spruce stump. The mycelium of the same fungus was cultivated in a solid and liquid media. Chitin, chitosan and glucosamine (450 mg/l) were used as elicitors. The fungal volatiles were identified from the hydrodistilled oils by GC and GC-MS analysis. In addition biological activity of the oil was tested using *Artemia test* and *Agrobacterium tumefaciens* (4).

The main volatiles *in vitro* cultivated fungus included linalool, citronellol, geraniol, methyl-*p*-methoxyphenylacetate or drimenol depending on the culture types and effects of elicitors. The same compounds apart from methyl-*p*- methoxyphenylacetate (33,5 %) occurred as minor compounds (5.4 %) in natural *G. odoratum*. The other constituents such as, santene, tricyclene, α-pinene, camphor, α-terpineol, borneol, bornylacetate are also typical for conifervolatiles. The fungal oil was toxic to larvae of *Artemia salina*, and inhibited the growth of gall tumors on potato discs caused by *Agrobacterium tumefaciens*. These results indicate that the oil may possess cytotoxic activity.

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SYNTHESIS, ANALYSIS AND EXTRACTION OF SELECTED MONOTERPENE GLYCOSIDES

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In the course of investigations on Melissa officinalis (1, 2) the occurence of monoterpene glycosides has been demonstrated several times (3, 4). Nevertheless to our knowledge their role and significance in monoterpene turnover is not answered conclusively. The use of indirect methods, i.e. enzymatic or acid hydrolysis, seems to be difficult for the solution of biological questions (high amounts of plant material, long sample preparation).

To overcome these problems we developed a direct method for extracting and analyzing the monoterpene glycosides of *Melissa officinalis* (L.) and other plant material.

We synthesized Neryl-ß-D-glucosid, Geranyl-ß-D-glucosid and (-)-ß-Citronellyl-ß-D-glucosid by conventional Königs-Knorr-Synthesis. After purification the identity was determined by GLC-MS of the peracetylated and the permethylated products (Table 1) according to literature data (5).

Table 1:

Permethylated Glucopyranosides	MS m/z (intensity)
Geranyl-gluc.	45, 115(91), 101(81), 41(63), 147(60)
Neryl-gluc.	45, 41(69), 101(67), 69(56), 115(56)
(-)-ß-*Citronellyl*-gluc.	88, 101(34), 187(27), 45(27), 41(24)
n-Dodecyl-gluc. (Sigma)	88, 101(34), 45(21), 71(18), 43(17)

(gluc. = ß-D-glucopyranosid)

Since it should be possible to analyze monoterpene glycosides on a GLC-System used for essential oil separation, we chose the method of permethylation (5). Permethylated glycosides have lower boiling points than trimethylsilyl- or acetyl-derivatives. Furthermore they can be readily separated from their reaction mixture and analyzed on a Durabond-Wax capillary column.

Using these monoterpene glycosides we developed a short extraction procedure: solid/liquid sample preparation on SepPak RP_{18}-cartridges (Waters, Millipore) eluting with methanol/H_2O. Quantification is possible by adding Phenyl-ß-D-glucopyranoside (Fluka) as internal standard.

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IDENTIFICATION OF ALLICIN IN GARLIC BY LIQUID CHROMATOGRAPH/MASS SPECTROMETRY/MASS SPECTROMETRY

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ABSTRACT

An on-line liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) techniques with thermospray (TSP) interface were used for the identification of allicin, a thermally labile compound of crushed garlic bulbs (*Allium sativum* L.). In this paper, the reverse phase column and mobile phase of methanol-0.1 M ammonium acetate (70:30) were used for the separation of garlic components. Systems with the thermosprary vaporizer at 100°C and the 125°C ion source temperature of the tandem quadruple mass spectrometer were connected to LC column for MS/MS analysis. This soft ionization technique can reduce thermal deomposition and obtain a chemical ionization (CI) type of spectrum with a protonated molecular ion $[M+H]^+$. Further structure information was obtained by means of collision-induced dissociation (CID) analysis. The mass fragments from CID technique were obtained from the daughter ion scan mode. Comparing to the identification from synthetic allicin compound, it is demonstrated that using the on-line LC/MS/MS methods have the accuracy and simplicity to the solution of the analytical problems in the thermally labile compound. The separation and characterization of LC/MS and LC/MS/MS methods for the analysis of allicin and the other components of garlic extracts are presented and discussed.

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Gas Chromatographic Investigation of the Monoterpene Fraction of Citrus Oils Using Hydrophobic Cyclodextrins as Chiral Stationary Phases - Detection of Adulterations

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During recent years great progress in the investigation of chiral constituents of essential oils has been made by the introduction of modified cyclodextrins as chiral stationary phases in gas chromatography (GC)[1]. The separation of the enantiomers of various terpene hydrocarbons, such as α-pinene, β-pinene, sabinene, Δ-3-carene, α- and β-phellandrene, camphene and limonene is rather remarkable. All chiral and non-chiral monoterpene hydrocarbons occuring in essential oils can be separated on a dual column system using two 25 m fused silica columns coated with heptakis(6-O-methyl-2,3-di-O-pentyl)-β-cyclodextrin and with octakis(6-O-methyl-2,3-di-O-pentyl)-γ-cyclodextrin[2]. Some of the monoterpene hydrocarbons are eluted in reversed order on both columns. The described system may be used as a good method to help the analyst in detecting citrus oil adulterations. The information obtained by enantioselective GC of the volatile fraction of citrus oils is sufficient to ascertain authenticity. Our investigations confirm in essential parts the conclusions of G. Dugo et al.[3].

Further applications of hydrophobic cyclodextrins in the examination of essential oils will be presented.

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ENANTIOMERIC RATIO OF SELECTED OXYGENATED TERPENOID COMPOUNDS IN ESSENTIAL OILS

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Chiral terpene alcohols and carbonyl compounds considerably contribute to the characteristic flavour and fragrance of an essential oil. In many cases the perception of odour or taste differs greatly depending on the configuration. The determination of the enantiomeric ratio may therefore be indicative of the origin, purity, age and authenticity of a specific essential oil. We have prepared selectively substituted cyclodextrin derivatives suited for enantiomeric resolution of many terpene alcohols and carbonyl compounds [1]. Some examples are: linalool, citronellol, terpinen-4-ol, α-terpineol, cis- and trans-sabinene hydrate, menthol, neomenthol, nerolidol, bisabolol, fenchone, menthone, isomenthone, piperitone, verbenone, pulegone, pinocamphone, isopinocamphone, camphor and citronellal. The determination of the enantiomeric ratio of these compounds as a fingerprint in a variety of essential oils shows typical differences and clearly demonstrates that this technique is suited to detect adulterations.

