21ST INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

LAHTI, FINLAND
July 26–28, 1990

Programme, Abstracts

21ST INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

LAHTI, JULY 26-28, 1990

Organization:	Prof. Raimo Hiltunen (University of Helsinki) with the aid of Mr. Timo Linnakylä and Mrs. Senja Heikkinen (Lahti Research and Training Centre), Mrs. Pia Härmälä and Dr. Heikki Vuorela (University of Helsinki)
Secretary:	Dr. Yvonne Holm (University of Helsinki)

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PROGRAMME

WEDNESDAY JULY 25, 1990

18.00 Registration, Adult Education Centre, Kirkkokatu 16
19.00 Get-together party, Adult Education Centre

THURSDAY JULY 26, 1990

09.00 Welcome, S. Koivula, Director of Lahti Research and Training Centre
Opening of the Symposium, R. Hiltunen, University of Helsinki

09.20 H. Schilcher, Berlin, FRG
Recent clinical, pharmacological and toxicological studies on essential oils

10.00 Coffee break

Chairman: H. Schilcher

10.20 P. Kaspar, Bad Reichenhall, FRG
Effect of an inhalatory therapy using essential oils on the ciliar activities in humans

10.40 S. C. Garg, Sagar, India
Some pharmacological actions of the essential oil of *Buddleia asiatica* Lour

11.00 M. Gaisbauer, Berchtesgaden, FRG
Über den Einfluss von Minzöl (JHP Rödler) auf die Immunität der Schleimhaut

11.20 K. P. Svoboda, Auchincruive, U.K.
Variability of rosemary and sage volatile oils obtained from various geographical sources and antioxidative properties of solvent extracts

11.40 M. T. Lis-Balchin, London, U.K.
Pharmacological studies on *Pelargonium grossularoides* and *Erodium cicutarium* (Geraniaceae)

12.00 Lunch

Chairman: M. von Schantz

14.00 G. Buchbauer, Vienna, Austria
Aromatherapy: evidence of sedative effects of the essential oil of lavender and some other oils

14.20 S. C. Garg, Sagar, India
Antifungal activity of *Capillipedium foetidum* oil

14.40 M.-L. Riekkola, Helsinki, Finland
On-line coupled LC-GC techniques and their applications to the characterization of essential oils

15.00-16.00 Poster presentation

19.00 Town reception, Town Hall of Lahti, Harjukatu 31

FRIDAY JULY 27, 1990

Chairman: A. Baerheim-Svendsen

09.00 J. J. C. Scheffer, Leiden, The Netherlands
New essential oil plants and new essential oil constituents

09.40 M. H. Boelens, Seville, Spain
The chemical composition of essential oils from *Pistacia lentiscus* L. (Mastic gum tree)

10.00 Coffee break

Chairman: E. Stahl-Biskup

10.20 K. Bruns, Düsseldorf, FRG
Turpentine oil - raw material for the synthesis of aroma chemicals

11.00 C. Ehret, Grasse, France
New components from cassie absolute (*Acacia farnesiana* Willd.)

11.20 C. Bicchi, Torino, Italy
Chromatographic separation and identification of irones and related precursors from *Iris pallida*

11.40 B. Galambosi, Mikkeli, Finland
Yield and quality of herbs grown in Lapland

12.00 Lunch

Chairman: K.-H. Kubezcka

14.00 T. Talou, Toulouse, France
Truffles extracts: novel bases for perfumery

14.20 E. Stahl-Biskup, Hamburg, FRG
Essential oils of primary and secondary oil ducts in Apiaceae roots

14.40 P. Weyerstahl, Berlin, FRG
Comparative analysis of some Brazilian wood oils

15.00 O. Ekundayo, Ibadan, Nigeria
no title available

15.20 Coffee break

15.40 Poster presentation

20.30 Symposium dinner and boat cruise, starting from Passenger Harbour

SATURDAY JULY 28, 1990

Chairman: J. J. C. Scheffer

09.00 K.-H. Kubezcka, Hamburg, FRG
Developments in the essential oil analysis

09.40 L. Jirovetz, Vienna, Austria
Automatic evaluation of essential oil components based on multivariate analyses of GC-MS, GC-FTIR, GC-AES and derivatisation data

10.00 G. Petri, Budapest, Hungary
Instrumental analysis of *Conyza pinnata* essential oil

10.20 F. Tateo, Milan, Italy
Influence of drying processes on essential oil quality of *Artemisia absinthium*

10.40 K. B. Yaacob, Bangi Selangor, Malaysia
Taxonomy and chemistry of *Pandanus amaryllifolius* Roxb.

11.00 Closing of the Symposium, R. Hiltunen

13.00 Bus tour to Helsinki via Porvoo.

* omgewisseld wegens ziekte van W.

POSTER PRESENTATIONS

1. A. Balinova
   About the dynamics of *Salvia sclarea* extraction

2. K. H. C. Baser, G. Tümen, T. Özek and M. Kürkcüoglu
   The essential oil composition of *Origanum sipyleum* collected from four localities in Turkey

3. K. H. C. Baser, G. Tümen and E. Sezik
   Composition of the essential oil of *Origanum minutiflorum* (Lamiaceae)

4. R. A. Clery and J. D. Ross
   The effect of nutrients and growth regulators on the essential oil of *Melissa officinalis* L. (Lamiaceae)

5. A. M. Eklund, I. Wahlberg and T. Pettersson
   New lactones from tobacco

6. S. Frazao, M. M. Carmo and F. Venancio
   Concrete of coffee flowers. Determination of some constituents.

7. S. Gabr, A. S. Hamed and A. El-Gabi
   Chemical constituents and antimicrobial effect of some essential oils as influenced by the extraction methods

8. B. Galambosi, Zs. Szebeni-Galambosi, J. Svab and E. Dorman
   Biological value of Asteraceae seeds collected from the nature in Hungary

9. B. Galambosi, Y. Holm, Zs. Szebeni-Galambosi, M. Repcak and P. Cernaj
   Yield and essential oil of some chamomile cultivars grown in Finland

10. Zs. Szebeni-Galambosi, Y. Holm and B. Galambosi
    Growth, yield and essential oil of lovage cultivated in Finland

11. L. Jirovetz, G. Buchbauer, A. Woidich, A. Nikiforov and V. Raverdino
    Application in essential oil analysis by GC-MS, GC-FTIR and GC-AES

12. D. Joulain, K. B. Yaacob, R. Laurent and J. P. Fourniol
    The essential oil of *Ruta angustifolia* L. from Malaysia

13. K. Kahlos, I. Laakso and R. Hiltunen
Composition of hydrodistilled fungal oil from *Fomitopsis pinicola*

14. D. Kustrak, J. Kuftinec and N. Blazevic
Analysis of the essential oil from *Origanum heracleoticum* (Lamiaceae)

15. W. A. König, D. Icheln, T. Runge, R. Krebber, G. Bruhn, E. Wilhelm and E. Stahl-Biskup
Stereochemical analysis of constituents of essential oils by enantioselective capillary gas chromatography using cyclodextrin derivatives as chiral stationary phases

16. L. Lelik, E. Bihatsi-Karsai, G. Vitanyi and E. Lemberkovics
Gas chromatographic and mass spectrometric determination of the essential oil from *Leonotis mollissima* (Lamiaceae)

17. P. Manninen, Y. Holm, R. Hiltunen and M.-L- Riekkola
On-line coupled SFE-SFC for the analysis of aromatic plants

18. F.-J. Marner and W. A. König
Separation of enantiomeric irones by gas-liquid chromatography on a modified cyclodextrin

19. M. Mata-Rico and A. Velasco Negueruela
Analysis of the essential oil of *Achillea tomentosa* (Anthemideae) collected in Spain

20. M. Mata-Rico and A. Velasco Negueruela
Composition of the essential oil of Spanish *Achillea thracica*

21. O. A. Onayade, J. J. C. Scheffer and A. Baerheim Svendsen
Polynuclear aromatic compounds and other constituents of the herb essential oil of *Salvia coccinea*

22. O. A. Onayade, J. J. C. Scheffer and J. Schripsema
6-Hydroxycarvotanacetone and other constituents of the essential oil of *Laggera alata*

23. R. Piccaglia and M. Marotti
Chemical composition of the essential oil of an Italian *Thymus vulgaris* L. chemotype

24. U. Ravid, A. Eshel, D. Carmeli, E. Putievsky and I. Katzir ≠ HP Schenke
The essential oil of *Artemisia judaica* chemotypes

25. I. Salamon and A. Orinak
Effect of various distance of the plant rows on the quantitative-qualitative characteristics of the essential oil of chamomile-variety "Bona"

26. E. Sarer
Essential oil from *Salvia potentillifolia* Boiss. et Heldr.

27. S. Sonnenberg, T. Müller and P. Weyerstahl
Structure-odor correlation of cascarilla acid derivatives

28. H. Ziegler and G. Spiteller
Cnidicin - a new compound in lemon oil

S. Hose

W. Schultze J. Koch-Heitmann, S. Hose, A. Abou-Mandour,
A. Zänglein, J. Schulz, F.C. Czygan, K.H. Kubeczka.
Volatiles from callus cultures of Melissa officinalis

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ABSTRACTS OF LECTURES

CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF IRONES AND RELATED PRECURSORS FROM IRIS PALLIDA

C. Bicchi[1], C. Frattini[1], G. Pellegrino[1] and D. Joulain[2]

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The extracts of roots of Iris pallida are widely and successfully used in the perfume industry. The olfactive value is due to the presence in the iris absolute of irones, a class of compounds which derive by oxidative degradation from triterpenic precursors, the iridals, contained in the freshly collected roots. To ensure the transformation of most of the iridals, the roots must be stored for at least 30 months after harvesting.

