SOCIETÀ ITALIANA DI FITOCHIMICA

22ND INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

FINAL PROGRAMME
ABSTRACTS
LIST OF PARTICIPANTS

SEPTEMBER 11-14, 1991
CENTRO CONGRESSI GRAND HOTEL BILLIA
ST VINCENT - AOSTA (ITALY).

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FINAL PROGRAMME

THURSDAY SEPTEMBER 12, 1991

Morning session

8.45 - 9.45	Opening Session
	Chairmen: A.Baerheim-Svendsen, K.- H. Kubeczka
9.45 - 10.35	*R. Kaiser* - Givaudan Research Comp. Ltd. - Duebendorf (CH) **Investigation of natural scents, an important stimulus in fragrance and flavour research**
10.35 - 11.00	Coffee break
	Chairmen: P.Manitto, A.F. Thomas
11.00 - 11.20	*P. Weyerstahl,* H.C. Wahlburg, H. Marschall-Weyerstahl, A. Rustajan * - University of Berlin - Berlin (D), *University of Tehran, Tehran (IR) **Constituents of the essential oil of *Pulicaria gnaphalodes* from Iran**
11.20 - 11.40	*M.H.Boelens,* R.Jimenez - Destillaciones Bordas Chinchurreta S.A. - Seville (ES) **New chemical aspects of volatiles from *Cistus ladanifer* L.**
11.40 - 12.00	*R.Tabacchi,* D.Joulain * - University of Neuchatel - Neuchatel (CH), * Robertet S.A. - Grasse (F) **The essential oil from the fruits of *Sphaeranthus indicus* L.: further results**
12.00 - 13.00	Poster Session

Afternoon session

Chairmen: L. Peyron, C. Franz

14.15 - 15.05 *B.V. Charlwood* - University of London, London (UK)
Essential oil production by plant cell cultures, fact or fiction?

15.05 - 15.35 *E. Stahl-Biskup,* J.Holthuijzen, F.Intert*, M.Stengele
University of Hamburg, Hamburg (D), * University of Kiel, Kiel (D)
Monoterpene glycosides - New aspects?

15.35 - 15.55 *M. Maffei,* University of Torino, Torino (I)
Herbicide effect on monoterpene metabolism in peppermint and spearmint

15.55 - 16.20 Coffee break

Chairmen: A. Bruni, R. Hiltunen

16.20 - 16.50 *B.M.Lawrence* R.J. Reynolds Tobacco Company - Winston-Salem (USA)
Chemical components of *Labiatae* oils and their exploitation

16.50 - 17.10 *G. Buchbauer,* W. Jager, L. Jirovetz - University of Wien, Wien (A)
New results in aromatherapy research

17.10 - 17.30 *M.T.Lis-Balchin,* S.Hussain*, S.Hart** - South Bank Polytechnic, London, (UK), *University of Reading, Reading, (UK), **Kings College, London, (UK)
Correlation of essential oil composition and pharmacological action of four species from Zimbabwe

17.30 - 17.50 *F. Tateo,* G. Brusotti - University of Milano - Milano (I)
Kinetics of the formation of cyclic chetals during the storage of flavorings and the production of some foods

18.00 For Italian SFI delegates only: Meeting of Società Italiana di Fitochimica

FRIDAY SEPTEMBER 13, 1991

Morning session

Chairmen: F.Chialva, J.J. Scheffer

8.45 - 9.35 *P. Sandra* - University of Gent, Gent (B)
Chromatography in essential oil and flavour analysis

9.35 - 9.55 *J. R.J. Paré*, J. M. R. Bélanger*, C. Chambon**,
Environment Canada, R.R.E.T.C., Ottawa (CDN),
* Agriculture Canada, L.R.R.C., Ottawa (CDN),
** C.E.A.M.S., Avignon (F)
MAP (microwave-assisted process): a novel extraction process for the essential oil industry

9.55 - 10.15 *Jian Qin Cu*, F.Perineau, A.Gaset - Ecole Nationale Superièure de Toulouse, Toulouse (F)
Studies on the solvent extraction mechanism for aroma raw materials

10.15 - 10.40 Coffee break

Chairmen: D. Lamparsky, I. Morelli

10.40 - 11.10 *B. Kolb* - Bodenseewerk Perkin-Elmer GmbH, Uberlingen (D)
Quantitative routine analysis of flavour compounds in liquid and solid samples by automated equilibrium headspace-GC with cryofocusing

11.10 - 11.30 *F. Andreolini* - Carlo Erba Instruments - Milano (I)
On-line coupled HPLC- Capillary GC for the characterization of essential oils and other natural products

11.30 - 11.50 P.Chatzopoulou, A.De Haan*, *S.T.Katsiotis*
University of Thessaloniki, Thessaloniki (GR),
* University of Delft, Delft (NL)
Comparative study on the CO_2-extraction of the volatile constituents obtained under different treatments from *Juniperus communis* berries

11.50 - 13.00 Poster Session

Afternoon session

	Chairmen: D.Joulain, G.M.Nano
14.20 - 14.40	*F. V. Schurig* Universitat Tubingen, Tubingen (D) **Enantiomer separation by inclusion gas chromatography on cyclodextrin derivatives**
14.40 - 15.00	*K. Bruns*, H. Rahnenfuhrer - Henkel KGaA, Duesseldorf (D) **Separation of racemic aroma chemicals by GLC in chiral cyclodextrin-phases**
15.00 - 15.20	*E. Shaaya*, U. Ravid - The Volcani Center - Bet Dagan (IL) **Fumigant toxicity of essential oils against stored food products, moulds and insects**
15.20 - 15.40	*L G. Bergstroem*, University of Goeteborg, Goeteborg (S) **Pollination attractants/excitants from flowering plants**
15.40 - 16.10	Coffee break
	Chairmen: C. Bicchi, K. Bruns
16.10 - 17.00	*W. Francke*-University of Hamburg, Hamburg (D) **Odoriferous compounds in insect/plant and insect/insect interactions**
17.00 - 18.00	Closing Session

ABSTRACT

PL1 - INVESTIGATION OF NATURAL SCENTS, AN IMPORTANT STIMULUS IN FRAGRANCE AND FLAVOUR RESEARCH

Roman Kaiser,
Givaudan Research Company Ltd.,
Ueberlandstrasse 138, 8600 Duebendorf, Switzerland

Out of a wealth of highly attractive natural scents, only a limited number can be captured in adequate quality and on a commercial scale as an essential oil or absolute. These natural products important to the fragrance and flavour industry have been extensively investigated during the past decades. As a logical completion of "essential oil research", more and more emphasis is given today to the trapping and subsequent investigation of natural scents as they are emitted by the respective flower, fruits, plants. After a brief discussion on experimental as well as biological aspects to be considered in connection with the application of such techniques, special emphasis is given to new or unusual compounds identified in preconcentrated headspace samples of rose species, entire uninjured citrus fruits and especially of orchid species. Finally, some practice orientated remarks to the reconstitution of flower/plant scents or even more complicated natural olfactory systems, based on such "headspace investigations", are made.

carvão activado

Recipiente especificos para o tamanho
de planta (localizaç cuidada
evitar incidência
do sol)

L1 - CONSTITUENTS OF THE ESSENTIAL OIL OF *PULICARIA GNAPHALODES* FROM IRAN

P. Weyerstahl, H.C. Wahlburg and H. Marschall-Weyerstahl
Institut für Organische Chemie, Technische Universität Berlin, Strasse des 17. Juni 135, D-1000 Berlin 12, Germany

A. Rustaiyan
Department of Chemistry, Shahid Beheshty University Tehran, Iran

The essential oil of *Pulicaria gnaphalodes* (Vent.) Boiss. (tribus Inulineae, family Compositae) was investigated by means of GC, NMR and MS. Main constituents of the 114 identified compounds are α-pinene (34%), 1,8-cineole (12%) and a new sesquiterpene alcohol, cadina-1(10),4-dien-8ß-ol (11%). The latter is a key compound for further cadinane alcohols and for two novel 1,8-oxidocadin-4-enes. The bisabolanes are represented by nuciferol, the new γ-curcumen-12-ol and some esters of these alcohols. Furthermore, several oxygenated derivatives with italicene and khusiene skeleton could be isolated. Obviously, these are [2+2]- and [4+2]-cyclization products of the corresponding γ-curcumenes. Some compounds responsible for the typical herbaceous, spicy, leathery, lemon-like, peppery and earthy odour have been evaluated.

cadinane ethers (podem ser ou não artefactos de destilaçã por acçã de ac. fórmico)

ciclo triene!! - cadinane — copaenos — muurolenos

G-curcumene → G-curcu → italicenos

↳ estrut 3-cíclicas

L2 - NEW CHEMICAL ASPECTS OF VOLATILES FROM *CISTUS LADANIFER* L.

Mans H. Boelens and Rafael Jimenez
Destilaciones Bordas Chinchurreta S.A. P.O. Box 11, Seville, Spain.

DATA BASE

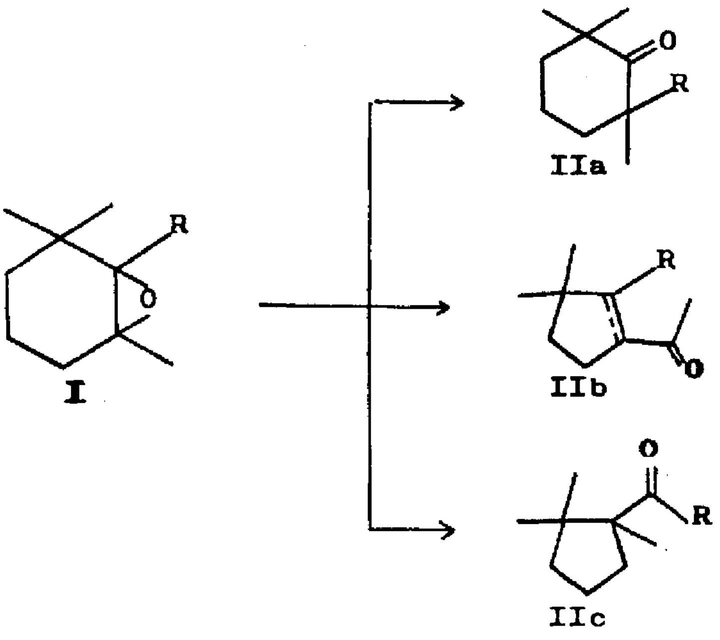
The chemical composition of hydrodistilled, esterified and vacuum-distilled oils from the plant exudate of *Cistus ladanifer* L. was studied. Over 250 constituents were detected in the oils. The main groups of compounds are: mono - and sesquiterpene hydrocarbons and oxygen-containing derivatives.

Two special groups of constituents are internal ethers/epoxides and acids. Representatives of these groups are alpha-pinene oxide, rose oxide, beta-ionone epoxide, amber oxide and cinnamic acid, phenylpropionic acid and labdenic acids.

The formation and rearrangement of the epoxides under influence of the acids will be discussed. The rearrangement of beta-ionone epoxide in vitro has been known (1,2). An analogy of the formation of new constituents on labdanum plants and the formation of known functionalized carotenoids in paprika will be demonstrated.

The rearrangement of functionalized trimethylcyclohexane epoxides (I) into functionalized tetrasubstituted cyclohexane- (IIa) and cyclopentane-derivatives (IIb,c) seems to be of general importance for the formation of volatile compounds and of natural colourants in plants.

1. K. L. Stevens, R. Lundin and D.L. Davis, Tetrahedron 31, 2749, (1975)
2. D. de Rijke, R. ter Heide, and H. Boelens, Perf. Flav. 7, (Febr./March 1982), 31-37.



L3 - THE ESSENTIAL OIL FROM THE FRUITS OF *SPHAERANTHUS INDICUS* L.: FURTHER RESULTS.