We want to thank K.-D. Protzen, Paul Kaders GmbH, for providing samples of essential oils for these investigations.

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ENANTIOMERIC DISTRIBUTION OF α-CURCUMENE, α-BISABOLENE AND β-BISABOLENE IN SELECTED ESSENTIAL OILS

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Only one gas chromatographic study about the enantiomeric distribution of some selected sesquiterpene hydrocarbons has appeared in the literature so far [1]. This seems to be mainly due to the lack of racemic reference compounds. After the synthesis of racemic α-curcumene, α- and β-bisabolene we succeeded in resolving their enantiomers on a capillary column with heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin [2] and showed that some closely related essential oils may contain either one of the enantiomers. Thus, the oil of *Curcuma longa* contains only (+)-α-curcumene, while *Curcuma xanthorrhiza* contains the (-)-enantiomer. (-)-β-Bisabolene is present in ginger oil and in *Curcuma longa* oil, the (+)-enantiomer was identified as a constituent of the oils of *Pimpinella anisum* and *Levisticum mutellina*. The (-)-enantiomer of α-bisabolene is present in spike lavender oil. The racemic standard compounds were prepared by synthesis according to known methods. Peak assignment was achieved by packed column preparative gas chromatographic enantiomer separation with subsequent determination of the optical rotation of the collected fractions [3].

It may be worthwhile to investigate the enantiomeric composition of sesquiterpene hydrocarbons more systematically considering the importance of these compounds for flavour and fragrance. These investigations may also give new insights in biosynthetic pathways of terpenoid compounds and may be useful for studying the stereochemistry of rearrangements.

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ENANTIOMERIC COMPOSITION OF TERPENIC HYDROCARBONS IN ESSENTIAL OILS FROM *Pinus sp.* AND *Abies sp.*

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α -Cyclodextrin (CD) complexation under appropriate conditions of partition gas chromatography (1) has been applied for chiral discrimination of hydrocarbon fraction of Pine oils and of Abies oils of various origin.

It has been found out previously (2) that the ratio of terpenic hydrocarbons enantiomers depended on the plant species. This commmunication refers to our attempts to apply the option using α-CD for the study of Pine oils and Abies oils of various species (*Pinus sylvestris* L., *Pinus montana* Mill., *Pinus nigra* Arnold, *Pinus pinaster* Aiton, *Pinus cembra* L., *Abies alba* Mill. and *Abies sibirica* Ledeb.).

The essential oils under investigations were obtained from needles and cones cultivated in Austrian, Italian, Korean and Polish plantations.

It has been found that the ratio of S to R enantiomer may vary largely depending on the species, on the origin of material i.e. part of the plant and place of its cultivation.

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A new procedure for the enrichment of headspace constituents versus conventional hydrodistillation.

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With the aid of a new dynamic headspace extraction technique the volatile constituents of small amounts of plant material have been separated and analyzed by capillary GLC. Under the applied conditions a micro scale steam distillation of the sample is performed using a 10 ml headspace vial containing the material to be investigated. This vial, which is placed in a heating block, is connected with a cooled receiver vial by a 0.25 mm i.d. quartz capillary. By temperature programmed heating of the sample the volatile constituents are vaporized and passed through the capillary into the cooled receiver vial. The volatiles as well as water, which has been added to the sample before heating, are condensed and collected in n-pentane within the receiver vial.

Because of its relative complex essential oil composition pine needles from *Pinus sylvestris* have been taken as a representative example to demonstrate the potential of the applied technique. The collected volatiles of 50 mg pine needles dissolved in approximately 200 µl n-Pentane could be injected directly into a capillary GC and were sufficient for numerous investigations of the composition.

The received analytical results are compared to data from identical samples obtained by conventional hydrodistillation, showing a good correlation of the qualitative and quantitative composition.

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Headspace Constituents of Opium

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The odour determining volatile constituents of opium (dried milky latex of Papaver somniferum L., Papaveraceae) are still unknown until today, although just the olfactoric properties of opium are important additional criteria for an identification of this drug in various national pharmacopeias as well as by trained police dogs. To get more information about the chemical structures of the relevant odour constituents of opium, GC-FID, GC-FTIR-MS and GC-MS (high resolution) as well as the determination of Kovats indices in combination with the GC-sniffing-technique and nitrogen-specific detection were used.

After trapping of the headspace sample by means of a commercial pumping system and desorption of the enriched volatile components with carbondisulfide, the GC-FID data allowed the detection of over 100 compounds of which 70 were elucidated by means of GC-FTIR-MS and GC-MS(HR). Using GC-Sniffing-Technique only a small part (about 5.5 min elution range of the whole 40 min.) of the chromatogram showed interesting olfactoric properties caused by 36 of the 70 identified constituents.

These volatile components are mainly short chained aldehydes (e.g. hexanal to tridecanal), alcohols (e.g. hexanol, octanol), γ-lactones, esters (e.g. methyl and ethyl lactate), acids (e.g. acetic acid) and hydrocarbons (straight chained as well as branched). Besides, especially N-containing heterocycles, above all pyrazines, were identified and elucidated as the main odoriferous substances.

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HEADSPACE-ANALYSIS OF MUSHROOMS USING GC/FID AND GC/FTIR/MS

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Headspace samples, steam distillates and dichloromethane extracts of well liked edible mushrooms (Agaricus campestris and A. bisporus, Lepiota procera, Armillaria mellea, Boletus edulis and Cantharellus cibarius) were investigated by means of GC/FID and GC/FTIR/MS.

The aim of these analyses was the identification of constituents responsible for the typical mushroom odor of each species. To relate the gaschromatographic-spectroscopic data with olfactoric data the GC-sniffing-technique was used.