This communication describes the analytical results of a research project aiming to evaluate the olfactive value of the iris roots in terms of their precursors i.e. whether it is possible to correlate the quali/quantitative iridal pattern to the final irone formation.
The main topics illustrated are:

- identification of irones in the Iris absolute through GC/MS and GC/FTIR
- development of an HPLC method to analyse iridals and to identify them through HPLC/Particle Beam Interface/MS. The MS iridal identification was carried out through their MS spectra recorded with both electron impact and negative and positive chemical ionization using different reactant gases.

L-2

THE CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM PISTACIA LENTISCUS L. (MASTIC GUM TREE)

Mans H. Boelens and Rafael Jimenez

Destilaciones Bordas Chinchurreta S.A., P.O. Box 11, Seville, Spain

The chemical composition of the essential oils isolated by extraction of Mastic Gum and by hydrodistillation of leaves, unripe and ripe fruits of Pistacia lentiscus L. was studied.
Up to 250 constituents were detected in the oils. From these components about 70 could be identified and quantified, comprising 99 % of the oils.
Gum oils contained 85 % and leaf oils 50 % monoterpene hydrocarbons, oxygenated monoterpenes (18 %), sesquiterpene hydrocarbons (25 %) and oxygenated sesquiterpenes (5 %); whereas fruit oils consisted of monoterpene hydrocarbons (96 %) and sesquiterpenoids (3 %).

The main constituents were:

- in mastic gum oil	75 - 80 %	α-pinene;
- in leaf oils	10 - 15 %	α-pinene and
	15 - 20 %	myrcene
- in unripe fruit oils	20 - 25 %	α-pinene and
	50 - 55 %	myrcene;
- in ripe fruit oils	10 - 15 %	α-pinene and
	70 - 75 %	myrcene.

α-Terpineol and 4-Terpineol (together ca 15 %) were the dominant monoterpene alcohols in leaf oils. 2-Undecanone (0.2 - 0.7 %), seems to be an olfactively important constituent of the oils. Dimyrcene (4 isomers) occurs in all the oils.

TURPENTINE OIL - RAW MATERIAL FOR THE SYNTHESIS OF AROMA CHEMICALS

K. Bruns

HENKEL KGaA-TFC/Riechstoffe, P.O. Box 1100, D-4000 Düsseldorf

For approximately 30 years, turpentine oil has been used for the manufacture of aroma chemicals as a renewable raw material, whose constituents can be converted by partial synthesis into a large variety of aroma chemicals with isoprenoid structure. In this presentation, ways of synthesizing the major common aroma chemicals as well as new products will be discussed.

L-4

AROMATHERAPY: EVIDENCE OF SEDATIVE EFFECTS OF THE ESSENTIAL OIL OF LAVENDER AND SOME OTHER OILS

G. Buchbauer[1], L. Jirovetz[1], W. Jäger[1] and H. Dietrich[2]

[1] Institute of Pharmaceutical Chemistry, University of Vienna, Austria
[2] Central Laboratory of Animal Facilities, Medical School, University of Innsbruck, Austria

The sedative properties of the essential oil of lavender (Lavendula officinalis) and of its main constituents, linalool and linalyl acxetate, are shown in mice followed up in a series of experimental procedures. The significant decrease in the motility of female and male laboratory animals is found in close dependence of exposure time and drug concentration. Following the injection of caffeine an over-agitation of the mice was proven which could be reduced to normal motility values by inhalation of these fragrance drugs. The noticed effect is caused by a direct pharmacological action upon the central nervous system and not by a reflected influence. A pharmacokinetic drug decline is experimentally proven, thus furnishing evidence of the aromatherapeutical use of herbal pillows used in folk medicine since ancient times in order to facilitate falling asleep or to minimize stressful situations. Some other essential oils are also included in this study.

NEW COMPONENTS FROM CASSIE ABSOLUTE (ACACIA FARNESIANA WILLD.)

C. Ehret, P. Maupetit and M. Petrzilka

ROURE, Centre de Recherche, 34, Chemin de la Madeleine, B.P. 72, 06332 Grasse Cédex, France

The Absolute of Cassie, obtained via hexane extraction of the flowers of Acacia farnesiana Willd. followed by treatment with alcohol, exhibits a highly appreciated warm, strong, herbaceous, flowery odour with a tenacious balsamic dry out.

Like Mimosa and Narcissus, the Absolute of Cassie belongs to a group of Natural Products characterized by a complex composition and also by the absence of main constituents which contribute decisively to the overall odour impression. The olfactive character is the result of a very subtle equilibrium between numerous minor and trace components.

In this study the olfactively important part of this absolute was divided into neutral (11.5 %), carboxylic acid (32.8 %), phenolic (3 %) and nitrogen containing (0.16 %) fractions. Their GC and GC/MS analysis led to the identification of over 150 new constituents, most of which were found to contribute favorably to the odour of the Absolute of Cassie.

L-6

ÜBER DEN EINFLUSS VON MINZÖL (JHP RÖDLER) AUF DIE IMMUNITÄT DER SCHLEIMHAUT

M. Gaisbauer

Klinik in der Stanggass, Sonnleitstrasse 33, D-8240 Berchtesgaden, BRD

Zur Immunität des Menschen gehören neben systemischen auch lokale Immunprozesse z.B. der Schleimhäute des Respirations- oder des Gastrointestinalstrakts. Einen entscheidenden immunologischen Parameter für die jeweilige Funktionslage des lokalen Immunsystems stellt das sekretorische Immunglobulin A (secretory IgA, sIgA) dar, ein binäres IgA-Molekül. Seine Konzentration lässt sich mittels radialer Immundiffusion bestimmen. Es zeigt sich, dass die Inhalation von Minzöl die Konzentration von sekretorischem IgA im Bereich der Schleimhäute des Respirationstrakts signifikant erhöht. Untersucht wurde das Minzöl der Fa, Rödler (JHP), die die Untersuchungen auch gesponsert haben.

YIELD AND QUALITY OF HERBS GROWN IN LAPLAND

B. Galambosi[1], I. Biro[4], Y. Holm[3] and J. Kumpulainen[2]

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Growing possibilities of 12 herbs (marjoram, savory, sage, thyme, hyssop, lemon balm, dragonhead, lovage, dill, peppermint, yarrow and nettle) were studied in Kittilä, Finland (68°10' N, 25°46' E) during 1988-1989.
The effect of different growing methods (outdoor and indoor cultivation, plain soil, ridge and plastic mulch) were studied on growth, overwintering, contents of volatile oil and heavy metals. The length of the growing periods (June–August) was 92 days in both years. The effective heat sums of the growing periods were 761 and 634 C°, respectively. The winters caused 30–100 % frost damages in the perennials.

The fresh yields varied from 0.4 to 3.2 kg/m^2 depending on the species, year and growing technics. Higher yields were achieved under warmer growing conditions.

The volatile oil contents of herbs grown indoor were clearly higher than of herbs grown outdoor. The composition of the volatile oils did not differ from those that were distilled from the Central European reference samples. The average content of lead and and cadmium of herbs grown in Lapland were 1.5 times lower than the content in samples from South Finland and 3–4 times lower than in samples from Central Europe.

L-8

ANTIFUNGAL ACTIVITY OF CAPILLIPEDIUM FOETIDUM OIL

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The hitherto unworked essential oil from the inflorescence of Capillipedium foetidum Lisboa has been studied for in vitro antifungal activity against nine human and plant pathogenic fungi viz., Arthroderma benhamiae, Microsporum gypseum, Trichophyton mentagrophytes, Ctenomyces serratus, Aspergillus niger, A. fumigatus, A. flavus, A. candidus and A. ochraceous. The oil has exhibited excellent to very good activity against the test organisms when compared with Griseofulvin (1000 ppm). The susceptibility of the oil towards dermatophytes is interesting and it can be exploited as a natural fungicide.

SOME PHARMACOLOGICAL ACTIONS OF THE ESSENTIAL OIL OF BUDDLEIA ASIATICA LOUR

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Buddleia asiatica Lour. belongs to the family Loganiaceae. The hitherto unworked essential oil obtained from the shade dried leaves of the plant by hydrodistillation in an yield of 0.3 %, has been found to be CNS depressant on the basis of decrease in spontaneous motoric activity, potentiation in the hypnosis induced by pentabarbitone sodium, effect of rotarod performance, blockade of conditioned avoidance response, analgesia and hypothermia in albino rats. The oil produced sudden fall in blood pressure followed by increase in respiration in anaesthetised dogs with quick and complete recovery from the effect. The oil has been found to cause cardiac depression in isolated heart of frogs and rabbits. The depressant effect was not blocked by atropine. The effect of the oil was more specific on smooth skeletal muscles than cardiac muscles. Investigation on different sets of experiments revealed that the oil had a caffeine type of action on these muscles. A part of its action was also dependent on extracellular calcium. The contraction produced by the oil has been found similar to that of caffeine.