R.Tabacchi[+] and D. Joulain[++]
[+]Université de Neuchâtel, Institut de Chimie, Av. de Bellevaux 51, 2000 Neuchatel, Switzerland.
[++]P. Robertet S.A., B.P. 100, 06333 Grasse/Cédex, France.

In continuation of an in-depth analysis of a commercial essential oil from the fruits of *Sphaeranthus indicus* L. (Compositae) (1), we have carried out a closer examination of both the hydrocarbon and the sesquiterpene alcohols fractions,. that strongly account for the peculiar odour of this oil. Besides the previously identified sesquiterpene hydrocarbons, including caryophyllene and the unusual modephene (major), isocomene, silphinene etc., we focused our attention on several hydrocarbons with molecular weights ranging from 154 ($C_{12}H_{10}$) to 188 ($C_{14}H_{20}$, major). The fraction containing the sesquiterpene alcohols is responsible for the intense woody note of the oil. In addition to the already known T-cadinol, several other alcohols, including the very fragrant valerianol (major) have been isolated.

(1) R. Tabacchi and D.Joulain, Paper presented at the 11th International Congress of Essential oils, 12-16 nov. 1989, New Dehli (India)

PL2 - ESSENTIAL OIL PRODUCTION BY PLANT CELL CULTURES FACT OR FICTION?

B. V. Charlwood
Kings College London, Campden Hill Road, London W8 7AH, England

Study of the accumulation of essential oils by plant cell, tissue and organ culture dates from the 1950s, but early anticipation that the technology would have immediate commercial application was unfounded. This was due mainly to a lack of understanding of developmental gene expression which controls oil production, and of the fundamental physiology of oil storage.

In particular, essential oils are relatively toxic to plant cells even at low concentrations and are often sequestered in the intact plant in specialised storage sites. In undifferentiated cell cultures, such storage sites are absent and compartmentation of the product cannot occur. Under these circumstances repetitive cell culture will lead to selection of cell lines that either accumulate low levels of the toxic product or have increased activity of catabolic pathways designed to detoxify the essential oil produced.

In order to obtain high yields of essential oils in plant culture either alternative storage sites must be provided for oil accumulation, or the natural storage sites themselves must be retained *in vitro*. Both of these strategies have been investigated and the results reported so far are reasonably promising.

Assuming that a satisfactory tissue culture system is available then the next challenge is genetically to manipulate an increase in carbon flux through the chosen pathway and focussed on the target product. Methods for the formation of fast-growing organ cultures have recently been developed using *Agrobacterium* vectors and such transformation systems also permit the stable incorporation of new genetic material into the plant genome. The far-away dream of being able to engineer alterations to existing pathways, and perhaps even to design new ones, is perhaps about to become a reality. This paper will consider the developments in this area that may provide a basis for the future exploitation of this area of plant biotechnology.

a > quanti, 0.15% todas as restantes @ <
e a formaç de shoots apenas por induç hormo-
nal > a produç
a adiç de terp às cult < a viab mas este
efeito é < e – fase estacionária e > na lag

L4 - MONOTERPENE GLYCOSIDES - NEW ASPECTS ?

E. Stahl-Biskup* , J. Holthuijzen* , F. Intert** and M. Stengele*
* Lehrstuhl für Pharmakognosie der Universität Hamburg,
Bundesstrasse 43, D-2000 Hamburg 13, Germany
** Institut für Pharmazeutische Biologie der Universität Kiel, Grasweg 9,
D-2300 Kiel 1, Germany

Five years ago, an explosion of research activity in the field of terpene glycosides and other glycosidically bound volatiles prompted scientists to review the state-of-the-art (1,2). At that time, only a limited number of terpene glycosides were known and only little could be reported on the distribution of those compounds in the plant kingdom.
In the meantime many interesting results of individual studies in this field have been published. They concern structurally diverse new terpenoic and non-terpenoic glycosides, their occurrence in different plant species and organs of essential oil bearing plants as well as of some oil free plants.
In summary, the close connection between the glycosides and the essential oils as it has been usually discussed should to be thought over again. Furthermore, in view of publications dealing not only with the identification but with the physiology of these compounds, the significance of former hypotheses will be discussed. New experimental attempts are on the way to yield not only analytical data but more facts to clear up the role of the glycosides.

(l) Stahl-Biskup, E., Flavour Fragr. J. 2, 75 (1987)
(2) Mulkens, A., Pharm. Acta Helv. 62, 229 (1987)

L5 - HERBICIDE EFFECT ON MONOTERPENE METABOLISM IN PEPPERMINT AND SPEARMINT

M. Maffei
Dept. Morphophysiology. University of Turin. Viale P. A. Mattioli, 25 10125 Turin, Italy.

The metabolism of C-3 and C-2 hydroxylated compounds in maturing peppermint (*Mentha piperita*) and spearmint (*Mentha spicata*) leaves respectively has been shown to involve NADPH-dependent oxido-reductions of the related monoterpenes. The synthesis of these compounds requires fixed carbons, ATP and reducing equivalents mostly provided by primary biosynthetic pathways (Maffei M. and Codignola A., J. Ess. Oil Res., 2, 275,1990). The relationships between primary and secondary metabolism are still matter of controversy even though a direct link between these two pathways has been suggested (Haslam E., Nat. Prod. Rep., 3, 217,1986). Findings based on analytical investigations with specific inhibitors of the light phase of photosynthesis are described which confirm a direct link between NADPH production and monoterpene oxido-reductions in peppermint and spearmint.
Results of a series of in vivo experiments are provided to elucidate the pathways in these respective plants for the conversion of menthone to menthol and limonene to t-carveol to carvone.

A adição de herbicidas e inibição do PSI ou PSII tem efeito na composição do óleo

Paraquat gera OH^+ q atacam a memb (por ataque aos ac. gordo) o q justifica a alteração do óleo n1 planta mas @ na outra

L6 - CHEMICAL COMPONENTS OF LABIATAE OILS AND THEIR EXPLOITATION

B. M. Lawrence
R. J. Reynolds Tobacco Company, Bowman Gray Technical Center,
Winston-Salem, NC 27102 USA

The Labiatae family contains about 180 genera of which 40 percent are thought to possess aromatic properties. Essential oils of more than 30 selected species have been items of commerce for more than 75 years. The chemical composition of some of these commercially important oils will be discussed with reference to their origin, variation and infraspecific differences. Particular attention will be given to basil oil (*Ocimum basilicum* L.), an oil of commerce that is found to vary depending upon its origin. The concept of using pollen grain morphology to determine whether a taxon is oil-rich or oil-poor will be a further topic of discussion. Examples using a wide range of genera will be presented. Finally within the oil-rich genera, a large diversity of volatile constituents are encountered in the essential oil. After briefly examining the biosynthetic relationships of a number of them, some useful sources of specific components will be identified.

shikimic ac
via
fenilalani
↓
metilchavicol
↓
Met Eug
↓
Eug
↓
Met. cinam

L7 - NEW RESULTS IN AROMATHERAPY RESEARCH

G. Buchbauer, W. Jager and L. Jirovetz
Institute of Pharmaceutical Chemistry, University of Wien, Wien, Austria

In continuation of our studies about the biological (mainly sedative) properties of essential oils and fragrance compounds new results in aromatherapy research are presented. The decrease of motility of mice after inhalation of the fragrance compound is shown for a series of hitherto unchecked essential oils and some of their main constituents as well as their influence upon caffeine induced overagitation of these laboratory animals. The study also includes a detailed analysis of the essential oil of Herba Passiflorae (*Passiflora incarnata* L., Passifloraceae) in context with aromatherapy. Finally the high skin permeation rate of essential oils in combination with massage oils after massage is discussed.

L8 - CORRELATION OF ESSENTIAL OIL COMPOSITION AND PHARMACOLOGICAL ACTION OF FOUR SPECIES FROM ZIMBABWE.

M. T. Lis-Balchin[1], S. Hussain[2] and S. Hart[3]
[1]Biotechnology Department, South Bank Polytechnic, Borough Road, London SE1 OAA, England.
[2]Agrobotany Department, University of Reading, Whiteknights, Reading Berks RG6 2AS, England.
[3]Pharmacology Department, Kings College, Chelsea Campus, Manresa Road, London SW3 6LX, England.

The pharmacological action of the essential oils of the following species from Zimbabwe was studied: *Nidorella auriculata* (Compositae), *Helichrysum lepidissimum* (Compositae), *Leucas neufliseana* (Labiatiae) and *Hoslundia opposita* (Labiatiae). The essential oil profiles were obtained using GC/MS.

All four species exhibited a spasmolytic action on the isolated guinea-pig or rabbit ileum in vitro. A correlation between the spasmolytic action and the main essential oil components was investigated.

L9 - KINETICS OF THE FORMATION OF CYCLIC KETALS DURING THE STORAGE OF FLAVORINGS AND THE PRODUCTION OF SOME FOODS

F. Tateo, G. Brusotti
DISTAM - University of Milan, Milan, Italy

The formation of ketals during food production and storage can lead to significant problems with regard to the quality of flavourings, the safety of use and the legal implications. Indeed, in the majority of cases ketals cannot be classified as "natural-identical", and toxicological data are not available for most of these compounds. This paper considers the problem in terms of the modifications that can occur in a flavouring as a result of the formation of ketals during both technological transformation and storage. Furthermore, the desirable production of ketals for flavouring formulations is discussed in terms of the possibility of producing some important cyclic derivatives starting with essential oils and their fractions. Naturally, reference is also made here to the hydroxylate compounds that are responsible for reactions leading up to ketal formation.

PL3 - CHROMATOGRAPHY IN ESSENTIAL OIL AND FLAVOUR ANALYSIS

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The new trend in analytical chromatography is miniaturization . Micro-chromatography is the subject of intense research. Advantages of microchromatography over conventional chromatography methods include higher column efficiency, higher inertness, speed of analysis, higher detectability, easier hyphenation with spectroscopic methods etc . Main disadvantage is the small sample capacity which renders trace analysis difficult .

The state of the art in miniaturization of the different chromatographic techniques will be discussed in this contribution and emphasis will be given to the everyday applicability of Microchromatography in essential oil and flavour analysis.

Capillary Gas Chromatography already is the miniaturized form of Gas chromatography and CGC is a well established technique. Nowadays, the keyword in CGC optimization is selectivity. New phases have been introduced; particularly interesting for essential oil analysis are the derivatized cyclodextrins for enantiomer separation. New methods for selectivity enhancement before the chromatographic column include head space collection on open tubular traps and supercritical fluid extraction. The coupled chromatographic techniques CGC-CGC and HPLC-CGC are becoming more and more in use to unravel the complex essential oil profiles. Last but not least, hyphenation with universal and specific detectors and with the spectroscopic techniques mass spectroscopy, Fourier transform infrared spectroscopy and atomic emission detection provides the selectivity and sensitivity to elucidate most of the compounds. On the other hand, decreasing the internal diameter to 100µm, drastically reduces analysis time thus enhancing on detectability. Normal sample sizes can be introduced via split or PTV injection. Further reduction of the internal diameter demands for new instrumentation.

Developments in Micro Liquid Chromatography diverge into three mainstreams : Microbore LC, Micropacked LC, and Open Tubular LC. The latter seems to be the most interesting approach. Its introduction however, will be subject to improved injector and detector performance. The further evolution of Microbore and Micropacked LC is dependent on the commercial availability of columns and instrumentation. Continued optimization of packing material is a must. The characteristics of silica materials urgently need to be standardized. The wide variability in polarity, selectivity and inertness of different straight and reversed phase materials is an acute problem. Microbore and Micropacked LC are interesting alternatives to HPLC in essential oil and flavour analysis as coupling with other chromatographic techniques and hyphenation with spectroscopic methods is easy.

Although the technique is still young, Micellar Electrokinetic Capillary Chromatography (MECC) looks very promising in the analysis of flavour and fragrances. We expect a lot of chromatographers "to discover" in the near future the benefits of electrochromatography. A lot of efforts will have to be spent in sampling methods, column technology and detection optimization before the technique reaches a stage of maturity. Developments however are moving very fast and MECC seems an excellent alternative to Micro LC as open tubes are used!