First results, especially of the headspace samples, showed that 1-octen-3-ol, a well-known mushroom compound, is of basical importance for the top-note of the investigated mushrooms. More than forty identified constituents additionally cause an effect (qualitatively and quantitatively) on the odor impression of these species. Octane-derivatives (saturated and unsaturated hydrocarbons, alcohols, aldehydes, ketones and esters) contribute in a significant way to the odor of the headspace samples. Also alcohols, aldehydes, ketones, acids and esters (C4-C14) play an important role (eg. anisaldehyde in Agaricus samples) in the headspace, distillate and extract samples. Some distillates as well as extracts have none-typical side-notes. Odor impressions of extracts with high percentage of fatty acids were only in low agreement with the odor of the genuine or headspace mushroom odor.

For the cluster characterization of various mushroom samples the Multivariate Data Analysis (MDA) was additionally applied using the concentration values of most important aroma compounds.

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HEADSPACE-ANALYSIS OF AROMA CONSTITUENTS OF SUNFLOWER STEMS

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The constituents of various parts of sunflower (Helianthus annuus L.) were extensively investigated by different groups, however the volatiles of sunflower stems are still unknown until now. Especially various pharmaceutical interesting sesquiterpene lactones as well as di- and triterpenic compounds of this agricultural precious plant have been increasingly analyzed in the last years. Only one patent (Austrian Patent 375 258/1981) discussed the use of sunflower stems for cosmetic uses (skin and muscle regeneration), but the aroma constituents were not analyzed by gas-chromatographic-spectroscopic systems. The aim of this investigation was therefore not only to detect and identify the headspace constituents of sunflower stems by means of GC/FID and GC/FTIR/MS analyses but also to use the GC-sniffing-technique in order to get information on aroma compounds contributing and responsible for the interesting and agreeable odor (natural green, hexenol-like, light acidic and weak terpenic).

After volatile-trapping of cutted sunflower stems (dynamic headspace method) the GC/FID measurements showed twenty main as well as twentyfive minor compounds. Using GC/FTIR/MS analyses the structure of all 20 main and of 12 minor constituents could be established (mass and IR spectra correlation with library spectra and coinjection of pure compounds with retention time correlation). As dominant components of the H. annuus headspace sample alpha-pinene (86.2%), beta-pinene (4.1%), butyl acetate (2.3%) next to further monoterpenes and also minor aliphatic alcohols were identified. The comparison of olfactoric literature data of pure aroma compounds identified in the headspace showed a significant conformity with the odor impression of these components in the genuine headspace sample obtained by the GC-sniffing-technique. This result leads to the conclusion, that for the interesting green note especially also identified (Z)-3-hexenol, (E)-2-hexenol and 2-nonanol, and for the terpenic odor impression the identified terpenes (alpha- and beta-pinene, camphene, alpha-thujene, limonene, beta-phellandrene, linalol, bornyl acetate, geranyl acetate, fenchone, alpha-terpineol, beta-thujone and beta-caryophyllene) must be responsible.

This statement was proven by odor correlations of the headspace sample (nearly the same odor as of the genuine stems) with a mixture of all identified constituents (same percentage of each compound determined in the headspace). The odor of the test mixture was in high agreement with that one of the headspace (for six odor trained panelist used), but for the professional perfumer the green-note impression was slightly different from that one of the genuine headspace sample. Therefore further not identified trace compounds of the investigated sample with very low odor thresholds (green-note) apperently exhibit also synergistic effects on the green odor impression.

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Antibacterial, antimycotic and antioxidant activities of five South African essential oils

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A selection of five indigenous plants from Southern Africa was used in an investigation into the bioactivities of their essential oils. The plants selected were ***Cymbopogon plurinodes, Helichrysum sp [Kidd's Beach], Pteronia incana, Salvia stenophylla*** and ***Tagetes minuta***. There is evidence linking the chemical nature of the oil constituents and their biological activities.

Steam distilled oils were evaluated for their antibacterial activity by the agar well-diffusion test of Deans & Ritchie [1987] using a collection of 25 test bacteria, a number of which are of public health significance. The essential oils from ***Salvia stenophylla*** and ***Tagetes minuta*** were particularly effective at preventing the growth of most of the test bacteria to a significant extent. In contrast, the oil from ***Cymbopogon plurinodes*** was notable in eliciting only a small inhibitory effect. The other two oils were of intermediate but consistent activity, being inhibitory to the entire 25 bacteria. As in a number of similar studies, there was no obvious pattern of susceptibility as reflected in the Gram reaction of the individual strains : both Gram +ve and Gram -ve strains were inhibited.

In contrast to the mixed levels of activity in terms of antibacterial properties, the oils were found to be potently antimycotic in nature, with a number of oils exhibiting marked levels of growth inhibition even when tested at relatively low concentrations. As an example, the oil from ***Tagetes minuta*** at a concentration of 1μl/ml culture broth resulted in 80% growth inhibition in the plant pathogen ***Fusarium culmorum***. However, an interesting feature was noted with this oil and fungus, namely that even when the oil concentration was increased ten-fold, the level of antimycotic activity increased to only 81.5%. One possible explanation this suggests is that the active ingredient is clearly active at low levels but realises a 'saturation effect' at higher concentrations, perhaps being effective in some key metabolic event. In contrast to this curious effect, the same oil inhibited the spoilage fungus ***Aspergillus niger*** to only a very slight degree [1%] at concentrations of 1 and 2 μl/ml culture broth, but increasing to 92 and 96% at the higher concentrations of 5 and 10 μl/ml respectively. Activity against the mycotoxin-producing mould ***Aspergillus ochraceus*** was most noticeable in the case of the oil from ***Salvia stenophylla*** where inhibition levels of greater than 97% were realised at concentrations of >5 μl/ml culture broth.

Lastly, the five oils were tested for antioxidant capacity using the simple diffusion test of Araujo & Pratt [1985]. In this test, small volumes of test substances are placed in wells punched in agar containing ß-carotene and linoleic acid. When placed in an incubator operating at 45°C, within four hours discolouration will be complete in those plates where there is no protection against peroxidation. However, in those samples with pronounced antioxidant properties, a zone of colour retention was observed immediately around the perimeter of the well. This can be measured and a subjective judgement made as to the degree of colour retention. In this manner, it was determined that the ***Helichrysum sp*** from Kidd's Beach and ***Salvia stenophylla*** oils were overall the most effective in protecting the linoleic acid from undergoing peroxidation.

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Antimicrobial Activity of Dalmatian Sage Oil from Different Regions of Croatia

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Antimicrobial activity of essential oils as well as of Dalmatian sage oil / Salvia officinalis L. / depends on their chemical composition /1/. Individual compounds of essential oil /1,8-cineole, thujone and camphor / depend on the conditions of soil and climate /2,3/. Since each chemical component has its own biological activity, the total antibacterial activity of a given oil sample is different. Therefore we studied the antimicrobial activity of various oils taken from different locations along the Adriatic coast.