L-10

AUTOMATIC EVALUATION OF ESSENTIAL OIL COMPONENTS BASED ON MULTIVARIATE ANALYSES OF GC/MS, GC/FTIR, GC/AES AND DERIVATISATION DATA

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With the increasing use of GC coupled spectroscopic methods such as GC/MS, GC/FTIR or GC/AES the problems of efficient handling, correlation and at least partial automatic or semiautomatic interpretation of these data sets become more and more important.
With the help of self-developed personal computer operating programmes the data sets of different GC coupled methods were first transferred to the central-AT-computer and afterwards semiautomatically converted into hypothetical "common time base" data sets. These converted sets were used subsequently for constructing either "intermethodical" or "intra-methodical" oriented classifiers based on logical component evaluation of both the classical (ion profile, selected wave chromatogrammes) and the multivariate higher order (derived from cluster analysis or Gram-Schmidt orthogonalisation) entities.
In applications the analytical data of GC/MS (EI and CI), GC/FTIR and GC/AES (O- and C-trace) investigations of four lavender oils (genuine samples and samples after hydrogenation/deuteration) and four thyme oils of different origin were correlated by multivariate analysis and used subsequently for the comparison of the samples. In addition to the correlation of the spectral data, the multivariate correlations of the chromatogrammes of the GC/MS, GC/FTIR and GC/AES data sets were also used for the comparison of the samples.
With multivariate correlation profiles the direct differentiation of the individual oil types and partial automatic interpretation of spectral data was possible.

EFFECT OF AN INHALATORY THERAPY USING ESSENTIAL OILS ON THE CILIAR ACTIVITIES IN HUMANS

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The mucociliary clearance (MC) is an important part of the defence mechanism of the bronchial mucosa. The aim of our study was to prove the effect of essential oils (Aerosol Spitzner®) on the MC and on the ciliary beat frequency (CBF).

We took a brush-biopsy from the nose of patients with COLD and brought the cells into Hanks solution. The CBF was measured using the phase-contrast-microscopy and photo-multiplier-technique, the MC was measured with the Saccharine-methode.

To examine the influence in vitro we added increasing doses of essential oils to the buffer and found no change of CBF.

For in-vivo-study we measured the CBF and the MC before and 40 minutes after an inhalation of the essential oils. We found a significant increase of the CBF and a significant decrease of saccharine-time. This decrease is comparable to the effect of an inhalative treatment with theophylline.

We conclude that essential oils may be useful in the therapy of disorders with reduced mucociliary clearance.

L-12

PHARMACOLOGICAL STUDIES ON PELARGONIUM GROSSULARIOIDES AND ERODIUM CICUTARIUM (GERANIACEAE)

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Extracts of the leaves of Pelargonium grossularioides (L'Herit) and Erodium cicutarium (L'Herit.) have both been used as folk medicines in South Africa to procure abortions. The pharmacological activities of these two species were initially investigated using in vitro preparation of guinea-pig ileum. Contractions were produced by crude extracts of both species. Strong contractions were also produced in guinea-pig uterus preparations in vitro. There is therefore a strong correlation between the reported medicinal property and the effect on smooth muscle.

As there was great similarity in pharmacological activities in the two species some similarity in chemical composition was expected. Following steam distillation of the leaves the essential oil profiles of P. grossularioides and E. cicutarium were therefore compared using GC/MS.

INSTRUMENTAL ANALYSIS OF CONYZA PINNATA ESSENTIAL OIL

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The Conyza species (e.g. C. ulmifolia, C. obscura, C. linifolia, C. viscidula, C. balsamifera, C. canadensis) can be characterized by their essential oil composition. The essential oils of C. canadensis and C. aegyptiaca are rich in sesquiterpene hydrocarbons, oxygenated sesquiterpenes and acetylenic ester constituents (1,2). The oil of C. canadensis is used occasionally in perfumery and also as a flavouring agent (3). Lenfeld *et al.* (4) established that the C. canadensis oil has anti-inflammatoric activity. The aim of our investigation was to characterize the composition of the essential oil obtained from C. pinnata by steam distillation.
The separation of the essential oil components was carried out by GC. We used three stationary phases of different polarities: OV-17, HP-101 and HI-FFAP. For the identification of each component standard addition, relative retention factors (5,6,7) and GC-MS were used.
We established that C. pinnata oil contains beside mono- and sesquiterpenes also aromatic compounds. The main components of the oil are ocimene and 3,7-dimethyl-1,3,7-oktatrien monoterpene hydrocarbons.

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L-14

ON-LINE COUPLED LC-GC TECHNIQUES AND THEIR APPLICATIONS TO THE CHARACTERIZATION OF ESSENTIAL OILS

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Simplification of sample preparation by minimizing pre-separation and clean-up steps, efficient elimination of interfering components, improvement of quantitation, reduction of analysis times, better repeatability and more analytical information about the sample components than by conventional methods make on-line coupled LC-GC the method of choice for the analysis of complex samples.

There are at least three approaches for coupling LC with GC: 1) modification of LC to meet the requirements of GC, that is, the use of microcolumns in LC; 2) modification of GC to "accept" large sample volumes; and 3) reduction of the sample liquid introduced into the GC system by using effluent splitters.

It is possible to introduce large volumes of liquid into the GC system by using either on-column or loop-type interfaces. Long retention gaps used in conjunction with an on-column interface lengthen the analysis times and many parameters must be adjusted with conventional retention gap and concurrent solvent evaporation techniques. For the latter, only one parameter, the temperature of the oven during the introduction of the sample, needs to be adjusted. These techniques have also been applied to the characterization of essential oils. From the results it is clearly seen that on-line coupled LC-GC provides high potential for the analysis of complex mixtures such as essential oils.

NEW ESSENTIAL OIL PLANTS AND NEW ESSENTIAL OIL CONSTITUENTS

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A search through the Chemical Abstracts did not yield the information needed for a review on the subject in question.
Therefore, some selected journals have been screened for publications dealing with new essential oil constituents. Plants which contain volatile oils with an unusual composition or with a composition similar to that of well-known essential oils have also been reviewed.
The present paper is not intended to give a complete review of the literature, but some illustrative examples will be presented instead.

L-16

RECENT CLINICAL, PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES ON ESSENTIAL OILS

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Since our review: "Effects and side-effects of essential oils" in 1985 (1) more than 100 scientific papers are published on this subject. Most of these publications confirm in experimental and/or clinical studies the hyperaemic, antiinflammatory, antiseptic/desinfectant, granulation stimulating and deodorizing effects by external application as well as the expectorating, appetite stimulating, cholekinetic, carminative, spasmolytic, antiinflam-matory, antiseptic, sedative and circulation stimulating effects by internal application. More than 50 percent of the papers are engaged with the antimicrobial activities of different essential oils and underline the importance of these substances as effective antiseptics. 63 monographies of plants which contain more or less essential oils published by the Comission E at the Federal Health Office, Berlin (2) are also of great interest. They officially attest the above mentioned effects of essential oils. Recent papers additionally report on mucolytic and antiviral activities (3) as well as antidiuretic (4) and antipyretic (5) effects. The knowledge about the in vitro inhibition of the prostaglandin-biosynthesis by essential oils is discussed (6). The importance of the valepotriates for the sedative effect of valerian preparations is still contrary discussed (7,8). General and special pharmacokinetic data were improved with modern methods (9,10). Clinical trials at colon irritabile (11) and hypercholesteraemia are new (12).Toxicological studies on essential oils of European Acorus calamus gave neither teratogenic nor genotoxic side-effects. But the essential oils of Juniperus sabina showed a fetotoxic potential on mice (13). A subacute dermal toxicity study of the wood essential oils from Cedrus deodara in

rabbits gave no negative results (14). Contrary discussed is the toxicity of anethole and anise oil as well as cineole and eucalyptus oil.

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L-17

ESSENTIAL OILS OF PRIMARY AND SECONDARY OIL DUCTS IN APIACEAE ROOTS

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In the roots of Apiaceae seedlings, the essential oils are accumulated in triangular "primary" oil ducts between the pericycle cells. Later, when the diameters of the roots are increasing, in the cortex near the cambium "secondary" oil ducts continually develop, later moving outwardly. At different stages of development after germination, the changes of the essential oil composition of the roots of Carum carvi, Foeniculum vulgare ssp. azoricum, Pastinaca sativa, and Levisticum officinale were analysed parallel to microscopical observations.

In contrast to the "adult" oils, the essential oils of the primary oil ducts consisted of higher amounts of sesquiterpene hydrocarbons and did nearly not change until the beginning of the secondary thickening two weeks later. At that time, the changes of the oil composition started although the secondary oil ducts have not yet been developed. 8 to 12 weeks after germination, just when the secondary oil ducts have appeared, the oil amounts in the roots remarkably increased accompanied by intensive changes of the oil composition into the direction of the "adult" oils.

Only Levisticum officinale did not fit in this regularity. The phthalides, the characteristic compounds of the adult oils of this species, were produced in considerable amounts only 18 to 20 weeks after germination. At this time, many completely developed secondary oil ducts could already be observed.

VARIABILITY OF ROSEMARY AND SAGE VOLATILE OILS OBTAINED FROM VARIOUS GEOGRAPHICAL SOURCES AND ANTIOXIDATIVE PROPERTIES OF SOLVENT EXTRACTS

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The volatile oil profiles of 49 sage and rosemary samples (from Italy, Turkey, Dalmatia, Greece, England, Spain, Morocco and Tunisia) obtained from major suppliers in the U.K. were determined by GLC and GC–MS together with morphological characteristics.
Distilled samples showed a wide variation in oil yield: in sage, 0.4–2.5 % (v/w); in rosemary 1.0–2.0 % (v/w). Material from Auchincruive yielded 1.5 % and 2.0 % (v/w), respectively. Greek, Turkish and Italian sage had high levels (expressed as % of total volatile oils) of 1,8–cineole (22–58), camphor (6–15), camphene (2–8), α–pinene (3–10), and very low levels of thujone (1-2); English and Dalmatian sage had low levels of 1,8–cineole (3–12), high concentrations of thujone (7–10 for English, 20–30 for Dalmatian), camphor (12–22) and α–pinene (5–6). Sage grown at Auchincruive resembled English samples, but had a higher level of thujone (25). The profiles of some oil samples were confusing, suggesting either blending of oils or mis–labelling of the products. The major components of Spanish rosemary oil were 1,8–cineole (18–23), α–pinene (13–22), camphor (19–23), camphene (6–9) and linalool (6–10). Moroccan rosemary differed with a higher level of 1,8–cineole (41–42), lower α–pinene (12–15), camphor (15–16) and camphene (4). All oil samples tested were uniform in their composition. Rosemary grown at Auchincruive had high levels of 1,8–cineole (32), camphor (26), lower levels of camphene (4), α–pinene (7) and linalool (2).
The antioxidant properties of sage and rosemary extracts were determined. Sequential extractions were made from dried material using *n* –hexane, dichloromethane and ethanol. Maximum antioxidative activities were recognised with the ethanol extract. There was appreciable variation between samples of different geographical location: English sage was the most effective.