Capillary and Micro-Packed Supercritical Fluid Chromatography should make its decisive move from research laboratories to routine application laboratories. Further optimization of injection technology and detector interfacing (restrictions) is necessary to reach this goal. The inertness of packing mterials in packed column SFC requires special attention. Polymer coatings and materials will play an important role. New, "exclusive" applications need to further support the future Micro-SFC.

L10 - MAP (MICROWAVE-ASSISTED PROCESS): A NOVEL EXTRACTION PROCESS FOR THE ESSENTIAL OIL INDUSTRY

J.R.Jocelyn Paré[1], Jacqueline M.R.Bélanger[2], and Catherine Chambon[3]
1. Environment Canada, R.R.E.T.C., Ottawa, ON,Canada K1A 0H3;
2. Agriculture Canada, L.R.R.C., Ottawa, ON,Canada K1A 0C6; and
3. C.E.A.M.S., 23 rue d'Annanelle, 84000 Avignon, France.

Recent developments in the application of the Microwave-Assisted Process (MAP) to the extraction of essential oils will be presented. A series of examples will be used to demonstrate several of the advantages associated with the process in terms of greater yields (e.g. monardas), higher commercial value of the extracts (e.g. pepper mint), and novel components and novel extracts (e.g. garlic). Other examples will examplify the enhanced versatility and ease of use of the process over conventional methods in applications such as in *continuum* operation, extractions in series, the *in situ* concentration of the extracts and the direct determination of the oil contents of *in vitro* plant materials.

(na + solv) → bloc no microwave

L11 - STUDIES ON THE SOLVENTS EXTRACTION MECHANISM FOR AROMA RAW MATERIALS

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118, Route de Narbonne, 31077 Toulouse, France

Carrot seed, celery seed and lovage root have been extracted with various solvents (ethanol, hexane, cyclohexane, benzene, methylfuran, dichloromethane and 1,1,2-trichloro-1,2,2-trifluoroethane). Both yield and composition of extracts obtained differ in function of solvent.
From the point of view of chemistry, the solvents have always the positive selectivity for the odorant molecules and negative selectivity for the non-desirable molecules. This is related to the affinity between solvent and chemical composition of raw materials. The affinity solvent/solute has been studied by comparison of the results obtained and solubility diagram of the solvents used as well as the principal constituents extracted by means of their hydrogen bond (αh) and polarity (αp).
From the point of view of botanical structure, the diffusion and penetration of the molecules of solvents face to odorant compounds inside raw materials are related to the physico-chemical properties of solvents, particularly their molecular density, surface tension, viscosity and kinetic viscosity. For the purpose of interpreting the extraction mechanism, the anatomy of cellular structure of aroma matters were studied and the location of essential oils inside the plants were determined.
Finally, the variation of quantity and quality for the results obtained has been interpreted with the well-established theory of Ashton.

L12 - QUANTITATIVE ROUTINE ANALYSIS OF FLAVOUR COMPOUNDS IN LIQUID AND SOLID SAMPLES BY AUTOMATED EQUILIBRIUM HEADSPACE-GC WITH CRYOFOCUSING.

B . Kolb
Bodenseewerk Perkin-Elmer GmbH, D-7770 Überlingen, Germany.

For automated routine analysis the most simple instrumental analytical technique should be preferred. For highly volatiles, such as flavour compounds the various techniques of gas extraction are very popular and are known collectively by the term “headspace” sampling. In general the dynamic headspace techniques (purge & trap) are used for this particular application, while the benefits of the equilibrium or static headspace procedure are mostly neglected or its potential is not fully realized. A comparison of both headspace techniques, however, conceming sensitivity and automation shows that the static technique compares well with the dynamic techniques. The quantitative analysis rests on a distribution equilibrium, which is termodynamically controlled and which therefore can be reproduced very precisely, thus offering good prerequisites for any type of calibration technique. In principle, all calibration techniques common in gas chromatography can be applied, such as extemal or intemal standard calibration, while the standard addition technique is particularly useful. In addition to these well known techniques, both the multiple headspace extraction (MHE) and the gas phase addition techniques are specific to equilibrium headspace. Which of these possibilities are finally best applied depends much on the sample type and these aspects are discussed on several typical examples.

L13 - ON-LINE COUPLED HPLC-CAPILLARY GC FOR THE CHARACTERIZATION OF ESSENTIAL OILS AND OTHER NATURAL PRODUCTS

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Chromatographic analysis of natural products is usually carried out either to determine contaminants of different origin or to detect particular components which contribute to their characterization or to indicate their origin. Although capillary GC is well suited to the analysis of volatile natural product components, sample complexity often forces the analyst to perform some type of clean-up prior to GC .

Coupling HPLC to capillary GC is a new promising technique for rendering sample preparation prior to GC more efficient. Efficiency means higher resolution power in preseparation or clean-up of the sample, replacing open column LC with HPLC . But also it means higher sample throughput, integrating the sample preparation step into GC analysis in a completely automated way.

Instrumental aspects of the fully automatic on-line HPLC-GC coupling are discussed and illustrated with several examples. Emphasis is given to the interfacing systems and their application to the transfer of different type of analytes and HPLC fraction volumes . Furthermore, a unique automatic multitransfer procedure is shown for the complete characterization of complex mixtures, a lemon oil sample is taken as an example. Other problems in the field of natural product characterization are addressed.

L14 - COMPARATIVE STUDY ON THE CO_2-EXTRACTION OF THE VOLATILE CONSTITUENTS OBTAINED UNDER DIFFERENT TREATMENTS FROM *JUNIPERUS COMMUNIS* BERRIES

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This investigation reports the experimental data on the influence of the grinding of the plant material *Juniperus communis* berries, the recovery and the composition of the extract under the application of super critical fluid extraction technology. The extraction of natural substances using supercritical gases represents a major and wide field of application for which the treatment of products at low temperatures with physiologically safe solvents, such as carbon dioxide, offers particular advantages: all free of organic solvents, storage stability and purity. Carbon dioxide extraction of essential oils were conceived during the search for a complete replication of nature by extraction of plant materials without damage or degradation.The extraction of the oil from *Juniperus* berries using carbon dioxide was studied by investigating the influence of the parameters of temperatures and pressure ranging from 35 to 50°C and 90 to 275 bar respectively in relation to the degree of comminution of the plant material -intact berries, particle size a)>2.5,> 1 mm and b) <1mm. The quality of the oil recovered from the berries by carbon dioxide extraction was found to be strongly dependent on the conditions of pressure and temperature applicated. The comminution affects greatly the total quantity of the oil recovered and furthermore the final composition of the extracts.

L15 - ENANTIOMER SEPARATION BY INCLUSION GAS CHROMATOGRAPY ON CYCLODEXTRIN DERIVATIVES

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The importance of the relationship between molecular chirality and biological activity is now well established in pheromone perception and flavour olfaction.

Thus, the determination of enantiomeric compositions (ee) and absolute configurations of biologically active compounds is highly warranted for insect pest control and in food analysis. In addition of enantiomerically active compounds is essential for establishing chirality - activity - relationships in biological and physiological trials.

The quantitative separation of enantiomers by gas chromatography on chiral (non racemic) stationary phases in connection with high - resolution - high - efficiency capillary columns represents a powerful tool for enantiomer analysis because of speed, simplicity, reproducibility and sensitivity.

We found that permethylated ß- cyclodextrin dissolved in a moderately polar polysiloxane such as OV-1701 offers excellent coating properties for small-bore open-tubular columns made from fused silica. On such columns chiral solutes ranging from apolar (cis-, trans-pinane) to highly polar (rac. butane diol, underivatized amines, alcohols and carboxylic acids) can be resolved.

It has also been possible to chemically link the cyclodextrins to a polysiloxane matrix and to immobilize the Chirasil-Dex stationary phases permanently onto the glass of fused silica surface. These chemically-bonded chiral phases can for the first time be used with supercritical carbon dioxide as mobile phase. Enantiomer separations in the SFC mode are applicable to nonvolatile and thermally unstable racemates.

The enatiomer separation of filberton, the aroma compound of the hazel nut, is discussed.

L16 - SEPARATION OF RACEMIC AROMA CHEMICALS BY GLC IN CHIRAL CYCLODEXTRIN-PHASES

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This paper reports on the gaschromatographic separation of racemic aroma chemicals and enantiomeric constituents of essential oils in different chiral cyclodextrin phases.

L17 - FUMIGANT TOXICITY OF ESSENTIAL OILS AGAINST STORED FOOD PRODUCTS MOULDS AND INSECTS

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At present, insect control in stored food products relies heavily upon the use of gaseous fumigants which pose serious hazard to warm blooded animals and the environment. Searching for alternative ways for insect control not involving insecticides, we have tested the fumigant toxicity of over 40 essential oils extracted from various spices and herb plants and their major constituents. Three groups of active materials were distinguished according to their activity against the test insects. The differences in activity of the various compounds were used in the search for more potent compounds for the control of the test insects. Recently, we were able to select a compound ZP51 with much higher potency than all the essential oils and the various compounds tested. A concentration of 3 µl/l air was enough to obtain 100% mortality of the test insects. This should be compared with 20-30 mg/l air of methyl bromide recommended for the control of these insects in the field. Data on the activity of these oils against food born moulds will be also reported. It should be noted that biologically active compounds of food plants are assumed to be environmentally more acceptable and less hazardous than other to humans. The results from this study, suggest that some oils or their major constituents could be efficient fumigants and also could be integrated with other pest management procedures.

L18 - POLLINATION ATTRACTANTS/EXCITANTS FROM FLOWERING PLANTS

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We have analysed the volatile compounds given off by flowers from a number of flowering plants belonging to ten families. They are potential pollination attractants/excitants. In some cases we have studied the behavioral response on pollinators of these compounds. The analytical results will be presented, including a summary of the methods/techniques used.

For nectar-producing flowers the compounds seem to act by signalling a food source to the pollinator, mostly insects. For flowers which do not produce nectar, they can act by chemical mimetism (*Ophrys*) or by food deception (*Cypripedium, Orchis,* etc.).

The most commonly found classes of chemical compounds which act as pollination attractans/excitants, are: fatty acid derivative, isoprenoids and benzenoid compounds. A theory-the so-called pollination syndrome hypothesis - predicts that some groups of plants give off some class of compounds which attract some groups of pollinators. This theory will be discussed in the light of the analytical results.

PL4 - ODORIFEROUS COMPOUNDS IN INSECT PLANT AND INSECT INSECT INTERACTIONS

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Odour signalling seems to be particularly important in insects, who have to orientate in a relatively spacious environment. Chemical communication, especially intraspecific attraction, over long distances requires specific signals which may be distinguished as true "chemical messages", from the "background noise" caused by odour compound of the environment. Generally, this is achieved on the basis of unique multicomponent mixtures of less specific compounds which are preferred over the production of species-specific substances which in turn would require the (costly) development of a species-specific enzyme. During a de novo synthesis the action of a less specific enzyme may even advantageously facilitate evolution through introduction of a "disposible variant".
Closely related species may produce bouquets which are chemically very similar, basing species specificity of a signal on quantitative differences (including enantiomeric proportions of chiral compounds) in the released mixture. Less specific enzymes may generate "by-product", which may add to the" fine tuning" of the bouquet . Such minor components need not necessarily be the same in related species and, though intraspecifically inactive, they may well be used as interspecifically active repellents which facilitate species discrimination.