The 12 samples of the plant material were collected from different parts of the coastal region / mainland and islands/. The essential oils were prepared by steam distillation in a Clevenger type apparatus and dried. The antibacterial properties of the oils were tested by the agar diffusion and dilution methods using the bacteria Bacillus subtilis /IP-5832/, Sarcina sp., Candida albicans, Staphylococcus albus, Klebsiella pneumoniae, Salmonella enteritidis, Proteus mirabilis, Shigella Schöttmiller and Escherichia coli.

The oils were found to be more effective against Gram positive bacteria than Gram negative bacteria. The results are listed in Table 1.

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The Antibiotic Activities of Essential Oils

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Our investigation was devoted to fix the price of antibiotic activities of essential oils from some species volatile oil plants, growing in St.-Petersburg Botanical Gardens. We produced essential oils by water distillation from follows species: Origanum tyttanthum Gontsch., O. vulgare L., Tanacetum vulgare L., Levisticum officinale Koch., Balsamita major Desf., Mentha x piperita L., some Monarda species, Lavandula angustifolia Mill., Myrrhis odorata (L.) Scop., some Heracleum species, and other.

The antibiotic activities of essential oils were fixed with respect to pathogenicity for humane Staphylococcus and Streptococcus species both in vitro and in vivo. We determined that essential oil of Origanum species were the most active, but ones of Lavandula - was the least active. The bacteriostatic and bactericidic effect took place when concentration of a essential oil in beef-extract agar were 50-100/200-400 mkg/ml. The essential oil from diferent parts of the plant possessed various of activities with respect to bacteria.

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Juniper berries oil: the fate of a traditional diuretic.

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Juniper oil from juniper berries, a traditional folk medicine up to modern times is a physiological and electrolyt saving diuretic, better called an aquaretic. Unfortunately, a shift in the appraisel of juniper oil is seen due to unreflected statements in pharmaceutical textbooks which still lasts on.

Covering the time from 1844 up to now we looked into the entire literature which was available to us using classic bibliographic methods and modern database retrival systems to find the source behind the warnings concerning juniper (SCHILCHER & HEIL, 1993). The first experiments with juniper oil were carried out with very high dosis with rabbits (up to 30 g per animal) in 1844 (SEMON). The dosage was sufficient to kill them and there kidneys were naturally effected too. This may have been the origin of the warning which was printed half a century later in Materia medica (POTTER, 1898).

"... may set up renal irritation, in large doses producing strangury, priapism, hematuria, suppression of the urin and uremic convulsions."

We only want to give the translation of that statement in one of the succeeding german textbooks (MADAUS, 1938) which obviously shows the source:

"... es kann die Nieren reizen, und in großen Dosen Strangurie, Hämaturie, Priapismus und urämische Konvulsionen verursachen."

Due to the given warnings, scientists had a special eye on that topic during the following decades. However, using therapeutically adequate low dosages, they could not prove the old statements. There may be an explanation for the observation which is taken for kidney irritation: Clouding of the urin. High dosages of juniperus oil lead to clouds in the urin which may be mistaken for proteine (GMEINER, 1906). If such clouds are not verified by further analysis (dilution with ethanol makes the juniper oil-clouding desappear from urin, proteine-clouding would stay), one may judge the therapy with juniper oils as kidney damaging.

A toxicological study on rats with high dosages of a standardized juniper oil with low content of α- and ß-pinen and a sufficient amount of terpinene-4-ol (unpublished results) has shown no damage of the kidneys.

As some kidney irritation (as terpinene oil would do) may result from high contents of α- and ß-pinen in juniper oil, due to provenance, co-destilled parts of the bushes (i.e. needles,branches) or ripening grade of the berries, the oil choosen for therapeutical usage should have a reliable source. Taken into account these informations, there should be no hindrance to use juniper oil as a traditional and physiological diuretic, save in cases of acute kidney inflammation. On the other hand, some stimulation has to be, because the juniper oil works by enhancing the blood flow through the kidneys.

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In vitro Inhibition of Prostaglandin Biosynthesis by a Combination of Secretolytic Drugs

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Various essential oils and drugs containing saponins are established in the treatment of airway diseases (e.g. sinusitis). The secretolytic effect of Sinuforton^{R} - a fix combination of extracts from *primulae radix* and *thymi herba* plus *oleum anisi* - is well known and has been documented even in monographs, whereas the antiinflammatory effects are still discussed. In former investigations herbal drugs as well as some of their main constituents have been shown to inhibit prostaglandin biosynthesis (Wagner et al., Planta Med 184, 1986). In this study we investigated the inhibitory effect of extracts from *primulae radix, thymi* herba and of *oleum anisi*, individually as well as their combination, on prostaglandin synthesis.

As test systems a prostaglandin-synthesizing cyclooxygenase (CO) system from sheep seminal vesicles, a leukotriene-synthesizing 5-lipoxygenase (5-LO) system from leukocytes, and a reversed-phase HPLC sepration technique for the metabolites of arachidonic acid were used, respectively.

Oleum anisi induced a slight concentration-dependent inhibitory effect on CO (50-250 µg/ml I.A.). The inhibitory effect on CO amounted to about 34 % (50 µg/ml I.A.). *Primulae radix* and *thymi herba* had no effect on CO. Accordingly the fix drug combination exerted a small inhibitory effect on CO, due to *oleum anisi*. Similar results were found with the 5-LO system.

The results show that *oleum anisi* is a dual but slight inhibitor of the CO and 5-LO system, supporting the view that essential oils might induce antiinflammatory effects. However, the secretolytic activity of this herbal drug combination seems to be the most effective mechanism in the treatment of sinusitis.

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DEVELOPMENT OF THE MALE GAMETOPHYTE IN SALVIA SCLAREA L. (LAMIACEAE LINDL.)

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Salvia sclarea is a well known essentialoil and medicinal plant. The creation of more perspective cultivars based on the heterosis selection suggested the finding out of malesterile forms.