L-19

TRUFFLES EXTRACTS: NOVEL BASES FOR PERFUMERY

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Truffles are the fruiting bodies of hypogeous fungi which grow in symbiosis with certain trees, especially oaks. The production areas are limited to several regions of Southern Europe, particularly France. Due to its typical flavour, the black Perigord truffle (Tuber melanosporum Vitt.) is the most famous truffle species. It was wery much appreciated by gourmets and traditionally used by the french food industry to flavour its products.

Referring to our previous studies, truffles alcoholic extracts appeared to have no interest as flavourings. But accordiong to a french supplier of perfume compounds, such extracts seem interesting for perfumery purposes.

Alcoholic extractions of the three more common french species of truffles, i.e. black Perigord truffle (Tuber melanosporum Vitt.), winter truffle (Tuber brumale Vitt.) and summer truffle (Tuber aestivum Vitt.) are carried out. Capillary gas chromatography, with both FID and sniffing detections, and combined capillary gas chromatography-mass spectrometry are used in order to identify the major volatile components of these extracts. Additional comparative sensory analyses and tests in perfume formulations are carried out in order to complete the analytical characterization.

INFLUENCE OF DRYING PROCESSES ON ESSENTIAL OIL QUALITY OF ARTEMISIA ABSINTHIUM

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The modernization of production structures in the sector of officinal plants is very important nowadays and, particularly, it is necessary to fit mechanical equipment to the particular requirements of officinal cultures. For this purpose, the teams of two Institutes with different functions started co-operation; the results of one of the experiments carried out are presented in this paper.

The quality of Artemisia absinthium volatiles is very important from a commercial point of view since this species is one of the most used sources of bitter and aromatic elements for the preparation of alcoholic and soft drinks. Also, the use of A. absinthium is provided for in law for the preparation of Vermouth. This note presents the results of drying experiments carried out by the following methods:

i) traditional spontaneous drying at room temperature
ii) artificial ventilation by air at room temperature
iii) artificial ventilation by heated air.

Some comparative sensory evaluations on dried vegetables and some comparative analytical (GC-MS) and sensory evaluations on extracted essential oils were carried out. Also, relationships between the results of chemical analysis and sensory evaluation were determinated. It was possible to define a quality range with respect to the three different drying techniques examined.

L-21

COMPARATIVE ANALYSIS OF SOME BRAZILIAN WOOD OILS

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The essential oils from the Brazilian trees Aydendron barbeyana (Ayou oil), Oreodaphne porosa (Phoebe oil) and Cabralea cangerana (Cangerana oil) were analyzed by means of fractional distillation and chromatography. Most of the constituents could be isolated, identified by combination of all spectroscopic methods, and olfactively evaluated. Particularly, the Phoebe oil contains several new compounds with an unusual structure.

TAXONOMY AND CHEMISTRY OF PANDANUS AMARYLLIFOLIUS ROXB.

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The leaves of Pandanus amaryllifolius (local name: pandan) are used as flavouring additives in many traditional culinaries and cakes. Previous works on the chemical composition of the volatile oil indicated the presence of 2-acetyl-1-pyrroline as the compound responsible for giving the characteristic pandan odour. The complete taxonomy of the plant and also the chemical constituents of the volatile oil, anyhow, has not been examined in detail. We report here some of our latest works on the taxonomy and chemistry of the volatile oil of P. amaryllifolius.

ABSTRACTS OF POSTERS

ABOUT THE DYNAMICS OF SALVIA SCLAREA EXTRACTION

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Observations have been made on the dynamics of the solvent extraction of Salvia sclarea accomplished with petroleum ether.

It has been found that the largest amount of extractable substances produced within the first ten minutes is about 60 % of the total yield obtained by sevenfold extraction for 120 min. With each subsequent treatment the yield continuously diminishes and after 40 minutes its reduction is 4 % on average for every subsequent 20 minutes' period.

The products obtained from the individual extraction procedures differ considerably in composition and odour. The extract of the first ten minutes has the highest content of the major components sclareole and linalylacetate and the lowest content of paraffins. It is characterized by a pure, fresh and typical odour.

P-2

THE ESSENTIAL OIL COMPOSITION OF ORIGANUM SIPYLEUM COLLECTED FROM FOUR LOCALITIES IN TURKEY

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Turkey has a rich flora of plants used in folk medicine. Origanum sipyleum L. (Lamiaceae) is such a plant which is endemic throughout Anatolia. It is called with various names in different regions. For example: in Balikesir region the plant is called "Bayirçayi", in Ankara region "Güveyotu".

Flowering parts of O. sipyleum were collected near Balikesir at an altitude of 250 m (Sample I), near Manisa at an altitude of 750 m (Sample II), near Eskisehir at an altitude of 300 m (Sample III) and near Ankara at an altitude of 950 m (Sample IV).

The air-dried flowering parts were subjected to hydrodistillation and the oil was studied by capillary GC and GC-MS. The composition of four essential oils were compared by means of GC analysis. The oils consisted mainly of monoterpene hydrocarbons: γ-terpinene (11-27 %) and p-cymene (4-40 %) except for Sample I which contained methyl thymol (20 %) as the main component.

COMPOSITION OF THE ESSENTIAL OIL OF ORIGANUM MINUTIFLORUM (LAMIACEAE)

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Origanum minutiflorum O. Schwarz & P.H. Davis is an endemic plant in southwest Anatolia. The plant is commonly called "Togkakekigi, Yaylakekigi" in Antalya. The oil from the herbs is used in folk medicine for gastrointestinal disorders. The samples were collected in Antalya and Isparta provinces. This is the first report on the chemical constituents of O. minutiflorum.

Essential oils of the aerial parts were prepared by hydrodistillation and studied by capillary GC and GC-MS. The identification was based on mass spectral analysis and subsequent confirmation by gas chromatographic retention indices.

Among the identified compounds in the oils of O. minutiflorum, up to 75-82 % was carvacrol. In addition p-cymene (3-9 %) and α-pinene (1.0-1.2 %) were present.

P-4

THE EFFECT OF NUTRIENTS AND GROWTH REGULATORS ON THE ESSENTIAL OIL OF MELISSA OFFICINALIS L. (LAMIACEAE)

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This experiment was set up to examine the effect of the major plant nutrients nitrogen and phosphate, and the growth regulators C.C.C. and ABA on the essential oil of Melissa officinalis. Different amounts of nitrogen and phosphate were applied weekly as nutrient solutions. The growth regulators were sprayed onto the foliage of the plants.

Biomass and photosynthesis measurements showed the physiological effects of the treatments; and yield by hydrodistillation and compositional measurements provided data on the essential oil.

The percent dry weight (% DW) yield of oil tended to decrease at high nitrogen levels and was negatively correlated to the amount of chlorophyll. Phosphate levels had less effect on the yield of oil but a greater effect on the composition of the oil. The main compositional changes associated with increased phosphate were a lower proportion of citronellal, and a higher proportion of citral and geraniol.

NEW LACTONES FROM TOBACCO

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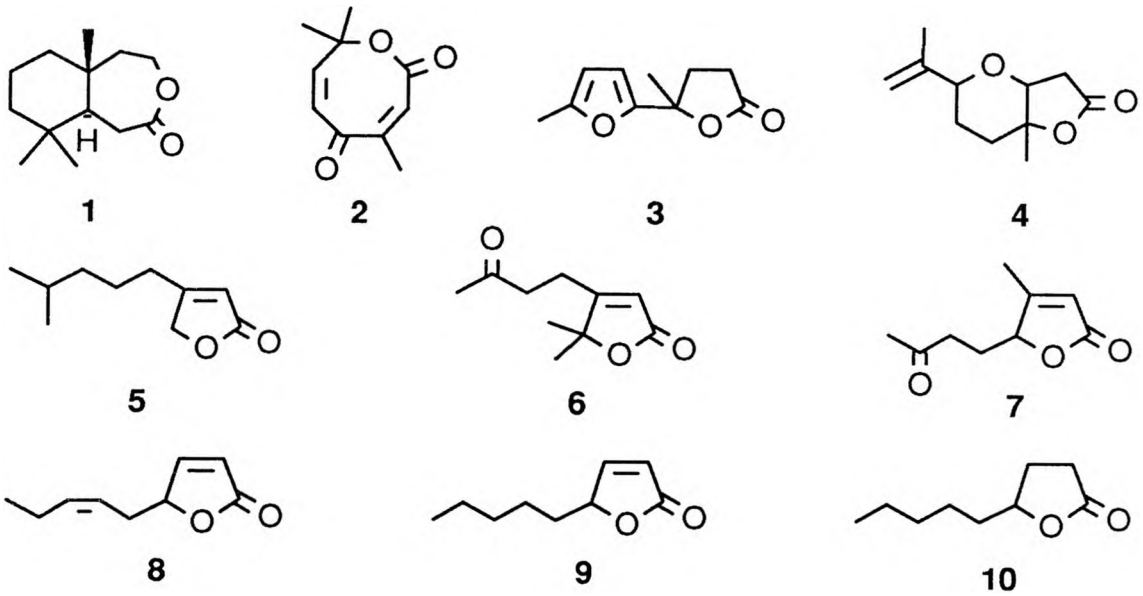
During our search for aroma compounds we have isolated a series of new lactones from sun-cured leaves of Greek tobacco (1 - 7). Their structures have been determined with the use of spectral methods. Work is now in progress to synthesize the new compounds.