Besides a genuine contribution to the principle development of a "chemical language" through de novo synthesis, coevolution of plants and insects may also form a basis for the origin of communication systems. Plant constituents which attract both sexes will cause mate finding at the plant site; storage and release of secondary plant constituents used by animals for their own benefit in order to attract or deter others will induce evolutionary processes which involve the use of metabolites of food and/or host compounds (or appropriate imitations thereof) to specify (or imitate) a chemical message. Such mechanism will include direct use of plant allelochemicals or corresponding metabolites in odour communication.
Compounds synthesized de novo may reflect a specific physiological state of the emitter, while signals made up by sequestered host components may provide information on the quality of emitter's habitat.

Striking similarities in odoriferous compounds both from animals and plants point to common roots and basic concepts in "semiochemistry". Considerations on biogenetic relationships may be useful in structure elucidation of unknown compounds ("biochemical analysis").

- Acetogenins show unbranched carbon skeletons with oxygen or nitrogen containing functional groups and/or one or more double bonds. They may form carbocyclic or heterocyclic rings, and according to their origin, often occur as series of bishomologues.
- Polyketids, derived from propanoate units, show characteristic -1, 3-dimethylbranchings. Mixed biogenesis from acetate and propanoate forms branched compounds with an uneven number of methylene interruptions. The oxygen of the subunits may be kept in the final product. Relative configuration at chiral centers often is the same in different compounds.
- Mevalogenins like mono-,sesqui-and diterpenoids form another ubiquitous group. Compounds showing an allylic oxygen function are common among the class.

POSTERS

P1 - ENANTIOMERIC SEPARATION OF SOME MONOTERPENE HYIDROCARBONS IN PINE NEEDLE OIL

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A high and low 3-carene chemotype is known in Finnish Scots pine populations (*Pinus silvestris* L.)(1). The distribution pattern of 3-carene has been explained by 3-carene inheritance at a single locus with two alleles c and C and varying dominance at the locus. The three genotypes, homozygous cc and CC and heterozygous Cc, produce low and high levels of 3-carene. The variation of α-pinene has shown to be closely dependent on the variation of 3-carene and related compounds. In order to obtain more information about the biogenesis of monoterpenes in Scots pine, the needle oil was analysed using a chiral phase in gas chromatographic separations.

Hydrodistilled oils from grafted trees of high (E 80) and low (E 736) 3-carene chemotypes and their reciprocal crosses (N = 32) were analysed on a heptakis-ß- cyclodextrin column. Identification was based on the GC retention times of authentic samples and spectral comparison by GC-MS. On the basis of different enantiomeric distributions of (S)(-)-α- and (R)(+)-α-pinene, both the low and high 3-carene types could be separated into two subgroups: 1) low 3-carene with either high (S)(-)-α-pinene or (R)(+)-α-pinene, 2) high 3-carene with high (s)(-)-α-pinene or (R)(+)-α-pinene (see Table).

Enantiomeric ratio (%) of α-pinene in the needle oil.

Chemotype	S(-)-	R(+)-	Chemotype	S(-)-	R(+)-
Low 3-carene 1.	84	16	High 3-carene 1.	72	28
2.	25	75	2.	18	82

As indicated by the multivariate analysis the amounts of tricyclene, myrcene, camphene, (S)(-)-ß-pinene and (S)(-)-limonene were closely related to the proportion of (S)(-)-α-pinene. The increase in the amounts of 3-carene was found to occur at the expence of (S)(-)-α-pinene. The variations in the amount of sabinene, E-ß-ocimene, γ-terpinene and terpinolene were highly dependent on those of 3-carene.

Reference

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P2 - ANALYSIS OF MATRICINE IN CHAMOMILE BY SFE/SFC

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Matricine in chamomile flowerheads is usually determined indirectly as chamazulene by GC. It can be converted into chamazulene during isolation of the essential oil by hydrodistillation or in a headspace vial after addition of water and heating. Because matricine has anti-inflammatoric properties it is of great interest to know the content of matricine in the drug. Therefore a direct method of matricine determination by on-line coupled static SFE/SFC was tested.

The analyses were carried out using a Lee Scientific Series 600 supercritical fluid chromatograph equipped with FID and a self-made extraction system placed in the chromatographic oven. The extraction system consisted of a 3-port valve, two 10-port valves and an extraction vessel (120 µl). The supercritical fluid was CO_2 and the column SB-Phenyl-50 (Lee Scientific, 10 m x 50 µm i.d.). The plant material (cultivated in Malacky, Czechoslovakia in 1988) was finely ground and 25 mg weighed into the extraction vessel. Extractions were made at 60, 65, 70, 75, 80 and 85° C using densities of 0.4, 0.5 and 0.6 g/ml. Matricine was identified by spiking with pure matricine (ASTA Pharma AG). The best recovery of matricine was obtained at the highest temperature and the highest density. In the other conditions the proportion of matricine was lower and the proportions of α-bisabolol higher, respectively.

P3 - EXTRACTION OF VIOLET LEAVES IN A CLOSED CONTINUOUS SOLVENT FLOW SYSTEM: STUDY ON THE INFLUENCE OF MAIN PARAMETERS ON THE YIELD AND COMPOSITION OF THE EXTRACTS

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Violet leaves were subjected to extraction with 1,1,2-trichloro-1,2,2-trifluoroethane in a closed continuous solvent system on a pilot of 100 liters during harversting season of 1989 and 1990. Such parameters as ratio between raw material and solvent, extraction time, solvent flow, number of successive extractions, etc, have been studied.

For the purpose of quality control, the composition of the concretes which are considered as representative according to the variation of the experimental conditions were determined after their fractionation either in the form of absolute or in the form of volatile fraction by means of Likens/Nikerson apparatus.

The olfactory tests were appreciated on the different samples by perfumers. This drives us to chose the best extraction conditions for violet leaves. The results showed that both quantity and quality of the concretes vary a great deal according to the experimental parameters. The optimal conditions for industrial extraction of violet leaves are given.

P4 - FLAVOUR TRACE ANALYSIS BY LARGE VOLUME HEAD SPACE INJECTION WITH SAMPLE CRYOFOCUSSING AND MULTIPLE DETECTION

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Direct analysis of head space using high resolution capillary columns may be very useful in determining volatile compounds present as traces in complex mixtures, such as flavour and fragrance. High level of sensitivity can be reached by splitless injection of large gaseous volumes followed by the cryotrapping of the sample in the initial part of the capillary column. The sample enrichment effect is obtained with a short trap (17 cm length) cooled by liquid nitrogen and connected directly to the injector. After the injection the cooled column section is rapidly heated to start with the analysis of the trapped compounds.

In this paper, the design and operation of a new head space autosampler is presented for analysis of the volatile aromatics compounds in natural products. The increase in sensitivity obtained through efficient on column cryofocussing and by using selective and sensitive detectors after chromatographic analysis is also showed.

The results are particularly valuable to establish a “fingerprint” of natural products for quality control analysis.

P5 - AUTOMATIC ON LINE SFE-HRGC ANALYSIS OF FLAVOURS AND PESTICIDES FROM SOME NATURAL PRODUCTS.

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Until recently, the use of supercritical fluid extraction (SFE) has generally been confined to relatively large-scale processes. The use of supercritical fluids for analytical extraction has recently received increased attention and a number of dedicated instruments are now available. In comparison with classical liquid-solid extraction methods, supercritical fluid extraction offers many potential advantages: faster and more efficient extractions, increased selectivity, easier coupling with on line analytical methods.

SFE can be used for preparative purposes only or combined on line with a separation technique as capillary GC or SFC for achieving in one run the sample extraction and the analysis of the extract.

The SFE analyser mod.300 is designed for multipurpose operations:

- on line SFE monitorisation,
- preparative purpose,
- analytical tasks by on line combination of SFE with capillary GC or SFC.

The capabilities of the instrument for on line analysis of flavours and pesticides in natural products is illustrated in the present paper. The SFE consists of an accurately controlled oven using air circulation where up to six extraction cartridges can be mounted. Sequential analysis of these six samples then take place completely automatically. The processes which influence the quantitative transfer of the extract into the analytical system are discussed and illustrated.

P6 - THE OLFACTORY PROPERTIES OF CARVONE AND FENCHONE ENANTIOMERS

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Carvone and fenchone enantiomers were subjected to a series of olfactory discrimination tests in humans and on mole rats. It was found that humans cannot discriminate between the enantiomers of fenchone. There was however a clear indication on the percentual differences between the two enantiomers of carvone. A different response towards the two carvones was also observed with the mole rats. As to the fenchones, the mole rats preferred the R (-) enantiomer. Optically pure (S)(+)-carvone and (R) (-)-carvone were isolated from the essential oils of the fruits of caraway and the leaves of spearmint, respectively (1).

Reference

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P7 - SUPERCRITICAL FLUID EXTRACTION OF ESSENTIAL OILS. THEORY AND APPLICATIONS

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A supercriical fluid is formed under conditions equal to or above its critical point. Under a supercritical conditions, a gas can be compressed to a density that approximates that of a liquid. This makes a supercritical fluid unique in that it possesses the chemical properties of its liquid state and the physical properties of its gaseous state.

The HP7680A SFE uses supercritical carbon dioxide, either alone or with modifiers, to prepare samples. Carbon dioxide in its supercritical state has very desirable properties for extraction:

- easy to create and maintain in its supercritical state;
- easy to remove due to its boiling point;
- easy to dispose of and non toxic.

The variablesolvent power of supercritical CO_2 permits removal of fractions of different polarity from the sample matrix. Due to automated choice of several different parameters which affect extraction conditions, users can work with a wide range of sample sizes, sample matrices, analyte concentrations and properties for flexibility in sample preparation.

In the field of essential oil, SFE has been successfully applied to accomplish the need to either analyze thermally labile compounds or handle complex matrices. The HP 7680A SFE offers a fast method for the extraction of substances from either fresh (wet) or dry plant materials and derivatives. Different specific application will be presented.

P8 - ENANTIOSELECTIVE ANALYSIS OF MONOTERPENE HYDROCARBONS FROM CONIFER NEEDLE OILS OF DIFFERENT ORIGIN

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Natural chiral flavour components seem to appear with characteristic enantiomeric relations which can be traced back to stereoselective, enzyme catalysed biosynthesis. The knowledge about the biological variation of the enantiomeric ratio permits stereodifferentiation to be used as a valuable analytical criteria to ensure the natural origin of essential oils. In the course of our studies of several needle oils, the enantiomeric compositions of monoterpene hydrocarbons in fir and pine needle oils were investigated. Fresh needles from the botanical garden of Hamburg and from Punkaharju in Finland were distilled for two hours according to Juvonen, who already stated in 1966 that about 90% of the total oil could be obtàined within the first 35 minutes. Besides this, for the determinàtion of the enantiomer ratio no quantitative isolation of the oil is necessary because isomerisation and racemisation can be neglected. A number of commercial needle oils were also investigated by means of GC on achiral and chiral stationary phases. Stereochemical analysis of α-pinene, ß-pinene and limonene was carried out on Heptakis-(6-O-methyl-2,3-di-O-pentyl)-ß-cyclodextrine and Octakis -(6-O-methyl-2,3-di-O-pentyl)-γ-cyclodextrine, both chiral stationary phases with reverse elution order, tested with optical pure reference substances (1). Relative amounts and enantiomeric excess (EE%) of all investigated compounds are given. We found that the enantiomeric proportion of (+)- and (-)-α-pinene varies considerably in both the natural oils and the commercial oils. (-)-ß pinene occurs in high enantiomeric purity; in most of the oils only traces of (+)-ß-pinene could be detected. Our results agree with the generally accepted hypothetical mechanism for the biosynthesis of the pinane skeleton (2).

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HRC (in press)
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Chemical Reviews, 1972, 72, (2), p.115-155

P9 - A RAPID METHOD FOR THE MONITORING OF ESSENTIAL OIL-LIKE EXTRACTS

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Steam distillation, headspace, microwave-assisted extraction, as well as a novel concentration process based upon microwave technology have been used to produce essential oil-like extracts from various plant materials. The new process is described in details in function of its simplicity and of its capacity to produce such extracts in extremely short periods of time. The qualitative composition of the extracts has been defined from gas chromatographic and mass selected data. It served to establish the degree of viability of these methods at being used as rapid monitoring and screening techniques to determine the pre-processing aromatic quality of fresh plant materials.