As a result of detailed cytoembryological investigation on the plants from the collection of the experimental field of the Institute of Botany we found out that the sterilisation processes in the malegenerative organs are wide spread, not only in the malesterile but also in the fertile plants. There was established an interesting not known yet phenomen for this species. The mature pollen grains in the anthers of the fertile plants are only twocelled by well known in the literature threecelled pollen grains. For explaining of this phenomen an in vitro concerning spermiogenesis was carried out on a nutrient medium.

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ON THE STRUCTURE OF THE ANTHERS IN HYSSOPUS OFFICINALIS L. SSP. ARISTATUS (GODR.) BRIQ. (LAMIACEAE LINDL.)

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Hyssopus officinalis ssp. aristatus is a new aromatic plant, wild grown in Bulgaria. From the air dried herb it has been obtained 1,35 % essential oil with pleasant odour.
The cultivation of this plant suggested also a cytoembryological investigations. This study concirning the histological structure of the anthers. They are predominantly twolobed with markedly expressed placentoids.

The anther wall is four-layered comprising of an epidermis, an endothecium, an ephemeral middle layer and a secretory tapetum. The mature pollen is threecelled, like those described by other authors, and formation of pollen diades, tetrades and polyades of various configuration was observed. Degeneration processes of the pollen grains and their germination in the anthers were a common phenomen.

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The effect of some essential oils on colorado potato beetle Leptinotarsa decemlineata of potato and eggplants

A. Mateeva *

The effect of different dozes of essential oils from wormwood Artemisia mariatinia L. and Lavender - Lavandula vera L. was investigated under field conditions against the Colorado potato beetle - Leptinotarsa decemlineata say on potato and eggplants.

The effect of essential oils on insect pests and plant damage was compared with those of a commercial insecticide like decis 25EK - 0,03 %, and bacteriology preparat Novodor 0,4 %.

The studying oils from wormwood and lavender 0,1 and 0,15 % caused repellent effects in colorado larvae`s and adults. However the repellent effects did not last long. And the same time the oil from basil had significant antifeedant effects in the larvae of the both pests wormwood.

Plots treated only with oil from wormwood and decis 2,5 EK 0,03 % were relatively less damaged and had some lower incidence of insect pests campare the central plat`s and the variance with preparat novodor 0,4 %.

Preliminary results indicate that wormwood oil have potentials in giving good protection of potato and eggplant from one of the most importan pest - Lepti notarsa decemlineata say.

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PLANT PROTECTION POTENTIAL OF SOME ESSENTIAL OIL REFUSE PRODUCTIONS

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Ecological agriculture proposes first of all the use of unchemical plant protection methods like antifeedants, plant extracts and different refuse productions.
Essentials oil refuses from rose and basil for plant protection against spide milben Tetranichus urticae Koch. and green peach louse Myzodes persicae Sulz. on paper and cucumber was used.
In our investigation has been studied the influence different dozes of oil refuse productions in connection of plant age and pest density.
Good insecticides and acarizides effects until 5-6 days after spray shown some higher dozes of oil refuse productions on different pests - 3-5 mobile forms on leave by spide milben and 6-10 larvae on leave by green peach louse - Myzodes persicae Sulz.
There was no correlation between pesticides effects by of oil refuse productions and plant age.
In conclusion it was found that this rose and basil oil refuse productions can involve like alternative plant protection methods.

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Selective biotransformation of essential oil components by cell cultures of *Peganum harmala*.

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Cell cultures of *Peganum harmala* have been widely studied with a view to investigating their ability to produce amines and β-carboline alkaloids [1]. However, we have isolated a non-alkaloid producing cell line, which is able to metabolise a wide range of plant volatiles.

Callus cultures of *Peganum harmala* were established from cotyledons, and healthy suspensions grown using Murashige and Skoog medium. Biotransformation reactions were carried out using exogenous terpenoids and phenyl-propanoids. No bioconversion was detected when feeding hydrocarbons, acids or ketones but there was widespread metabolism of other oxygenated derivatives. All nine α-acetates showed hydrolysis during the first 24 hours after feeding, and eleven aldehydes were also converted to their corresponding alcohols. However, no biotransformation was detected using two ortho-substituted exogenous aldehydes. A similar result was previously reported using 2-oxo-p-menthane derivatives in *Nicotiana tabacum* suspensions [2]. These results give further evidence regarding the importance of the nature of the functional group in the substrate administered, and the structural moieties in the vicinity of the functional group.

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COMPUTER-ASSISTED STRATEGIES FOR FLAVOR CREATION

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Abstract

Blending the artistic and creative elements of flavor development with the mathematical precision of experimental design techniques will be presented in a poster format. Enhancing the creative flavor development and optimization process through computer software called Echip will be described. An example will be given to illustrate the basic principles of the method.

Flavor Overview

The performance of a flavor system depends on the ratio of specific ingredients to each other. A particular flavor profile depends not only on the ingredient proportions of the blend (mixture experiment) but also on the total usage level (mixture-amount experiment). For example, ingredient ratios can determine flavor characteristics for strawberry ranging from a jammy, seedy type to a fresh, sweet, fruity type. Knowing how to combine the ingredients into just the right mixture to yield the best possible flavor profile requires specialized training, time and energy. The level of flavor expertise depends on the developer's knowledge and understanding of flavor chemistry. The software is not a substitute for flavor skills. It functions as a catalyst in flavor creation by linking talented people with enhanced information to accelerate the development process and therefore increase overall productivity by saving time and energy. Decision-making skills are improved because the inefficiencies of trial and error procedures are avoided.

Computer Application

The strategy of using Echip software is to provide a systematic approach to flavor development. Because Echip software specifically addresses mixture experiments, it has a tremendous application in flavor creation because flavors are blends of different ingredients. A feature of the mixture experiment is that because the ingredients represents proportionate amounts of the mixture they are restrained and must add up to 100%. Based on ingredient input by the user, the software constructs an experimental mixture design which is then used as a batch sheet for producing flavor prototypes. The flavor mixtures are blended according to the design and each one is tasted by the developer and given a flavor score. This information from the flavor evaluations is used to validate equations for generating the contour maps. The software processes this specialized mixture data through sophisticated computer models where the mathematics are completely transparent to the user. Echip is user-friendly and interactive. It allows for "what if" analysis so that the user can simulate how a change in one ingredient can impact the overall flavor profile. The contour maps enable the developer to focus his or her creative flavor skills on ingredient combinations that are likely to deliver the most promising flavor options. Once an area has been determined, the developer can select flavor prototypes from that region for further refinement and fine-tuning.