We have also isolated a few lactones, which are new to tobacco but which have previously been found in other sources, e.g. 8 in tuberose absolute and 9 in corn oil.

Tobacco also contains chemically simple γ-lactones such as 4-nonanolide (10). As shown by GC on a chiral column these lactones are present as racemates. It is noteworthy that the γ-lactones occurring in fruits are normally enantiomerically pure.



P-6

CONCRETE OF COFFEE FLOWERS. DETERMINATION OF SOME CONSTITUENTS

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The wild flowers of the coffee trees of the developing countries can be considered as suppliers of raw material for production of aromatic extracts. The coffee flowers are very fragrant and their odour is like jasmine and tuberose.

This first study about the extraction and chemical composition of coffee flowers concrete and absolute is made with samples of Coffea arabica L. and Coffea robusta L. cultivated in greenhouses of the IICT/CEFC (Centre for Research of the Coffee Tree Rust), at Oeiras, Portugal.

The ethereopetrolic concrete and absolute were analysed by GLC, using capillary columns of different polarities and by GC-MS. Due to the complexity of the extracts we only identified a few compounds: limonene, *cis* - and *trans* -β-ocimene, methylantranilate, methyl and benzyl benzoates, anisaldehyde, fenchyl alcohol, farnesol and series of esters like methyl derivated from C_{16}-C_{24} acids, were detected.

CHEMICAL CONSTITUENTS AND ANTIMICROBIAL EFFECT OF SOME ESSENTIAL OILS AS INFLUENCED BY THE EXTRACTION METHODS

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Essential oils of some umbelliferous plants were isolated by solvent extraction using cold n-hexane for maceration at room temperature and hot n-hexane in a continuous extraction apparatus, as well as by hydrodistillation. The influence of the isolation method on the composition of the isolated essential oils was studied. The constituents were identified and determined using GLC and IR. Chemical and physical constants were also determined. Cyclic terpenes, aliphatic hydrocarbons and aliphatic alcohols, phenols, phenol ethers and cyclic terpene ketones were identified and determined.

For comparative study of the bacteriostatic and fungistatic activity of the isolated oils and their active principles, the agar diffusion method using the disc-plate technique was applied. The antimicrobial effect was tested on five bacteria, one mould and two yeasts. The major difference between the n-hexane extracts and the hydrodistilled fruit oils was the proportion of the hydrocarbons, which was markedly higher in the extracted oils. Among all the tested the essential oils, the n-hexane extracted oil (cold or hot) of caraway fruit proved to be the most effective against mould and yeasts, possessing remarkable biostatic activity. On the other hand, the hydrodistilled oil of coriander fruit had a high content of alcohols and was effective against bacteria.

P-8

BIOLOGICAL VALUE OF ASTERACEAE SEEDS COLLECTED FROM THE NATURE IN HUNGARY

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Due to the environmental pollution the importance of natural plant resources has increased during the last decades. Introduction to culture, preservation in genebanks and the use as propagation material for reproduction of the native flora in highly industrialized areas are vital methods in this kind of work.

Since there is very little knowledge of the germination characteristics of the wild medicinal plants in Hungary a long term study was started in order to collect and evaluate information about their germination features. The seeds of seven species belonging to Asteraceae and growing in the central parts of Hungary were collected during the years 1984-1989. The seeds were cleaned by machine and stored in paper sacks at room temperature. The germination tests were started 6 months after the collection and continued every 6th month up to 24-60 months. The tests were carried out on Petri dishes using the top paper method under continuous light at 22/18° C (day/night) temperature. The tests were repeated four times.

The average germination of the seeds was quite high and stable; Artemisia absinthium L. 71 %, Artemisia vulgaris L. 76 %, Matricaria inodora L. 78 %, Tanacetum vulgare L. 76 %, Taraxacum officinale Web. 74 % and Solidago gigantea Ait. 66 %. Only the germination of Centaurea cyanus L. was low (36 %). The year-to-year variation in the germination capacity was low and the viability of the seeds was maintained for 24-36 months, after which it decreased rapidly.

YIELD AND ESSENTIAL OIL OF SOME CHAMOMILE CULTIVARS GROWN IN FINLAND

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Chamomile (Matricaria recutita L.) is a very rarely occurring plant nowadays in Finland and all the chamomile flowerheads on the market are imported. The possibilities to cultivate chamomile in the open field was studied in Puumala, southern Finland during the period 1984-1989. Four different cultivars were studied. These were Bona (Czechoslovakia), Budakalaszi-2 (Hungary), Csömöri (Hungary) and Degumille (FRG). Budakalaszi-2 is a tetraploid cultivar and the other three are diploid. The flower yield depends very much on the wheather conditions during the 10-15 first days after the sowing, which is usually made in May. Dry wheather, but also very heavy rain during that period gives a low flower yield. Usually two harvests could be performed during each growing period, the length of which varied from 71 to 115 days. In 1988 three harvests could be carried out. Degumille gave the highest fresh flower yield (0.54 kg/m^2, average of four years) and Bona the lowest (0.28 kg/m^2). The essential oil content varied slightly from year to year both within and between the cultivars. The highest oil content was found in Degumille (1.1 %, average of three years) and the lowest in Csömöri (0.9 %). Degumille, Csömöri and Bona were (-)-α-bisabolol-types and Budakalaszi-2 a bisabololoxide A-type.

P-10

GROWTH, YIELD AND VOLATILE OIL OF LOVAGE CULTIVATED IN FINLAND

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Lovage (Levisticum officinale KOCH., Apiaceae) originates from West Asia, the Orient and South Europe. It is cultivated in Europe and North America as a spice and medicinal plant. The root is an official drug and a content of at least 0.4 % essential oil is required (DAC). The leaves are used as a spice.

Lovage was cultivated in Puumala, South Finland during the years 1984-1989. The plants overwintered well and produced fertile seeds every year with a germination capacity of 68 % on average. Some damages caused by bugs (Lygus sp.) were observed. The plants reached a height of 2-2.5 m in the second year and the root weight kept on increasing while the plants grew older. Data from plants older than five years are not available. Nitrogen fertilization increased both the root and leaf weight, the latter more than the former. Leaves could be harvested starting from the second year two or three times during one growing season. The heavy metal content (Cd and Pb) of lovage leaves from plants grown in Finland, Hungary and Czechoslovakia was studied in 1989. The content was 3 times lower in Finland than in Central Europe. The volatile oil content of 1-2 years old lovage roots was 0.2 % and of 2-3 years old roots 0.8 %. The leaves contained 0.2 % volatile oil. The main component of the root oil was butylidenephthalide, followed by pentylcyclohexadiene and β-phellandrene. The proportion of phthalides was higher in the older roots. The leaf oil contained α-terpinyl acetate, β-phellandrene and phthalides. The oil composition is in accordance with earlier published results.

APPLICATIONS IN ESSENTIAL OIL ANALYSIS BY GC-MS, GC-FTIR AND GC-AES

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Essential oils and their aroma compounds show a lot of different pharmacological and olfactoric activities. The rapid development of established analytical techniques and new coupled systems (e.g. GC-AES) in community with their high sensitivity allow the identification of single compounds and lead to a deep insight into the complex mixture and activities of these oils.

In this presentation lavender oil (Lavandula officinalis; genuine oils, samples after chemical reactions - like hydrogenolysis -, and headspace-extracts) from different geographic origin (oils from different regions of France and Yugoslavia; lavandin oil) were investigated by coupled systems like GC-MS (CI and EI), GC-FTIR, GC-AES, GC-FID and the data were correlated with results of olfactoric measurements. The concentration of linalool, linalyl acetate, geraniol, camphor, 1,8-cineole, lavandulyl acetate in each oil-sample is very important for the top-note of the oil, while some other terpenic compounds in lower concentration have only a small olfactoric importance.

In addition to this, lavender oil and its main compounds linalool and linalyl acetate have also been investigated as sedatives by animal experiments. Blood samples of mice after inhalation of lavender oil or linalool or linalyl acetate were analysed by GC-MS (CI and EI, SIM), GC-FTIR, GC-FID and GC-AES in order to detect the effective sedative compounds. Linalool (genuine and conjugated to glucuronic acid) and linalyl acetate (for a great part as linalool after hydrolysis by esterase) were detected in blood samples in ng/ml concentrations.

P-12

THE ESSENTIAL OIL OF RUTA ANGUSTIFOLIA L. FROM MALAYSIA

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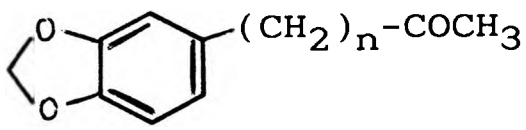
The hydrodistillation of the aerial part of Ruta angustifolia L. collected in Genting Highlands (Malaysia) provided an essential oil in moderate yield. The composition of the oil was investigated by using standard analytical methods, including GC/MS and GC/FTIR for the identification of individual components. Normal and branched chain aliphatic compounds that are commonly found in other Ruta species were identified: they include methylalkylketones (2-undecanone in majority), methylalkylcarbinols and the corresponding acetates (2-nonyl acetate in majority).