P10 - THE USE OF DIFFERENTLY DERIVATIZED CYCLODEXTRINS IN THE GC SEPARATION OF RACEMIC MIXTURES OF VOLATILES

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The behaviour of differently derivatized alpha-, beta- and gamma-cyclodextrins in the separation of racemic mixtures of volatiles are discussed. According to Schurig, columns were prepared by mixing the derivatized cyclodextrin with OV-1701 or OV-1701-OH terminated. About 130 racemates with widely differing structures were used to test the performances of 2,3,6 permethylated alpha-, beta- and gamma-cyclodextrins and 2,6-dimethyl-3-trifluoro-acetyl alpha-, beta- and gamma- cyclodextrins mixed with OV-1701 or OV-1701-OH terminated in different ratios. To evaluate column performances a text mixture containing 10 compounds with highly different structural characteristics is proposed. The influence of the different types of cyclodextrins in the separation of flavouring and perfumed racemates is also shown.

P11 - INFLUENCE OF DAY LENGHT ON MARJORAM OIL BIOSYNTHESIS

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The large diffusion area of wild marjoram and its cultivation at different latitudes from Egypt to Germany provokes to study the influence of photoperiodism on essential oil biosynthesis as it was done exemplarily with *Mentha piperita* by Holtzel (1964).
Three different day lenghts (10h, 13h, 16h) were used in a phytotron to grow some crossing descendants of marjoram. The plants were cultivated in Mitscherlich pots, temperature and air humidity were controlled. The following characteristics were examined: habitus, vegetative development, flower differentiation, essential oil composition.
At full blooming the sexual peculiarities and the oil composition of leaves and flowers from different nodes were determined using HT-HS solid sampling technique. The influence of day lenght on the growth and quality of marjoram is shown.

Cis · sabinene hyd + sabinene > 16h e 13h

linalyl acetate/cis - sab hyd acetate > 10h

Flowers têm + sabinene e lynaly acetal / cis - sabin
hydrate acetate q as folhas

P12 - NATURAL PLANT ANTIOXIDANTS FROM ESSENTIAL OILS : THEIR INFLUENCE UPON LIPID METABOLISM IN MAMMALS

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HungaryPlant essential oils have been known since antiquity to possess biological activities. Antibacterial and antifungal properties have been recognised in a number of different genera (Deans and Ritchie, 1987; Deans and Svoboda, 1991) while more recently, antioxidative activity has been the subject of study (Deans *et al.*, 1991).

Lipid metabolism is of vital importance at the two extremes of the life cycle: in neonatal and ageing animals. Many of the degenerative diseases associated with ageing have their origin in the lipid status of the animal. At the other extreme, inadequate lipid levels may be responsible for slow development in the young. Protection of the lipids, in particular the polyunsaturated fatty acids (PUFA's) and their intermediates is clearly desirable, and natural plant extracts have a role to play in this process of antioxidation.

Oil of thyme or oil of clove was fed three times per week in drinking water to a group of mice over a period of five months. After this time, the animals were sacrificed and the weights of several organs recorded. Samples were taken for lipid analysis, following weighing, from the brain, heart, liver, kidneys, spleen, testes, retina and serum.

Table 1 shows the phospholipid levels in the liver as a result of such feeding, illustrating the beneficial effect upon the arachidonic (20:4) and docosahexaenoic (22:6) acid profiles. Animals fed the oil of thyme have lipid levels equal or greater to that of the young animal. Studies are underway to determine the active constituent(s) in oil of thyme.

Table 1 Phospholipid profile in liver of mice fed oil of thyme or oil of clove over a five month period (% total lipid)

	Young	Old	Clove Oil	Thyme Oil
18:2	20.16±1.58	16.20±1.63	16.03±1.14	16.89±0.90
18:3	0.72±0.28	0.61±0.09	0.63±0.09	0.36±0.11
20:4	13.47±2.69	7.26±0.36	10.74±2.01	16.47±2.62
22:6	3.60±1.90	2.35±0.62	3.59±0.46	6.77±0.25
Total PUFA's	37.95	26.42	30.99	40.49

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P13 - ANTIMICROBIAL AND ANTIOXIDATIVE PROPERTIES OF THE VOLATILE (ESSENTIAL) OIL OF *ARTEMISIA AFRA*

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Artemisia afra Jacq. (family Compositae) is also known as African wormwood, wild wormwood or wild als. It is one of the most widely used plants in popular medicines in South Africa, mainly for coughs and colds, chills, stomach ache, dry dyspepsia and as a purgative (Palmer, 1985). The leaves are used in a tea, for a bath, as a leaf poultice or the vapours from boiling leaves can be inhaled. It has also been used as an insect repellant.

Wild als is a bushy, branched shrub growing up to >1 m in height, with feathery grey-green leaves (Buchbauer & Silbernagel, 1989). It grows easily, seeds itself readily and remains green all year round. It can also be propagated from cuttings and rooted pieces. The plant is indigenous to the mountainous regions extending from the south west Cape through to Abyssinia.

The main components of the volatile oil are alpha-thujone (52.5%), beta-thujone (13.1%), 1,8-cineole (13.0%), camphor (6.6%) with other monoterpenes and sesquiterpenes under 2% camphene, gamma-terpinene, para-cymene, alpha-terpinolene and alpha-pinene (Priprek *et al.*, 1982; Graven *et al.*, 1984). Volatile oil yield is between 0.3 to 1.4% (Buchbauer & Silbernagel, 1989). The volatile oil used in our study was obtained from Essentoil Industries (South Africa), and the main components were alpha- and beta-thujone (52%), 1,8-cineole (13%), camphor (15%) and alpha-pinene (2%). The antibacterial and antifungal properties of the essential oil of *A. afra* were determined by the method of Deans and Svoboda, 1991, using seeded agar plates with wells into which is placed the oil, and flasks of YES broth for mycelial growth of three filamentous fungi. The antioxidant effects were determined by the method described by Araujo and Pratt, 1985 and the oil was shown to be active. Of the 25 test bacteria, 15 showed some degree of inhibition of growth with *Aeromonos, Brevibacterium* and *Acinetobacter* most affected by the volatile oil from *A. afra.*

Table 1 Antifungal properties of volatile oil from *A. afra*.
Inhibition is expressed relative to the control flasks.

Test Organism	Inhibition Index (% relative to controls) Oil Concentration (µl ml^{-1} YES broth) 1	2	5	10
Aspergillus niger	12.0	24.0	87.0	98.0
Aspergillus ochraceus	63.0	90.0	99.0	99.0
Fusarium culmorum	10.0	80.0	92.0	92.0

P14 - IN VITRO PRODUCTION OF ESTRAGOLE IN *AGASTACHE FOENICULUM* O. Kuntze SHOOTS

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Agastache foeniculum O. Kuntze was cultivated in vitro in order to initiate a clonal selection of ecotypes and chemotypes.

The explants quickly adapted to the experimental conditions (temperature 24°C, 16 h of lighting), and the multiplication index resulted in 2-3 new shoots after 30 days in a medium of Murashige Skoog (MS) integrated with BA.

The radication rate (66-68%) was also satisfying in an MS medium integrated with IBA either alone or in combination with IAA.

The 2-3 cm shoots showed well-formed secretory structures on the leaf blade. Their average density, calculated on all the small leaves of the shoots, was 1 8.8/mm^2.

Tests on the patterns of production of estragole and of anethole were carried out on methanol extracts from shoots grown in vitro.

The quantity of estragole resulted in 1-1.5 mg/g d.w. in all the samples examined.

The analysis of methanol extracts of plantules rooted, aclimatized in mist and kept in a coldhouse, revealed a quantity of estragole to the order of 2.1 mg/g d.w..

The quantities of anethole, however, proved to be much lower: 0.0038-0.018 mg/g d.w. and 0.005 mg/g d.w. for the in vitro shoots and for the coldhouse plantules respectively.

P15 - PRESENCE OF α-PINENE IN PLANT CALLUS CULTURES OF *SMYRNIUM PERFOLIATUM* L.

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Callus cultures derived from oil-producing plants were assayed for ability both to synthesize and to accumulate mono- and sesqui terpenes. Callus of *Pinus radiata* Don, for example, accumulated α - and β-pinenes at levels comparable with those in the parent stem and needles.
Studies done in callus cultures derived from Umbelliferae family species failed in the detection of terpenes normally synthesized in the parent plants, even if the prenyltransferase and the isomerizing system have activities many times greater than those extracted from the mature parent plants (BANTHORPE, D.V. et al. Phytochemistry 25: 629-636 (1986); MIURA, Y. et al. Planta Medica 53: 95-96 (1987)). In the above mentioned experiments the callus cultures was extracted with Et_2O and the extracts subjected to GLC. In this first approach we carried out the research of the terpenes in callus cultures of *Smyrnium perfoliatum* L. using head space analysis; in this analysis it is possible to concentrate the volatile substances in the vapour phase with moderate heating (40°C). Floral axis of *Smyrnium* was cultured on MS agar medium containing $5 \, . \, 10^{-7}$ M kinetin.
The cross sections of the zone between callus and parent floral axis showed a neoformated tissue that recovered all the transverse section; this tissue closed the artificially cut secretory duct as well the vascular tissue. Above this layer, a few secretory structures begin to differentiate: a group of callus cells begin to differentiate and to separate in three adiacent groups of secretory cells. In the top layer of callus the secretory structures appear to be well-shaped surrounded by the secretory cell ducts.
Each explant was dissected in the callus and the parent floral axis and both were subjected to H.S.A. α-pinene was the main terpene present in the vapour phase and this is on an average 1.5 times higher in the callus than in the parent floral axis vapour phase. The presence of α-pinene in the species of Umbelliferae family derived callus cultures has not been reported previously in the literature. Moreover, these results indicate that the H.S.A. is a powerful method for the qualitative determination of terpenes produced in callus cultures. Besides, these first data show that the composition in the vapour phase of terpenes produced in the callus cultures is somewhat different from that of above the essential oil vapour phase that was hydrodistilled from stems of parent plants even if the α-pinene is one of the higher components of this oil.

P16 - BIOTECHNOLOGICAL PRODUCTION OF LACTONES

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Lactones are widely distributed in nature, and they have been isolated from all the major classes of food systems including fruits, vegetables, nuts, milk products or meat. They are associated with aromas described as "fruity", "nut-like", "coconut-like", "sweet" or "buttery". Organoleptically important lactones are mainly γ- and δ-lactones and macrocyclic compounds. Due to their flavour properties, lactones are widely used by the flavouring industry.

The use of microorganisms for production of lactones has its origin almost 30 years ago. Since then a number of processes for a biotechnological generation of lactones have been introduced including microbiological and enzymatic methods as well. Most of these approaches are bioconversions of suitable precursors.

In 1972, Collins and Halim (1) described the isolation and identification of 6-pentyl-α-pyrone from cultures of the soil fungus *Trichoderma viride* (Deuteromycotina). This compound with a strong coconut-like aroma also possesses interesting antifungal properties. Selection of suitable strains and optimization of the culture medium led to a laboratory process with yields in the g/l-range (2). Further attempts for an economically competitive production of 6-pentyl-α-pyrone and other lactones are discussed.

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P17 - SECRETORY TISSUE AND ESSENTIAL OIL IN *BUPLEURUM FRUTICOSUM* L.