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Influence of harvesting time of dill (Anethum graveolens) on the antimicrobial effect of the essential oil

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During growth of the dill plant, significant changes of the essential oil are observed. From the efflorescence to the ripeness of the seeds, the essential oil content increases from 0.4 % to more than 3 %.

During unfolding of the blossoms, further components will develop which decisively are changing the character of the essential oil, namely carvone and structure-related substances, such as dihydrocarvone and carveols. Their content in the essential oil amounts to approx. 50 % when the seeds are ripe.

Carvone is imparting an expressed cummin shade to the taste profile. The limonene content is increasing from 3 % to approx. 20 %. Other components also are undergoing significant changes. The content of α-phellandrene which is responsible for the freshness of taste, is reduced from 60 % to 10 %. The content of dill ether, the most important substance for the typical dill taste is increasing to more than 20 % together with the development of the seeds and decreasing to 10 % till ripeness of the seeds. The taste of the essential oil will change correspondingly from typical fresh dill taste to a cummin shade.

These facts are explaining the changes of the antimicrobial status of the essential oil which are resulting from the changes of the composition of the components.

We have proven that with increasing ripeness of the plant the antimicrobial effect is increasing significantly. Growth inhibition in all the tested bacteria, yeasts and moulds was observed, especially in the yeast variety Candida and the mould fungi Pen. ochraceum.

For the determination of the inhibiting substances the essential oil was fractionated by vacuum distillation and the effect of the fractions tested. The highest effect was observed in the high-boiling components. Comparisons with pure carvone in the two enantiomeric forms suggest that the decisive antimicrobial effect in the essential oil of dill has to be attributed to carvone. Synergistic effects of other components were not discovered.

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Influencing the level of glycosidically bound volatiles by feeding experiments with *Mentha x piperita* L.

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In the course of our studies of glycosidically bound volatiles in Lamiaceae *Mentha piperita* L. cv. Black Mitcham was investigated (1). The aglycone fraction consisted mainly of menthol, 1-octen-3-ol and 3-octanol. Neomenthol which, according to (2), was expected to be a main component, only occurred in portions up to a maximum of 4% in that fraction. The sugar fraction consisted of glucose, galactose and rhamnose.

The seasonal variation of the main essential oil components in *Mentha piperita* prompted Croteau to a detailed investigation of the menthone metabolism by tracer experiments in vitro (2,3). The ratio of neomenthyl glucoside to menthol was far greater than that observed in intact leaves. He tried to explain that by the fact that menthol and neomenthyl glucoside may arise in compartments differently accessible to exogenous menthone.

In the present paper we report on results obtained by feeding experiments with our *Mentha piperita* L. cv. Black Mitcham containing menthyl glucoside as the main glycosidically bound volatile. Following the pattern of Croteau's tracer studies, pieces of leaves obtained from outdoor peppermint plants were incubated with menthone for seven hours. An increase from 6.4 to 31.5% menthol and 0.1 to 1.3% neomenthol in the aglycone fraction resulted. That means the main portion of the exogenous precursor menthone was converted into a menthyl glycoside. This is inconsistent with the highly specific menthone metabolism postulated by Croteau (4), who found neomenthyl glucoside to be the only metabolite.

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Essential oil formation in caraway fruits in relation to development and sucrose availability.

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Caraway [*Carum carvi* L.] fruits are a source of essential oil containing S-carvone which inhibits potato sprout growth and may replace chlorinated hydrocarbons used so far [1]. To improve essential oil yield and quality, monoterpene formation in caraway fruits is studied. Carvone and the other major monoterpene in caraway essential oil limonene, are stored in oilducts in the fruit's pericarp. Analysis of essential oil accumulation during development of caraway fruits showed that only young fruits synthesize and accumulate essential oil [2].
Carbohydrates, particularly sucrose, are the most likely source of carbon utilized in the formation of monoterpenes. Essential oil metabolism is thought to be regulated by the balance between assimilate supply and utilization for growth and differentiation [3]. Moreover, secondary metabolism obtains its precursors from the primary biochemical reactions. Thus, the rates with which substrate can branch off from primary pathways and 'funnel' into secondary biosynthetic routes determines the size of secondary metabolism [4].

We investigated the relationship between sucrose availability and the accumulation of carvone and limonene in caraway fruits at different stages of development. To eliminate influences of the intact plant we developed a semi *in-vitro* model system, in which umbels were detached from the plant at increasing days after pollination and, after sterilization of the peduncle, placed in a tube with sterilized liquid medium, containing sucrose and nutrients according to Murashige and Skoog [5]. The umbel itself protruded, ensuring transpiration which may ease transport of components from the medium. Data obtained from this model system were compared with data obtained from fruits of similar age attached to the plant.
In fruits of undetached umbels limonene appears a few days before carvone. Use of [U-^{14}C] sucrose showed that exogenous sucrose supplied in our model system was incorporated in limonene and carvone. Therefore in further experiments we used unlabeled sucrose.
Increased sucrose availability stimulated the formation of limonene and carvone and increased fruit dry weight and carvone content. However, limonene content as percentage of dry matter was not influenced by exogenous sucrose at any developmental stage. In young fruits (5 to 12 days after pollination) carvone accumulated *in-vitro* when enough sucrose [$\pm$ 4 %(w/v)] was available but not in undetached umbels. Enzymes and structures required for carvone production seem present in young fruits but apparently monoterpene formation is source (carbohydrate) limited.
Carvone yields may be increased by breeding for altered source-sink relations, in which more carbohydrates are allocated to the fruits, especially during the early stages of development.

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Growing Conditions and Breeding of Chamomile, *Chamomilla recutita* (L.)Rauschert, from the View-point of Qualitative-quantitative Characteristics of Essential Oil in SLOVAKIA.

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Our effort in the research and breeding the chamomile was aimed at increasing the essential oil amounts with emphasis being put on both the content and the stability of effective components.

The breeding of diploid variety 'BONA '(' KOSICE - 3 ') and tetraploid variety 'GORAL ' (' KOSICE - 2 ') was realized within 1975 - 1990. The plants of diploid variety have the higher content of essential oil with decisive portion of /-/-α-bisabolol. Tetraploid variety 'GORAL ' has sufficiently high content of essential oil in the dry flower anthodia. Dominant essential oil components are /-/-α-bisabolol and chamazulene. These new improved varieties with their parameters are better that diploid variety 'BOHEMIA ' that was acknowledged chamomile variety in Czecho-Slovakia since 1952.