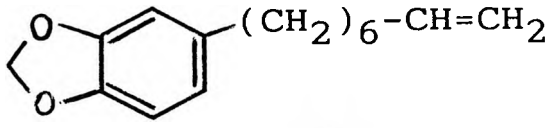
$CH_3-CHR^1-(CH_2)_n-CO-CH_3$ n = 4-9; R^1 = H or CH_3

$CH_3-(CH_2)_m-CH(OR^2)-CH_3$ m = 4-9; R^2 = H or Ac

The essential oil was also found to contain a number of compounds possessing the 3,4-methylenedioxyphenyl moiety: in addition to the known Moskachan A 1, Moskachan B 3, and Moskachan D 4 previously identified in an extract of R. angustifolia, new derivatives including 2 and 5 were identified.



1 (n = 0) ; 3 (n = 4)
2 (n = 2) ; 4 (n = 6)



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COMPOSITION OF HYDRODISTILLED FUNGAL OIL FROM FOMITOPSIS PINICOLA

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Fomitopsis pinicola (Sw.) Karst. (Basidiomycetes) is a brown-rot fungus common in Finland, usually found growing on dead trunks or stumps of conifers. Fungus has been reported to be used as a tonic and folk remedy in dermatology, for example. Volatile oils are therefore interesting since they often possess microbiological activities. The composition of the volatile oil was investigated in this study.

The identification of the compounds of the hydrodistilled oil was carried out using GC- and GC-MS analyses.

The results showed that the fungal oil was rich in lipid compounds (55.8 %) containing C_{14} - C_{18} fatty acids and their methyl esters. The main sesquiterpene alcohols (7 %) were nerolidol, α-, T- and δ- cadinols and cubenol isomers. The major sesquiterpene hydrocarbon was δ-cadinene (1.6 %). Alcohols such as 3- and 1-octanol (3.6 %) were also detected.

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ANALYSIS OF THE ESSENTIAL OIL FROM ORIGANUM HERACLEOTICUM (LAMIACEAE)

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Origanum heracleoticum L. is used as a spicy herb and its essential oil in the flavour and fragrance industry. It also possesses pharmacological properties and is applied as a spasmolytic drug for the gastrointestinal tract and as a digestive.

The samples of O. heracleoticum were collected in July 1986-1988 at the island of Hvar. The flowering plants were subjected to hydrodistillation for 4 h and a yellowish essential oil (3.10 - 3.76 %) was obtained. The oil was analysed by GC-MS. The components were identified by comparison with gas chromatographic retention times of standards and by mass spectrometric data. The main components of the oil analysed were thymol (53.19 - 67.88 %), carvacrol (3.99 - 14.02 %) and p-cymene (9.63 - 16.34 %).

STEREOCHEMICAL ANALYSIS OF CONSTITUENTS OF ESSENTIAL OILS BY ENANTIOSELECTIVE CAPILLARY GAS CHROMATOGRAPHY USING CYCLODEXTRIN DERIVATIVES AS CHIRAL STATIONARY PHASES

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Several hydrophobic derivatives of α-, β- and γ-cyclodextrins were synthesized and evaluated as chiral stationary phases for enantiomer separation of constituents of essential oils (1). The investigation of a selection of conifer needle oils, dill herb oils, caraway oils, lavender oils, citronella oils, geranium oils and others showed that chiral constituents may considerably vary in their enantiomeric composition. Only in exeptional cases pure enantiomers were found. The use of properly deactivated fused silica capillaries allowed the separation of underivatized alcohols (linalool, hotrienol, menthol, *trans* -verbenol, *cis* - and *trans* -carveol). In some cases for the separation of chiral alcohols trifluoroacetyl derivatives are preferable (citronellol, myrtenol, borneol, isoborneol). Excellent enantiomer separations were also obtained for α- and β-pinene, camphene, limonene, α- and β-phellandrene, carvone, fenchone, menthone, isomenthone, verbenone, isopinocamphone, piperitone and camphor. Some of the reference compounds had to be prepared synthetically, because only one enantiomer is readily available from natural sources.

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GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC DETERMINATION OF THE ESSENTIAL OIL FROM LEONOTIS MOLLISSIMA (LAMIACEAE)

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The genus Leonotis, Lamiaceae, is distributed in tropical regions of the world. Leonotis mollissima is an endemic plant of Brazil. Reports on biological activities could not be found.

The present work describes the chemical composition of the essential oil in which about 50 compounds were detected. Gas chromatographic analysis was carried out with an HP 5890A gas chromatograph using a FFAP fused silica column (50 m x 0,20 mm i.d.). The helium flow rate was 1 ml/min.

The GC was coupled with a VG TRID-2 quadrupole mass spectrometer by a direct capillary interface. The mass spectra were obtained with electron impact ionisation mode. Data acquisition was performed by a Digital Micro PDP 11/53 computer. The identification of the components were based on the retention times of standards and/or on the NBS library spectra.

ON-LINE COUPLED SFE-SFC FOR THE ANALYSIS OF AROMATIC PLANTS

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SFE is a selective sampling method, which in certain cases has advantages over conventional methods. For preliminary studies on the applicability of static SFE-SFC to the analysis of aromatic plants dragonhead herb was chosen. The main components of dragonhead herb are 2,6-dimethylocta-2,6-dien derivatives *e.g.* acetate, aldehydes and alcohols. The method used is comparable to static headspace gas chromatography (HSGC).
In order to find the best analytical conditions different extraction temperatures (60, 70, 80, 90 and 100°C) and densities of CO_2 were tested. The total and relative peak areas vary depending on the temperature. The maximum peak areas were obtained at 80°C and at this temperature the terpene composition is comparable to that of the distilled oil analysed by GC: The peak areas of the studied compounds increase while increasing the density of CO_2 from 0.2 g/ml to 0.6 g/ml at the chosen temperature. The increase of peak areas is exponential at densities from 0.5 g/ml to 0.6 g/ml, and at higher densities the increase is drastic.
The time required for static SFE/SFC analysis of dragonhead herb is comparable to HSGC. The resolution for the main compounds with SFC is satisfactory. The main advantage of using SFC-SFE is the more reliable quantification of oxygenated compounds in plant volatiles (1).

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SEPARATION OF ENANTIOMERIC IRONES BY GAS-LIQUID CHROMATOGRAPHY ON A MODIFIED CYCLODEXTRIN

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The irones, responsible for the violet like scent of the precious iris oil are derived from oxidative degradation of methylated bicyclic triterpenoids, the cycloiridals, which are found together with the monocyclic iridals in some Iris species (1). The irone moiety is formed by transfer of a methyl group to the homofarnesyl side chain of the iridals (2). This enzymatic step, however, does not lead to the specific formation of one product byt to diastereoisomeric (*cis/trans*) irones and α or γ double bond isomers. Additionally, different Iris species produce enantiomeric irones (1,3). The enantiomeric purity of the compounds so far only was estimated by extrapolation of their optical rotations (3). We were now able to separate the enantiomers by GLC on a WCOT fused silica column (25 m) coated with octacis(3-0-butyryl-2,6-di-0-pentyl)-γ-cyclodextrin (4) at 115°C. The investigation of irones from different origin showed that (-)-*cis* -α- and (+)-*cis* -γ-irones usually occur in almost 100% ee, whereas the (+)-*cis* -α- and (-)-*cis* -γ-irones possess a poor optical purity. The possible significance of these findings for the enzymatic environment during the formation of the irone moiety is discussed.

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ANALYSIS OF THE ESSENTIAL OIL OF ACHILLEA TOMENTOSA (ANTHEMIDEAE) COLLECTED IN SPAIN

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Achillea tomentosa L. (Asteraceae) is a perennial herb, tomentose to sericeous, which grows on dry hillsides and waste places of S.W. Europe. As there is no previous report concerning the composition of its essential oil, it seemed interesting to undertake this study.

The plant material was collected in July, 1982 (Cuenca, Spain, altitude 1300 m) on motley loams. The essential oil was obtained by hydrodistillation (cohobation) in a Clevenger apparatus. Physical and chemical constants of the oil were determined (d_{20}^{20} 0.931, n_{D}^{20} 1.4799 [α] + 13.41°), and the yield was 0.31 % (vol / dry wt).

The tentative identification of most of the individual components was based on gas chromatographic retention indices, and was confirmed by GC/MS analysis and its spectroscopic results from IR and ^{13}C-NMR. The main constituents of the essential oil were: limonene + 1,8-cineole (31.47 %), α-pinene (12.87 %), α-terpineol (12 %), terpinen-4-ol + *trans* -pinocarveol (6.19 %), artemisia ketone (4.58 %), caryophyllene oxide (3.33 %), artemisia alcohol (1.81 %), β-eudesmol (1.67 %) and yomogi alcohol (0.57 %).

The presence of some irregular monoterpenes, which may be used as excellent chemotaxonomic markers, confirms this species' place within the Anthemideae tribe.