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Bupleurum fruticosum L. (Umbelliferae) is an evergreen shrub which lives spontaneously in Sardegna, Sicilia, Liguria and Puglia (PIGNATTI, S. Flora d'Italia Vol.2°: 217 (1982)).
The hydrodistillation of stems yields an essential oil which has a very different composition from that of the hydrodistilled from leaf oil. The former contains ß-phellandrene (45,60%), myrcene (11,41%), ß-pinene (11,25%) and many other lower than 10% compounds; the later contains sabinene (35,78%), ß-phellandrene (34,85%) and many other lower than 10% compounds. Myrcene is lacking in the stem oil while the ß-pinene amounts to 0,38% (PEYRON, L. and ROUBAUD, M.. Plante médicinales et phyto . 4: 172 - 175 (1970))
The aim of our work was to investigate if the different composition between the stem oil and the leaf oil was related to anatomical differences in the secretory tissue of these two plant organs.
The transversal sections of living plants were stained with Safranine or Methyl violet or Sudan III stains.
The internodal secretory tissue was formed of 28 forming cortical ring ducts with an irregular section; in the nodal area, three secretory ducts accompany the vascular bundle (three bundles); in the petiole, the ducts were reduced to two or one and this configuration was maintained in the leaf. In the mesophyll there are other secretory ducts non related with the vascular bundle ones.
It is evident that the secretory ducts constitute a single network which has a very high concentration in the stem.
The difference in the composition between the leaf and the stem oil seems to have a different activity to the surrounding ducts of the secretory cells. Indeed, it seems very unlikely that a difference in the evaporation of the most volatile compounds from the stem and the leaf may determine the difference found in the composition between these two essential oils.

P18 - INCORPORATION OF RADIOACTIVE MEVALONE ACID LACTONE INTO ESSENTIAL OIL OF CAMOMILE CULTURES ORGANIZED

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Since the formation of metabolites, especially that of volatile oils, depends on the intact plants of a defined specialization of the cells and tissues, it seemed advisable to study besides the callus- and suspension-cultures the volatile oil synthesizing capacity of organized and isolated organs cultures too.
In the course of chemical screening test we analysed more profoundly the components of the therapeutically most important volatile oils by gas-chromatographical and spectroscópical methods (IR, mass-). To the investigation of biosynthesis isotopic technics were used. The components of the volatile oils of roots and herbs incubated with radioactive mevalon acid were separated from one another by preparative gas-chromatography and their activity was measured by the scintillation method. The root and herb of the camomile that we have got organized synthetize in a conspicuous large quantity of components which are to be found in the root of the intact plant too, as for instance trans-ß -farnesene, α-farnesene, spatulenol, bisabolol oxide II and besides cis- and trans-spiroethers; two new sesquiterpene components (MW 222 and MW 238). On the basis of isotopic tests it may be supposed that one of the new sesquiterpenes (MW 238, $C_{15}H_{25}0_2$) is an important precursor or an intermediate of the biosynthesis of volatile oil.

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P19 -ANTIHISTAMINERGIC EFFICACY OF CALAMUS OILS WITH DIFFERENT AMOUNT OF ß-ASARON

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Acorus calamus L. has traditionally been used in man because of its well-known beneficial effects on functional disorders of the gastrointestinal tract. Therefore, the essential oil preparation of the drug is used in appetizers, bitters, spasmolytics and antiulcer preparations. The essential oil contains variable amounts of ß-asaron or cis-isoasaron, a phenylpropan-derivative in relation to the genetics of the plants used for oil preparation (1). Moreover, it has been shown that ß-asaron is responsible for cancer induction in the duodenum of rats after chronic application of calamus oil (2). We were interested to know whether calamus oils are effective spasmolytics against histamine in the isolated guinea-pig ileum preparation and if ß-asaron is responsible for this putative therapeutic effect. After elucidation of concentration dependant spasms following addition of histamine to the isolated and equilibrated guinea-pig ileum we repeated the provocation of spasms in another set of experiments, where the organs had been pretreated with concentrations of the following types of calamus oil: a) with nearly no, b) with low (10%) and c) with high (95%) content of β-asaron. The standard antihistaminic drug pyril-amine maleate was used as a reference (10^{-5} M). Our experiments showed that all three types of calamus oil were able to antagonize the histaminergic spasms in concentrations of 10, 32 and 100 µg respectively. The calamus oil preparations with nearly no or with low content of ß-asaron were similar in their antihistaminergic activity in comparison to the above mentioned concentration of pyrilamine maleate and clearly exceeded the efficacy of the ß-asaron-rich calamus oil preparation. These findings as well as the reasons of drug safety speak for the future use of rhizomas of ß-asaron-free diploid or tetraploid *Acorus calamus* for the production of therapeutics from this drug.

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P20 - OCCURRENCE OF SOME VOLATILE CONSTITUENTS FROM CHITOSAN CONTAINING CULTURES OF *INONOTUS OBLIQUUS*

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Chitosan, the deacetylated derivative of chitin (a polymer of N-acetylglucosamine), is a constituent of fungal cell walls. It has been reported to reduce the growth of numerous fungi (1), and to be an elicitor on phenylpropanoid metabolism (2). In the white-rot fungus *Inonotus obliquus* (Pers.ex Fr.) Pilat., the lanostane-type triterpenes have been shown to have antitumour activity (3). Some volatile sesquiterpenes may also have such properties (4). Our study investigated the effects of chitosan on the volatile oil composition of *I. obliquus* cultures.

The strain of fungus was isolated from sporophores grown in rowan (*Sorbus aucuparia* L.), and the mycelia were cultivated on malt agar or on a liquid culture medium for 35-45 days. The chitosan concentration ranged from 50 to 2250 mg/l in the cultures. The volatile oil was isolated by hydrodistillation, and the constituents were identified by GC and GC-MS.

The composition of hydrodistilled oils from malt agar and liquid cultures was rather similar, whereas at chitosane concentrations clear differences were noted. The highest amount (2250 mg/l) obviously inhibited the formation of votatiles, since n-decane was the only major constituent followed by traces of monoterpene hydrocarbons. At a chitosane concentration of 1250 mg/l, the oil was characterized by thymol and *ar*-turmerone. The major identified constituents of the hydrodistilled oils obtained at a chitosane level of 250 mg/l included *ar*- turmerone, thymol, bisabololoxide-B, nerolidol and ß-caryophyllene. The occurrence of antitumour active *ar*-turmerone (4) in fungal culture is interesting, since it could not be identified in natural fungus *I. obliquus.*

These findings also suggest that chitosan is able to stimulate the formation of aromatic volatiles in this fungus.

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P21 - ON THE EXTRACTION OF *SALVIA SCLAREA* L.

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The extraction of clary sage (*Salvia sclarea* L.) in a closed periodical process is studied. The results about the influence of the factors: the continuance and the number of the extraction, the temperature of the process, the enflorescence development of the plant upon the yield and the composition of the concret are described in the report.

It was established that the enflorescence development influenced the content of the extractable substances more than the quantity of the main component sclareol.

The basic amount of concret (more than 70 %) was obtained during the first extraction. A considerable part of the remaining substances in the plant is yielded by means of a short second extraction.

The extraction of clary sage should be repeated 2 or 3 times. The quantity of sclareol and lynalylacetat in the concret decreased and paraffins increased when the process was repeated.

The temperature of the solvent was not a decisive factor for the yielding of concret and reducing the continuance of the process.

P22 - ESSENTIAL OIL OF *SALVIA* SPP.

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We report the essential oil composition of five Salvia species:
l) *S. canariensis* L., 2) *S. confertiflora* Pohl., 3) *S.* cfr. *mexicana* L., 4) *S. microphylla* H.B.K., 5) *S. somaliensis* Vatke.
Using HRGC and HRGC/EIMS more than hundred constituents were found;
among these:
Monoterpene hydrocarbons (MH): pinenes (α and β), camphene, Δ–3-carene and limonene
oxygenated monoterpenes (OM): 1,8-cineole, camphor, borneol, bornyl acetate
sesquiterpene hydrocarbons (SH): β-cariophyllene, γ -muurolene, germacrene B, α -copaene
oxygenated sesquiterpenes (OS): globulol, guaiol, spatulenol, α and β-eudesmol
are the main according to table

	SALVIA				
	1	2	3	4	5
E.O. yield % (V/W)	5.1	0.7	2.8	3.9	2.8
MH	31.24	24.53	20.96	16.46	45.04
OM	29.41	3.41	10.78	23.46	30.81
SH	17.11	56.30	16.15	22.98	11.10
OS	17.08	6.81	40.05	22.83	6.32
Others	0.68	0.25	0.13	0.69	0.18
Unidentified	4.48	8.70	11.93	13.58	6.55

Previous works are known on the E.O. of *S. canariensis* (1) and almost the whole of results has been confirmed. No previous works are known on the other four species of Salvia.

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Essential oil of *Salvia canariensis* leaves, Int. Symp. on Biology and Chemistry of Active Natural Substances, Bonn (F.R.G.), 1990

P23 - CHARACTERIZATION OF SAGE DESOLEANA, A NEW SAGE GENUS (*Salvia desoleana* - Atzei et Picci)

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A new Sage genus, Desoleana Sage, growing in Sardinia was identified and studied in botanic-chemical terms.
The essential oils were analyzed by capillary HRGC MS—FTIR.
The main difference between Sardinian Desoleana Sage and Clary Sage grown in different countries is the presence of noticeable amounts of alfa-Terpineol and alfa-Terpenil Acetate. The odour of these organoleptically powerful chemicals is probably responsible for the characteristic floral and sweet note associated with Desoleana Sage.

P24 - CHEMICAL COMPOSITION OF *SALVIA SCLAREA* ESSENTIAL OIL CULTIVATED IN PORTUGAL

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Salvia sclarea L. is a shrub belonging to the family of *Lamiaceae* from Mediterranean origin.

It is about 70cm high, though it reaches 1m if cultivated, with erect stems, ovate hairy trimmed leaves. It has mallow-lilac colour flowers with strong aromatic odour. It is cultivated in rocky and light soils and in some areas it is possible to harvest twice a year (July and September).

The preferential region for its habitat is the Italian alpine zone and the south of Central Europe.

The most common utilization of the essential oil of *Salvia sclarea* is for the alcoholic beverage industry (vermouth, aromatization of muscatel wine), perfumery and pharmaceutical industries.

The aromatic products obtained from *Salvia sclarea* are essential oil, concrete and absolute. In the beginning of our work we are studying the chemical composition of the essential oil obtained by steam distillation of plants grown in experimental fields.

The distillation was carried out from the whole plant. The obtained oil has a very light yellow colour and a typical animal odour similar to ambergris and muscatel wine.

The essential oil from plants acclimated in Portugal differs from similar oils mainly in the linalool content. The percentage of sclareol and linalyl acetate is according to other studies about oils of other origins.

Just the plants from one region of the country (Centre of Portugal) have been studied. It will be continued in experimental fields with different climate and soil conditions in order to establish a quality standard of *Salvia sclarea* of portuguese origin.

P25 - ESSENTIAL OILS AND GLYCOSIDICALLY BOUND VOLATILES OF SOME *THYMUS* SPECIES - A COMPARISON

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As part of our continous work describing the role of glycosidically bound volatiles in plants we are analyzing the composition of the glycosidic fractions from different *Thymus* species. Simultaneously the essential oils obtained by hydrodistillation are analized by GC and GC-MS. The aim of the present study is to find out whether in *Thymus* species characteristic oil constituents can generally be found as glycosides in the glycosidic fractions as previously described for *T. vulgaris* (1).

T. x *citriodorus*, a lemon smelling thyme, and the northern *T. praecox* ssp. *arcticus* have been chosen for our experiments. The hydrodistilled essential oil of the first has not been studied up to now. The only reference to lemon thyme deals with headspace analysis (2).

For analyzing the glycosidic fractions won by extraction with ethyl acetate two different methods were used. Besides the classical way (hydrolysis, GC of the aglycones), a second more simple method was applied separating the glycosides after silylating by means of GC and GC-MS. The latter additionally gives the chance to differentiate between alcoholic (intensity m/z 204 > m/z 217) and phenolic (intensity m/z 217 > m/z 204) glycosides. Comparising the glycosidic composition with the essential oils, the two *Thymus* species showed different characters. In *T.* x *citrodorus* whose main compound in the oil was geraniol, a high content of geranyl glycoside was found. In contrast, *T. praecox* ssp. *arcticus* containing mainly linalyl acetate in the oil (3) has no remarkable amount of linalyl glycoside in the glycosidic fractions.