Mainly in connection with large-scale chamomile cultivation and production, the effects of mineral nutrition, irrigation, pesticide application, various types of growth regulators and the mechanisms of population dynamics at qualitative-quantitative characteristics of essential oil have been studied more intensively in Slovakia.

The contribution is aimed at the presentation of information from these experiments.

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STUDIES OF THE AMOUNT AND QUALITY OF ESSENTIAL OIL OF COMMON JUNIPER (JUNIPERUS COMMUNIS L.) IN BULGARIA

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Studies have been carried out for finding the amounts and chemical compositions of essential oils in the needles and cones of common juniper growing at different altitudes.

The oil's amount in the needles varied from 0.28 up to 0.38 %, and in the cones - from 0.43 up to 0.57 %. The main components of the oils in the organs of the two kinds, were α- and ß-pinene - the first one being dominating in the oil of the cones, and the second - in the oil of the needles.

Common juniper essential oils have a characteristic odour of a raw material, with clearly sensed fragrance of oil terpene. With their specific aroma, they are of interest to the perfume-and-cosmetics and pharmaceutic industries.

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STUDIES ON THE EFFECT OF SOME HERBICIDES UPON YIELDS AND QUALITY OF CORIANDRUM SATIVUM L. PRODUCTION

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S u m m a r y

In the period 1990-1991, we studied in a field experiment the effect of some herbicides on the weed density, the yield and quality of Coriandrum sativum L. production. The following herbicides and herbicide combinations were tested: 1. Control without weeding; 2. Control with a single weeding of all weeds; 3. Trifluralin - 840 cm^3/ha + Linuron - 1000 g/ha; 4. Trifluralin - 840 cm^3/ha + Bentazone - 960 cm^3/ha; 5. Pendimethalin - 1320 cm^3/ha; 6. Metribuzin - 500 g/ha; 7. Pendimethalin - 1320 cm^3/ha + Metribuzin - 500 g/ha.

Eighteen weed species were found to be present in the experimental plot, of which 5 perennial and 13 annual weeds.

In result of the herbicides applied, the best weed control was observed in variant 7, where the lowest weed density was established. A bit higher was the weed density in variants 3rd, 5th and 6th. The least effective was the weed control in variant 4th.

The combination of Pendimethalin + Metribuzin applied at the tested rates exerted in both years a toxic effect upon Coriandrum sativum L., which was the reason for the lowest yield obtained from this variant. The highest seed yields were produced by variants 5th, 6th and 3rd.

A GC analysis of the essential oil from all variants was made.

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Pyridines as constituents of essential oils and plant extrakts Possible pathways by model reactions of reactive carbonyl compounds with ammonia.

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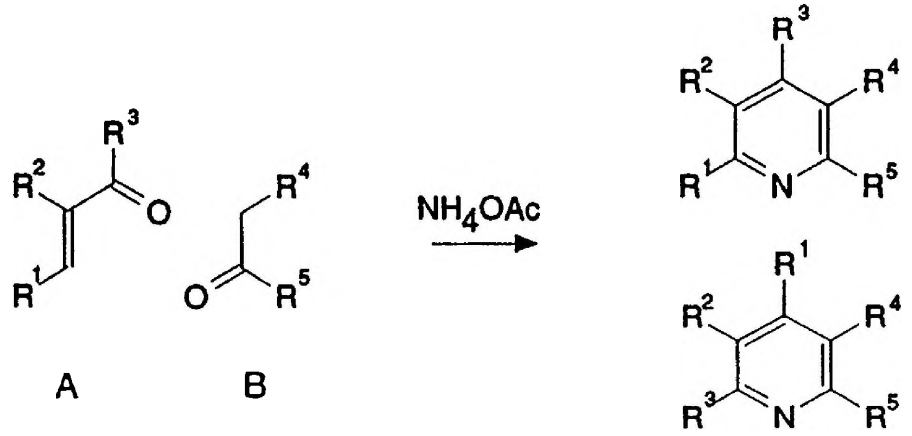
In the last time pyridines were frequently found in essential oils and plant extracts. Our interest was to find possible pathways for the formation of such compounds.

Retrosynthetic considerations led to the result that some of these pyridines might be formed by a cyclic aldol reaction between two reactive carbonyl compounds and ammonia. To prove this assumption, we performed several reactions with suitable carbonyl compounds and ammonium acetate. Compound **A** is always an α,β-unsaturated carbonyl compound, while compound **B** is either an α,β-unsaturated or a saturated carbonyl compound (Fig. 1) .

The reaction mixtures obtained by such model reactions show that many of the naturally occurring pyridines may be formed by this way. As an example, the (E)- and (Z)-Isomeres of 5-[1-butenyl]-2-propylpyridin and 3-[1-butenyl]-4-propylpyridin can be obtained by the use of 2-hexenal as carbonyl compound **A** and **B**.

Moreover, we used several other natural occuring aldehydes and ketones to obtain pyridines, which are potential natural compounds, as reference materials.