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COMPOSITION OF THE ESSENTIAL OIL OF SPANISH ACHILLEA THRACICA

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Achillea thracica Velen. (syn. A. filipendulina Lam. var. thracica (Velen.) Stoj & Stephanov) is a perennial herb that has been used as an ornamental plant. Because of its fast adaptation to environmental conditions, it has been spread toward larger areas, mainly in dry places.
Our aim was to analyse the essential oil of plants collected in Madrid (Spain), and to make a comparison between the chemical composition of the Spanish oil and that described in previous studies.
The inflorescence essential oils were prepared by hydrodistillation. We have obtained similar yields in all samples (0.27–0.28 % vol /dry wt). Physical and chemical constants were determined. The essential oils were analysed by GLC using columns of different polarities an by GC–MS. Most of the individual components were tentatively identified by comparing their retention times and mass spectral data, with those of pure standards and literature data. We have also used IR and ^{13}C–NMR analysis for further identification.
Trans –achillenol, borneol, limonene + 1,8–cineole, caryophyllene oxide, terpinen–4–ol and camphor + linalool were the main components. We haven't found *cis* achillene. But some irregular monoterpenes characteristic of the Anthemideae tribe: artemisia triene, yomogi alcohol, isolyratol and artemisia alcohol, have been found in this species.

POLYNUCLEAR AROMATIC COMPOUNDS AND OTHER CONSTITUENTS OF THE HERB ESSENTIAL OIL OF SALVIA COCCINEA

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Salvia coccinea Juss. ex Murr., family Lamiaceae, is used as an aromatic, ornamental and medicinal plant. It has many synonyms, and its common names include red sage or Texas sage.

Decoctions of S. coccinea are reported to be taken by Europeans for the relief of lumbago, kidney diseases and the cough of pulmonary tuberculosis, but the plant used in one case was suspected to be poisonous. No reports were found in the literature on the essential oil of S. coccinea, and because of the many biological activities of many of the 700 species of the genus Salvia, we were interested in the analysis of its essential oil.

Three herb samples (3 kg each) of S. coccinea, from plants growing at the same location on the campus of the Obafemi Awolowo University (Ile-Ife, Nigeria) were collected in August 1987, 1988 and 1989, respectively. A voucher specimen was deposited at the Herbarium of the Department of Botany of the same university. Plant materials were stored at -15°C until analysis. During each year of collection, frozen herb samples were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus (approximate yield 0.1 % vol/wt) and to distillation-extraction for 4 h using a Likens-Nickerson-type apparatus.

Capillary GC, LSC and GC-MS using an ion-trap detector (ITD) were used in the analysis of the essential oil samples. The method of isolation was found to affect the yield, composition and odour of the oil. About 40

components including Polynuclear Aromatic Compounds (PACs) were identified. The major components of the oil samples were acenaphthene (18 %), *trans* -hex-2-enal (11 %) and globulol (11 %) in 1987; globulol (65 %) and aromadendrene (11 %) in 1988, and 2,5-di-methoxy-*p* -cymene (29 %) and globulol (16.5 %) in 1989. Trace amounts of a compound which was found to be 1,2,3,4,5,6-hexachlorocyclohexane (= gammalin or lindane) were detected in the 1989 oil sample. The occurrence of acenaphthene in plants may very well be real; ryomenin, a novel sesquiterpene with a tetrahydroacenaphthene skeleton has been isolated from some ferns of Arachinoides species. The analysis and composition of the oil samples will be presented.

6-HYDROXYCARVOTANACETONE AND OTHER CONSTITUENTS OF THE ESSENTIAL OIL OF LAGGERA ALATA

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Laggera alata (D. Don) Sch. Bip. ex Oliv., family Asteraceae, is a strongly aromatic herb with a persistent thymol-like and sweet odour. Its leaves yield an essential oil, which is said to possess the odour of black currant, and may have some applications in perfumery.
The plant is eaten as vegetable in some parts of Nigeria. In other parts of Africa different preparations of the plant are used for treating intercostal and rheumatic pains and fevers. The leaf sap and root decoctions are taken by draught in the treatment of pneumonia. L. alata is a major ingredient in an ointment used in the treatment of skin tumour in Chinese medicine. The plant is used in Madagascar as a disinfectant. The antimicrobial activities of the essential oils of some Laggera species have been reported. Because of the various uses of the plant, we were interested in analysing its essential oil.
Herbs of L. alata growing wild at the Obafemi Awolowo University campus, Ile-Ife, Nigeria were collected in August 1988. The oil was isolated from frozen plant material by distillation-extraction for 4 h in a Likens-Nickerson apparatus, and analysed by means of GLC, LSC, GC-MS and ^{1}H-NMR spectrometry. Olfactographic analysis of the oil was also carried out. Amongst the identified components were phenolic ethers (about 45 %), monoterpenes (22 %) and sesquiterpenes (12 %). The main components were 2,5-dimethoxy-*p*-cymene (= thymoquinol dimethyl ether; 44 %), and sabinene (16 %). Another component (3 %) was found to

be 2-methyl-5-(1-methylethyl)-6-hydroxycyclohex-2-en-1-one (= 6-hydroxycarvotanacetone). This compound has until now not been reported to occur in nature. Another rare compound detected was 4-hydroxy-carvotanacetone-7-O-angelate (2 %).

In a recent report (1) on an hydrodistilled oil from pulverized leaves of L. alata also collected in Ile-Ife, much smaller quantities of thymoquinol dimethyl ether (11 %) and sabinene (6 %) were found, whilst 6-hydroxy-carbotanacetone and 4-hydroxycarvotanacetone-7-O-angelate were not reported. These observations suggest the occurrence of chemotypes, though some differences may be due to the isolation procedure used.

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CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF AN ITALIAN THYMUS VULGARIS L. CHEMOTYPE

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Thymus vulgaris L. grows wild in Italy, Spain and France. It is used, fresh and dried, as a culinary herb and its essential oil is employed in food industry, in the making of liqueurs and in perfumery. It possesses antiseptic, antifungal and vermicide properties and is used for medicinal purposes. In folk medicine it is employed to treat bronchitis, colic, fever, rheumatism and other disorders. The pharmacological action is mainly due to the phenolic components (thymol and carvacrol) of the essential oil. The essential oil composition can vary considerably arising from the existence of at least seven chemotypes, each characterized by the production of different major compounds. Therefore it is important to analyse T. vulgaris essential oil in order to define the chemotype.

The aim of this work was to determine the composition of the essential oil of an ecotype of T. vulgaris growing wild in Northern Italy. Essential oil, obtained by steam distillation, was analysed by GC and GC-MS. Forthy four compounds have been identified, fifteen of them sesquiterpenes. The oil consists mainly of monoterpenes (80 %), *p* -cymene, γ-terpinene and thymol being the major ones. They belong to the same biogenetic pathway and define the chemotype.

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THE ESSENTIAL OIL OF ARTEMISIA JUDAICA CHEMOTYPES

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Artemisia judaica L. is a perennial shrub which grows in desert wadi beds in eastern Sahara, Sinai, Israel (Negev), Jordan and Saudi Arabia. Distinct sub-species were described on the base of morphological differences. The distribution of the plant's population in the Negev suggests that it descended from the population in Sinai.

The GC-analysis of the essential oils of the plants from Sinai and Negev points on the existence of two chemotypes. More than 40 components have been identified in each of the two essential oils. The main constituents of A. judaica from Sinai are: chrysanthenone (5-10 %), camphor (16-23 %), piperitone (27-46 %), *cis* -ethyl cinnamate (5-6 %) and *trans* -ethyl cinnamate (8-13 %). The Negev type contained: artemisia ketone (18-42 %), yomogi alcohol (3-9 %), chrysanthenone (3-13 %), camphor (4-21 %), piperitone (5-20 %), *cis* -ethyl cinnamate (2-7 %) and *trans* -ethyl cinnamate (4-10 %). The main difference between the chemotypes is the occurrence of artemisia ketone and yomogi alcohol in the Negev type, and significantly higher levels of piperitone in the plants from Sinai.

Allelopathic and autotoxic effects of fresh leaves and essential oils were tested in the laboratory. Plant material and oil from Sinai were found to inhibit germination and radicle elongation of tomato and of A. judaica itself more than those from the Negev.

EFFECT OF VARIOUS DISTANCE OF THE PLANT ROWS ON THE QUANTITATIVE - QUALITATIVE CHARACTERISTICS OF THE ESSENTIAL OIL OF CHAMOMILE - VARIETY "BONA"

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A number of agricultural enterprises have introduced the large scale cultivation of chamomile. So far obtained results have shown that with the complex use of chamomile, its production with regard to contemporary price and expense relations is of great interest. Regarding the latter we are concerned with the investigation, quantity and quality of the essential oil in chamomile in relation to ecological-physiological agents of the environment where the plants grow. The aim of this work is to show that the search for the optimum organization of the chamomile cultivation leads to quantitative and qualitative changes of the essential oil of this medicinal plant.

We performed some field experiments with chamomile. The essential oil was isolated by steam distillation and the content was determined gravimetrically. Gas chromatography was used to characterize the main components of the essential oil.

Our work offers the results of quantitative and qualitative charcteristics of chamomile oil. The various distance of rows was found to have no marked influence on the essential oil content or composition.

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ESSENTIAL OIL FROM SALVIA POTENTILLIFOLIA BOISS. ET HELDR.

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

The flowering plants were collected in Elmali (Antalya, Turkey) in June 1986. The plant material was subjected to hydrodistillation for 2.5 h using a Clevenger apparatus and it yielded a yellowish essential oil (0.80 %).

The oil was fractionated by LSC on a silicagel column. The fractions were analyzed by GLC. Components of the oil were identified by comparison of the retention times on different columns with those of authentic compounds.