Detailed results from both methods and the composition of the essential oil of *T.* x *citriodorus* will be presented.

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P26 - ESSENTIAL OILS OF ENDEMIC *THYMUS* L. FROM PORTUGAL

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There are four endemic "taxa" of *Thymus* L. from Portugal: *Th. camphoratus* Hoffmanns. & Link, *Th. capitellatus* Hoffmanns. & Link, *Th. lotocephalus* G. López & R. Morales and *Th. villosus* L. subsp. *villosus.*
Those plants are not well studied and some of them are uncommon. As a contribution for their better knowledge and their conservation, taxonomic features and chemical composition of their essential oils are given.

T. capitellatus ≃ camporathus
↳ Chemotypes
| cineol
| camphene, cineol, borneol
| linalol / linalyl acetate

T. lotocephallus > cineol, camphor, linalol
linalyl acetat, α pinene

P27 - CHEMICAL POLYMORPHISM OF THE ESSENTIAL OIL OF *THYMUS MORODERI*.

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The composition of the essential oil of *Thymus moroderi* Pau ex Martinez (Lamiaceae), an Iberian Southeastern endemism belonging to the Section *Pseudothymbra* Bentham, has been previously investigated by us [1]. Preliminar analysis of its variability carried out in our Laboratory with a limited number of individual plants showed some differences between them, mainly in the sesquiterpene fraction.

In order to verify this variability, we have now studied the essential oils from ca. 80 plants of *T. moroderi* of two different localities.

Aerial parts were collected at the flowering stage in Murcia (sample A) and Alicante (sample B), Spain, in May 1987. Voucher specimens were deposited in the BCF Herbarium (Faculty of Pharmacy, University of Barcelona).

Qualitative and quantitative analysis of the volatile oils of the samples A and B were carried out by GC-FID an GC-MS as previously described [1]. The major components were shown to be 1,8-cineole, camphor, camphene, and borneol.

To study the infraspecific variability, ca. 40 individual plants were also collected in each place. The oil of each one obtained by hydrodistillation was analyzed by GC-FID and when necessary by GC-MS. The results obtained were submitted to a chemometric Cluster Analysis and a Principal Component Analysis as reported in [2].

The application of multivariate analysis techniques let us establish two groups of essential oils:

a) those having an unidentified oxygenated sesquiterpene, obtained from the plants collected in Murcia

b) those having α-eudesmol, ledol, α-elemol, and β-elemol, coming from plants collected in Alicante.

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P28 - ESSENTIAL OILS OF *THYMUS CARIENSIS* AND *THYMUS HAUSSKNECHTII*, TWO ENDEMIC SPECIES IN TURKEY

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As part of our continuing systematic phytochemical studies of the Turkish medicinal and aromatic plants, the constituents of the essential oils from two Labiatae plants (*Thymus cariensis* Hub.-Mor.et Jalas, *Thymus haussknechtii* Velen) were characterized by means GC and GC-MS. The two plants are used in folk medicine to treat bronchitis, asthma and colic in the local regions where they grow. Essential oils contained mono and sesquiterpenoid derivatives and also phenolic compounds in variable amounts. The significance of the compositional profile of each volatile oil shall be briefly discussed.

P29 - *TEUCRIUM ARDUINI* L. - ESSENTIAL OIL CONTENT AND COMPOSITION

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Content and composition of the essential oil of two samples of Yugoslav endemic plant *Teucrium arduini* L. was determined. Essential oil was extracted by hydrodistillation according to Ph. Jug. IV. Amount of essential oil was determined gravimetrically over in pentane and after that analyzed by gas chromatography. The oil content of shoots /predominantly young leaves/was 0.18% and 0.07%/v/w/ for samples of elder apical parts /leaves, calyces, fruits/.
The main component in the oil is allo-aromadendrene. Samples of shoots contained 57.8% and 23.4 % elder apical parts of plant of allo-aromadendrene. The second component in the both of the samples is caryophyllene /13.5% and 17.3%. In the samples were determined twenty three components of essential oil.

P30 - 3,7-DIMETHYLOCTAN-2-ONE, A NEW MONOTERPENE FROM *TEUCRIUM MASSILIENSE* L. (LABIATAE).

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Recent researches have evidenced the high interest of the genus *Teucrium*, both from the taxonomical and phytochemical point of view[1]. Owing to their active metabolites, many species are currently used in the folk medicine of many countries. Furthermore, an interesting "insect antifeedant" activity of some diterpenes was discovered[2] in some *Teucrium* species.
From a phytochemical point of view, the presence of flavonoids[3] and norclerodane diterpenoids[4] is well known, while the volatile compounds have been less studied.
In the present work, data concerning the essential oil composition of *Teucrium massiliense* (sect. *Scorodonia*) are reported. In parallel the micromorphology of the trichomes (both secretory and not) is also studied by SEM observations.
Plant specimens have been collected in Sardinia. Individual compounds were identified by GLC analysis and GC-MS spectroscopy.
The kind of trichomes found on *T. massiliense* is the same as already described in many Labiatae. The trichomes distribution in the different plant parts is very peculiar, expecially on the calyces, and is very interesting from a taxonomical point of view.
The essential oils have been separately examined for leaves, calyces and corollas to relate their compositions to the different secretory trichomes. They are characterized by the presence (up to a 5% expecially in leaves and calyces) of various aliphatic esters, such as isobutyl 2-methylbutyrate and 2-methylbutyl 2-methylbutyrate, which so far have not been found in other species of *Teucrium* of the same section. A very important feature is the presence of 3,7-dimethyloctan-2-one, a new acyclic monoterpene with an anomalous oxidation pattern, up to 20% in the leaves and calyces, together with minor amounts of the corresponding alcohol. This new product is less abundant in corollas, which, on the contrary are characterized by a greater content of normal acyclic monoterpenes (citronellol, nerol, geraniol and their esters). The new product has been isolated by preparative GLC [SE 30 5% on Chromosorb W 30-60 mesh, T_{Col} 140 °C, N_2 carrier gas flow 60 cc/min]. Its structure has been determined by MS and ^{1}HNMR spectroscopy

and confirmed by synthesis from citronellal[5] The sesquiterpene fraction show no sensible variation among the examined plant parts, and is characterized by both cyclic (e.g.: α-curcumene, ß-cubebene and bergamotene) and acyclic compounds (e.g. E- and Z-farnesene).

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P31 - THE ESSENTIAL OILS OF THREE SUBSPECIES OF *TEUCRIUM CHAMAEDRYS* FROM TURKEY

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The genus *Teucrium* comprises 349 species of which 27 species are found in Turkey (1,2) and used as a folk remedy. The essential oils of the genus *Teucrium* have been scarcely studied.

In this study we investigated the essential oils obtained from three subspecies of *Teucrium chamaedrys*. We collected flowering parts of T. *chamaedrys* L. ssp.*chamaedrys*, *T.chamaedrys* L. ssp. *tauricolum* Rech. fil., and *T.chamaedrys* L. ssp. *syspirense* (C.Koch) Rech.fil.from southern Turkey. The plant materials were subjected to hydrodistillation for 3 hours in a Clevenger apparatus, which yielded 0.19-0,43 % of essential oil. The oils were analyzed by GC/MS using 60m fused silica capillary columns, 44 components were detected.

The main components of the oils were caryophyllene (18.03-26.88 %), humulene(8.98-13.77 %) and caryophyllene oxide (5.90-13.60 %). But the oils have some differences in composition.

Further analytical data of the samples studied will be presented.

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P32 - IDENTIFICATION OF VOLATILES ISOLATED FROM *TAMBOURISSA LEPTOPHYLLA* SKIN FRUIT

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Tambourissa leptophylla (Tul.) A. DC. is a 80-30 ft. high tree growing in the mountain-woods of the Madagascar area. The local population uses the decoction of the leaves internally as an aborting agent and externally against dermatites. The pulp and the powdered skin of the fruits are also used externally as an antimicrobial agent and for healing wounds (1). In the context of a research project for identifyng the compounds responsible for the latter two activities of the fruits, we began a study of the components present in the skin which has a characteristic, pleasant smell. The methanolic extract of the skin of the fruits gathered by us during an expedition to island of Grande Comore, was fractionated by adsorbtion chromatography into different fractions. Each fraction was then analysed by GC-MS -DS..
So far n° 82 compounds have been identified, the main ones were:
α-pinene, δ-3-carene, p-cymene, limonene, α-cubebene, trans -α-bergamotene, α-curcumene, M.W. 204, geranyl acetate, α-bisabolol, ß-bisabolol
The use of GC-MS, coupled with a well stocked data-bank, was seen to be useful as a first approach to the identification of the volatile components of the *Tambourissa leptophylla* fruit skin which are likely to be responsible for the antimicrobial activity.

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P33 - CHEMOTYPIC VARIATION OF VOLATILE OILS IN THE GENUS *THAPSIA*

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The genus *Thapsia* (Apiaceae, Laserpitiae) includes perennial herbs widely distributed in the Mediterranean area and on the Iberian peninsula.
T. garganica has been used in folk medicine against rheumatic pains and some highly active irritants, called thapsigargines, have been isolated from this plant as well as from other *Thapsia* species.
According to Flora Europaea, the genus is divided into three species, *T. garganica* L., *T. maxima* Mill. and *T. villosa* L.
Flora of Portugal includes also *T. transtagana* Brot. which according to F. Europaea is synonimus of *T. garganica*. Other two species have been also recognized, *T. laciniata* Rouy. and *T. minor* Hoffg. & Link.
Screening of root extracts has revealed a marked inter - as well as infraspecific chemical heterogeneity within the genus *Thapsia*. Most of the differences are correlated with morphological and kariotypic diversity.
With the aim to obtain a broader insight in the chemotaxonomy of the genus we have investigated the variation in fruit volatiles from *Thapsia* plants with different chromosome numbers.
The typical components of the essential oils fall into two chemical classes: monoterpenoids and phenylpropanoids. According to their relative occurrence different chemotypes can be recognized. The influence of the ploidy of the investigated specimens on the mechanism of formation of the essential oils in these plants is also discussed.

P34 - COMPARATIVE STUDY ON PRODUCTIVITY AND ESSENTIAL OILS QUALITY OF CLONES OF *SALVIA OFFICINALIS* L. CULTIVATED IN DIFFERENT ITALIAN ZONES (+)

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Starting with genetic material coming from previous investigations, several clonal fields were constituted in 1988 in three different Italian zones, with the aims of individuating clones with the best productivity characteristics and quality of the essential oils. Twelve clones were choosen, and identified as FI1, FI4, FI7, FI10,FI12,FI20,TN58,TN75,TN132,TN191,TN600,TNB**. The plants needed for the formation of the three clonal fields were constituted in Trento; during 1989 the plants were displaced in these selected zones: Villazzano (Trento), Antella (Firenze) and Policoro (Bari). In 1989 a single harvest in all of the three localities, in 1990 three harvests in Policoro, two in Villazzano and one in Antella were made. The results can be so summarized: - the percentage of alive plants for each clone at the beginning of the third year was different in the three zones: FI12, TN58 and TN600 demonstrated to be more proof in the north, while TN191, TN75 and TNB** in the south;

- production of both fresh and dried vegetable matter in Policoro was higher than that of Villazzano and Antella. FI12 had the highest production in each of the three goegraphic zones, followed by TN191, FI10 and TN75.
- the clones cultivated in Bari had the highest content in essential oil. Distillation of TN75 and TN191 gave the maximum yelds; remarkable differences in composition of the essential oils were pointed out among the same clone in different harvests: the first harvest in Trento and Bari, together with the only one made in Firenze, was rich in terpenes, eucalyptol and sesquiterpenes, instead of thujones. Oils coming from the second harvest in Trento and those coming from the second and third in Bari were very similar; TN75 and TN600 revealed the highest content in α thujone, while TN75 had an highest content in the ß-isomer and was much rich in camphor. TN191, FI1 and FI4 were characterized by an appreciable content in thujones and a good balancing of the composition. Preliminar analysis of these data indicate TN191 and TN75 to be the most promising clones. Further examination will be developed in the next years.