A B

Fig. 1

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NEW BUILDING BLOCKS FROM β-CAMPHOLENIC COMPOUNDS

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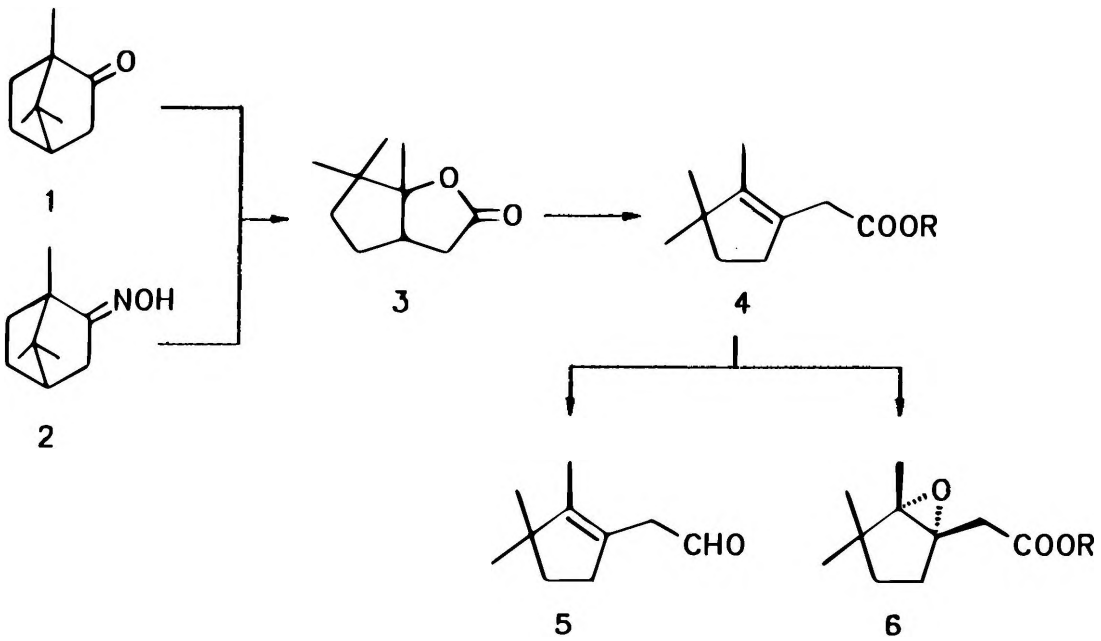
Since the first synthesis in 1895 by Tiemann und Behal [1,2] only little was known about β-campholenic compounds. In the last few years we developped three new methods for the preparative approach to β-campholenic compounds: The synthesis via Baeyer-Villiger-oxidation of camphor (1) [3], the preparation by a Beckmann-fragmentation of camphor oxime (2) [4] (both syntheses have the same intermediate the β-dihydrocampholene lactone (3)) and the third opportunity the rearrangement of α-campholenic derivatives in the presence of acids.

The obtained compounds are precursors for the functionalisation of the side chain and of the five membered ring. We report about the rearrangements of various β-campholenic epoxides and about the β-brahmanol compound.



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(+)-3-CARENE AS A KEY-COMPOUND IN SYNTHESES OF 6,6-DIMETHYLBICYCLO[3.1.0]HEXANE DERIVATIVES WITH OLFACTORY PROPERTIES

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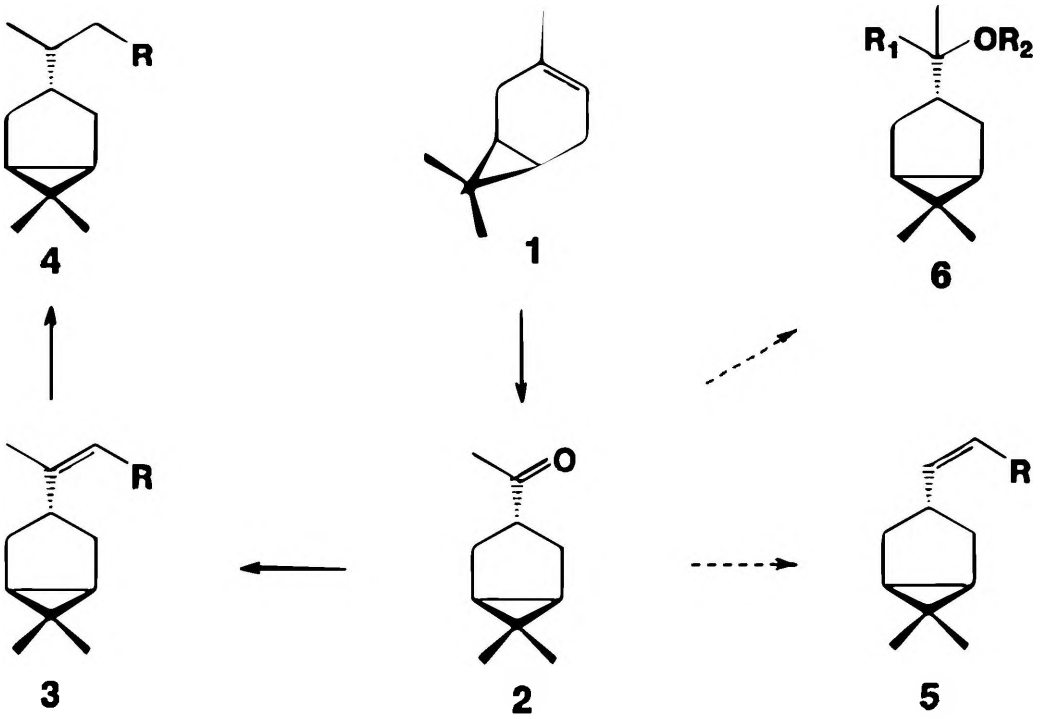
During several years of our studies on the stereochemistry and chemical transformations of the carane system, some simple preparative methods of synthesis of *gem*-dimethylbicyclo[3.1.0]hexane derivatives from (+)-3-carene were elaborated.

Using one of this method and the standard means of elongation of the side chain, a series of new compounds, containing the 6,6-dimethylbicyclo[3.1.0]hexane system with various substituents at the 3-position was synthesized.

The compounds obtained exhibited interesting odor properties. Total syntheses and odor chracteristics are presented.



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SYNTHESIS OF SOME NEW TERPENOIDS

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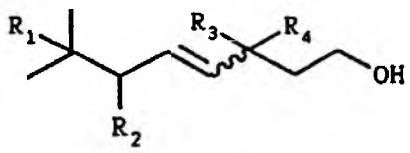
Searching for a new isoprenoid raw materials for synthesis of fragrance specifics and for synthesis of acyclic juvenoids we obtained five unsaturated (E or Z double bond at C_4 - C_5 position) alcohols 1a-e . Two of them (1a,b) can be considered as isomers of known terpene alcohols: citronellol and menthocitronellol. The next two, (1c,d), are homologs of 1a.



a,E, R^1, R^2, R^3 = -H; R^4 = $-CH_3$
b,Z, R^1, R^2, R^3 = -H; R^4 = $-CH_3$
c,E, R^1, R^2 = -H; R^3, R^4 = $-CH_3$
d,E, R^2, R^3 = -H; R^1, R^4 = $-CH_3$
e,E, R^1, R^2 = -C=C-, R^3, R^4 = $-CH_3$

Alcohols 1a,c,d,e were obtained by reduction ($LiAlH_4$) of corresponding γ,δ-unsaturated esters. These esters were afforded by Claisen rearrangement (orthoacetic modification) of corresponding secondary allyl alcohols. Alcohol 1b was obtained by isomerization of 1a.

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