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

STRUCTURE-ODOR CORRELATION OF CASCARILLA ACID DERIVATIVES

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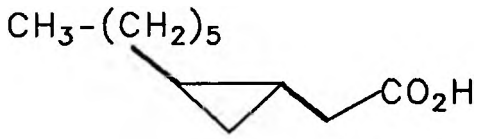
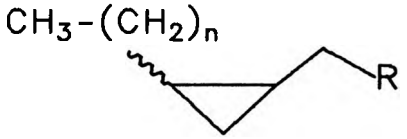
Cascarilla acid (1), the main constituent of the acid fraction of Cascarilla oil, is one of the many three-membered ring compounds possessing an interesting odor.
Now, we present the synthesis and olfactive properties of several acids, esters and alcohols (2) with cyclopropane skeleton. Structure-odor correlation can be made dependent on the stereochemistry, functional group and length of the alkyl side chain.



1



2

R = CO_2H, CO_2Me, CO_2Et, CH_2OH
n = 3 - 7

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CNIDICIN - A NEW COMPOUND IN LEMON OIL

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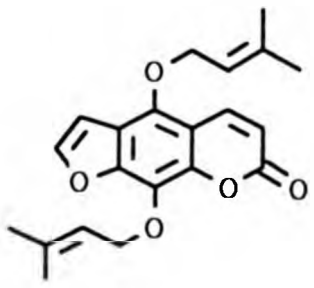
Investigation on the non-volatile constituents of cold-pressed lemon-oil led to the detection of a number of coumarines and psoralenes. In the current work we would like to present cnidicin (I) a so far unknown psoralene within the Rutaceae class.

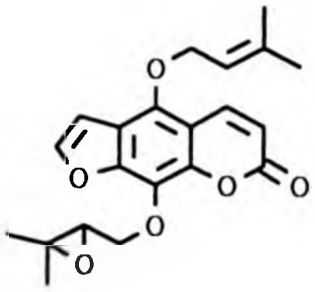
Detection and identification of cnidicin in Sicilian lemon oil was carried out according to a specific work-up procedure via GC-MS or HPLC, respectively. The compound was synthesized to confirm its structure. Spectroscopic data of this compound are reported for the first time.

The occurrence of cnidicin in lemon reveals this compound to be a relative to compound (II), recently detected in lemon oil:

- Compound (II) has hitherto been the only psoralene in lemon oil which possesses two five carbon alkoxy substituents.
- Cnidicin is therefore probably the precursor of the oxidized compound (II).

(I)





(II)

Analysis of Supercritical Carbon Dioxide Extracts from Cones and Leaves of a *Humulus lupulus* L. Cultivar

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Various cultivars of hop, *Humulus lupulus* L., are used in the brewing process to impart to beer a bitter taste and hop flavour, as well as a characteristic aroma. Breweries are known to use hop cones or hop pellets and hop extracts. These extracts are produced either by means of organic solvents or by means of liquid or supercritical carbon dioxide.
Because carbon dioxide is commonly used for extraction of hops in the industry and most of the procedures applied are patented, we analysed some extracts obtained by supercritical carbon dioxide extraction of cones and leaves of *Humulus lupulus* L. cv Wye Northdown.

The extractions, using 40 g samples of cones or leaves, were carried out at four combinations of temperature (40-60°C) and pressure (12.5-27.5 MPa). In each experiment three fractions were collected in liquid carbon dioxide and finally dissolved in pentane. Each of the fractions thus collected was divided into two parts; one was analysed by GC and GC-MS for volatiles (1,2), the second one by HPLC (3) and ^{1}H-NMR spectroscopy for the bitter compounds (α- and β-acids).
It was observed that the yield and the composition of the mixtures of the bitter compounds from the cones was largely influenced by the temperature and pressure applied during the extraction. Bitter compounds could not be detected in the extracts from the leaves. The most important volatile components identified were β-myrcene, β-caryophyllene and α-humulene. The extraction parameters selected, also influenced the composition of the mixtures of volatiles from the cones and leaves. Among the combinations of parameters tested, that of 40°C and 20.0 MPa was optimum for the extraction of both the bitter compounds and the volatiles.

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NEW METHODS IN ESSENTIAL OIL ANALYSIS

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In the past a great number of attempts have been made to optimize separation of essential oils and identification of the individual oil components. Recent developments in this field will be discussed according to the following classification:

1. Methods by which - in the ideal case - a separation to individual components may be accomplished. With regard to the complexity of the samples to be analyzed, almost exclusively chromatographic methods like GLC, HPLC, and more recently SFC including multidimensional and chromatographic coupling techniques are used.

2. The second group comprises the instrumental online couplings of chromatographic separation devices to spectrometers. Besides the commonly used GC/MS coupling, the GC/FTIR, SFC/FTIR, GC/UV and GC/AES couplings seem to be the most promising ones in the field of essential oil analyses.

3. The last group embraces methods that provide direct information about the composition of a particular multi-component sample without previous separation of individual substances. Foremost among these are spectroscopic methods like IR, MS and above all 13C NMR spectroscopy.

By means of selected examples mainly those analytical methods will be discussed, which have been developed and improved more recently.

STRUCTURE DETERMINATION OF VOLATILE PHENYLPROPANOIDS BY GAS CHROMATOGRAPHY AND SPECTROSCOPIC METHODS

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Essential oils are generally mixtures of mono- and sesquiterpenes but a smaller number, e.g. some oils from the Apiaceae, contain principally phenylpropane derivatives mixed with terpenes.
In contrast to terpenoids, up to now a comprehensive treatment of the chromatographic and spectral features of phenylpropanoids does not exist. We have therefore examined a great number of phenylpropanoids and related aromatic compounds occurring as constituents of essential oils and determined their gas chromatographic and spectroscopic properties (MS, UV, IR, 13C-NMR).
With the exception of a few phenylpropanoids, most of the investigated compounds with a certain substitution pattern of the aromatic ring exhibited on a polar GC capillary successive elution of the respective allylic, *cis* -propenylic and *trans* -propenylic derivative according to the prediction of Shulgin (1). Determination of the individual isomer with a propenylic side chain can be performed by UV, IR and 13C-NMR spectroscopy unambiguously, while the fragmentation pattern of the mass spectrum does not allow to distinguish these 3 isomers.
However, mass spectrometry is a valuable tool to differentiate most of the phenylpropanoids from other oil constituents and to determine their molecular weight but gives little information on the substitution pattern of the aromatic ring. This can only be achieved by NMR and to some extent by IR spectroscopy.

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THE ESSENTIAL OIL OF PETROSELINUM SEGETUM (L.) KOCH

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Petroselinum segetum (Apiaceae) is a biennial or annual plant growing at coastal localities in Western Europe from England, the Netherlands to Portugal extending eastwards to Central Italy.

This report deals with the essential oil composition of the herb, the fruits and the roots. The individual essential oils have been obtained by hydrodistillation from the different plant parts and were analyzed by means of chromatographic (LC, HRGC) and spectroscopic (IR, MS) methods.

The fruit oil is characterized by a high content of elemicin (75.5 %) besides lower amounts of mono- and sesquiterpene hydrocarbons, above all γ-terpinene (13.3 %) and β-pinene (5.4 %).

In contrast to the fruit oil the essential oils of the herb and the roots do not contain any phenylpropane derivatives in remarkable amounts. Major components of the oil of the herb are the hydrocarbons *E, E* -α-farnesene (27.8 %), *cis* -β-ocimene (21.1 %), germacrene D (14.2 %), and β-phellandrene (13.4 %), whereas the root oil is dominated by a high percentage of terpinolene (54.8 %). In addition, minor percentages of several ubiquiteous occurring mono- and sesquiterpenes were found.

Comparison of the essential oils from the different parts of Corn Parsley (Petroselinum segetum) and Garden Parsley (Petroselinum crispum) exhibited remarkable differences in composition of the respective fruit and herb oils. The root oils of the two species were different in composition, too.

ESSENTIAL OIL CONSTITUENTS OF SOME NIGERIAN ASTERACEAE AND ANNONACEAE MEDICINAL PLANTS

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As part of our continuing and systematic phytochemical studies of the Nigerian medicinal plants, the constituents of the essential oils from Asteraceae plants (Ageratum conyzoides, Blumea lacera, Laggera alata) and Annonaceae plants (Monodora tenuifolia and Uvaria chamae) were comprehensively characterised by means of GC and GC-MS. All the plants are widely used in the West African ethomedical practise. Virtually all their essential oils contained mono- and sesquiterpenoid derivatives in variable amounts. Additional uncommon constituents identified included chromones and phenolic ethers. The significance of the compositional profile of each volatile oil would be briefly discussed

GASCHROMATOGRAPHIC SEPARATION OF GLYCOSIDIC BOUND VOLATILES AFTER DERIVATIZATION

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Glycosidic bound volatiles have usually been separated by means of GC and GC-MS of their hydrolysis products. The result of hydrolysis are two fractions, an aglycone fraction and a sugar fraction. Unfortunately it is not possible to assign the sugar components to the aglycones in the aglycone fraction. Additionally, time-consuming sample preparation caused by the enzymatic hydrolysis may produce some artefacts.

In this paper we present some derivatization methods to analyse the glycosidic bound volatiles in total. At the moment aldonitrile acetates and especially trimethylsilyl ether derivatives are the best substances to guarantee not only the stability of the derivatives but the clarity of the chromatograms.

Ten different test substances are used to prove the successful derivatization of the glycosides. The resulting volatile derivatives are separated on a fused silica capillary column (30 m x 0,25 mm) coated with the apolar stationary phase DB 1. Good results are achieved as far as resolution and retention times are concerned. Quantitative estimation and GC-MS analysis of the derivatives are possible. The application of these methods on methanolic extracts from Thymus vulgaris L. are presented.

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