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P35 - PHENOTYPIC PLASTICITY IN *MENTHA VIRIDIS LAVANDULIODORA.*

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Plasticity is shown by a genotype when the expression of its individual characteristics is changed by environmental influences. Since all changes in the characters of an organism which are not genetic are environmental, plasticity is applicable to all intragenotypic variability (BRADSHAW A.D., Adv. Genet. 18, 115, 1965). The changing terpene composition from season to season is clearly a plastic phenomenon which has been thoroughly studied in many genera including the genus Mentha (MAFFEI M., Flav. Fragr. J., 3, 79,1988; MAFFEI M., Biochem. Syst. Ecol., 18, 493,1990).

Phenotypic plasticity, genetic variability and genetic variation for phenotypic plasticity were studied in F_1 hybrids coming from a seed population of *Mentha viridis* L. *lavanduliodora* Sacco. The plants were steam distilled in full bloom during two growing seasons: 1989 and 1990. The oils were analyzed by GC-MS and: limonene, 1,8 cineole, linalool, linalyl acetate, ß caryophyllene, α terpineol and germacrene D relative percentage values were used for the statistic analyses. The 1,8 cineole/limonene (R_1) and linalool/linalyl acetate (R_2) ratios were also calculated and statistically processed.

1) Two-Way ANOVA, 2) Cluster Analysys (CA) and 3) Principal Component Analysis (PCA) were used to evaluate: 1) genetic variation, phenotypic plasticity and genetic variation for phenotypic plasticity; 2) statistical linkage among plants in the same year and in pooled data; 3) pattern and structure of terpenes and partition among plants.

The results from the Two-Way ANOVA indicated high levels of genetic variation for linalool, followed by limonene, ß caryophyllene, α terpineol and R2. On the other hand high degrees of phenotypic plasticity were shown by linalyl acetate, R_1, and germacrene D, followed by 1,8 cineole. The greater variation among the genotypes in relation to environmental changes was found for R_1 and linalool. CA and PCA (Fig.1) discriminated plants having high linalool percentages from those having high levels of both linalool and R_1.

Discrimination was also found between plants with high linalyl acetate content lacking or not germacrene D. The factor distance calculated on the PCA indicated which plant was more or less plastic. The results of this study indicated that linalool is strictly dependent on the genotype, while linalyl acetate is much more affected by environmental factors showing higher levels of phenotypic plasticity. In the same way R_1 is much more plastic than R_2 and for this reason it should be used for checking stable plants.

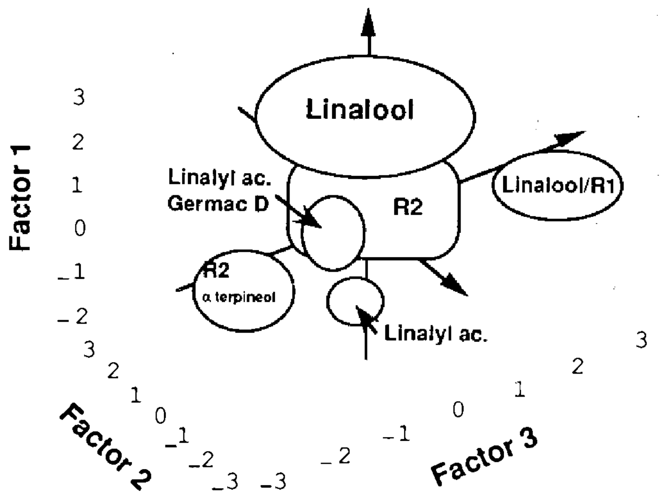

Figure 1

The evaluation of phenotypic plasticity can be considered a useful and important tool for selecting plants producing essential oils.

P36 - DISTRIBUTION AND SEASONAL VARIATION OF GLYCOSIDICALLY BOUND VOLATILES IN PEPPERMINT (*MENTHA PIPERITA* L.)

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Mentha piperita L. cv. Black Mitcham was the subject of Croteau's investigations into the function of glycosidically bound monoterpenes in plants. He found that the bulk of labelled 1-menthone used as an exogenous precursor was converted to d-neomenthyl-ß-D-glucoside in leaf discs obtained from plants grown under controlled lab conditions (1). Further tracer experiments indicated that metabolic turnover of foliar terpenes represents a mechanism for recycling carbon and energy (2).
In this paper we present for the first time the complete pattern of the glycosidically bound volatiles in outdoor *Mentha piperita* L. cv. Black Mitcham in comparison to the essential oil. The glycosidic content amounted to 0.003% in the fresh plants, the aglycone fraction consisting mainly of oct-1-en-3-ol, 1-menthol, and octan-3-ol. d-Neomethol which, according to (1), was expected to be a main component only occurred in portions up to a maximum of 4% in that fraction. The content and the composition of the glycosidically bound constituents as well as their distribution within the plant and their seasonal variation lead us to the conclusion that one cannot strictly transfer Croteau's results to outdoor plants. Therefore the role of those compounds in the plant must be reflected on again.

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P37 - ONTOGENETIC VARIATION OF THE ESSENTIAL LEAF OIL OF *MELISSA OFFICINALIS* L.

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Melissa officinalis L., Lamiaceae is an old medicinal plant, which is used due to its essential leaf oil. Therefore, several studies deal with the investigation of the volatiles gained from this plant (1,2).
A comparison of these studies reveals remarkable differences concerning the composition of the essential oil, esp. the main components citronellal and citral. Several reasons for the variation of the constituents, i.e. origin, date of harvest, storage conditions and the age of the plant material have been discussed (3), but information on the ontogenetic variability of the essential oil of balm is scarce and not very detailed (4). In order to check, whether variations whithin one plant can be responsible for the reported variability we analyzed its verticils separately in regard to 51 identified volatile compounds. The results show drastic quantitative changes between the essential oil of leaves from the basal region and those from the top. This is especially pronounced for the monoterpene aldehydes citronellal and citral, the monoterpene esters methylcitronellate and geranylacetate and the amount of sesquiterpenes (Table 1).

Table 1: Investigated verticils of one plant

	basal (3rd)	middle (6th)	top (11th)
Citronellal	52.4	25.7	1.1
Citral	0.5	10.3	37.2
Sesquiterpenes	10.0	46.0	39.0
Methylcitronellate	17.9	1.9	0.0
Geranylacetate	0.0	0.0	10.2

From the top (young) to the basal (old) region of the plant citral is decreasing whereas the amount of citronellal increases in the same direction. The sesquiterpene content has a maximum in the middle verticils of the shoot. Geranylacetate decreases sharply within the 3 top verticils from 10.2 to 0%. In a roughly opposite manner methylcitronellate increases in the basal region.
The question arose, whether the specific oil composition of each verticil is defined by its position on the stem or whether it is depending on its age.

The analysis of comparable verticils from sister plants during a six week interval proved that young and old leaves differ in the same manner as observed for top and basal region.
To our knowledge it is still in discussion whether changes in oil composition of Lamiaceae leaves are based on the development of new or a change of the content of already existing glands. By analyzing individually mapped glands from leaf tips of a defined age we proved dramatic changes of the oil composition of corresponding glands e.g. from 0 to 71% citronellal within two months.

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P38 - COMPARATIVE STUDIES ON THE COMPOSITION OF THE ESSENTIAL OIL OF COMMERCIAL SAMPLES FROM THE CRUDE DRUG "PETROSELINI RADIX"

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The crude drug "Petroselini Radix" is obtained from the turnip-rooted form of parsley (*Petroselinum crispum* (Mill.) A.W.Hill, Apiaceae), a biennial plant which is cultivated throughout the world in countries with a temperate climate. Besides the common use as a condiment the crude drug is employed in medicine primarily as diuretics and stomachics. This effect can be mainly attributed to the essential oil, but it is strongly dependent on oil composition.

We have therefore analyzed the essential oils obtained by hydrodistillation from 15 commercial samples of the crude drug "Petroselini Radix" by means of chromatographic and spectroscopic methods. Besides more or less high amounts of ubiquiteous monoterpene hydrocarbons, characteristic constituents of all these oils are the phenylpropanoids apiole and myristicin, the different content of which allows to classify three different types of crude drugs. In the first one apiole is dominating (82 - 88% of the total amount of phenylpropanoids) and the content of myristicin is lower than 18%. The second type yielded an essential oil with high percentual portions of myristicin (72 - 99%) while the apiole content was very low (0.1 - 5 .2 %). As a third group samples containing remarkable amounts of apiole (15 - 24%) and in addition high percentages of myristicin (52 - 75%) may be considered. Additionally, in a few of the investigated samples higher amounts of elimicin and tetramethoxyallylbenzene were found.

A considerable range of variations could be observed concerning the content of single monoterpene hydrocarbons. Thus, we investigated the influence of drying on the oil composition. As a result a significant increase of the phenylpropanoids at the expense of the more volatile monoterpenes is registrated; however, the relative proportions of the individual members within the group of the phenylpropanoids are scarcely affected.

P39 - ANALYSIS OF THE FRUIT ESSENTIAL OIL OF *XYLOPIA AETHIOPICA* (ANNONACEAE) AND THE INFLUENCE OF DIFFERENT ISOLATION PROCEDURES ON ITS COMPOSITION

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Xylopia aethiopica (Dun) A. Rich. - synonyms: *Annona aethiopica* Dun. and *X. eminii Chev.* -, Annonaceae, is referred to as Ethiopian, Guinea or Negro pepper, spice tree, or as African cubeb. Its fruit is fragrant and spicy, and possesses a very strong and persistent odor, like that of cinnamon. The fruits are used for flavouring food; they give it a mildly hot, and spicy note.

Essential oil yields of *X. aethiopica* were reported as 2% from whole fruits, 6% from fruit hulls and 1.3% from seeds, and as 3.9%, 4.7% and 0.4%, for the respective parts of the same species. The composition of essential oil samples of *X. aethiopica* from various geographical origins, which were isolated by various methods, from different morphological parts and from different forms of plant material, could be found in the literature. Some of the data were summarized in a recent review on the volatiles of the Annonaceae; and because of the wide variation in the composition of the reported oils, we were interested in the effects of the isolation procedures on the composition of the essential oil.

The oil samples were isolated either by distillation-extraction in a Likens-Nickerson-type apparatus after pre-soaking of the fruits in a solvent or a solvent mixture, by distillation-extraction without pre-soaking of the fruits, or by hydrodistillation in a Clevenger-type apparatus. The samples were analyzed by means of GLC, LSC and GC-MS.

A comparison of the percentage composition of the samples revealed that they were qualitatively similar but quantitatively different. The samples consisted mainly of terpenes; major components of the oil samples were sabinene, α- and ß-pinene, 1,8-cineole, terpinen-4-ol, α-terpineol and linalool. The different isolation procedures were found to influence the oil composition.

Reference

Onayade, O.A., Scheffer, J.J. C. and Baerheim Svendsen, A. (1991). Analysis of the Fruit Essential Oil of *Xylopia aethiopica* (Annonaceae) and the Influence of Different Isolation Procedures on its Composition (Manuscript submitted)

P40 - CHANGES IN QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF ESSENTIAL OIL IN CHAMOMILE DRUG-THE THREE HARVESTS OF A YEAR

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The aim of this contribution is to show what qualitative and quantitative changes in essential oil constituents occur in chamomile grown in Slovakia, after three production periods within a year.
Flower drugs of diploid variety /"Bona"/ and tetraploid cultivar /"Kosice-2"/ from field experiment in Nova Lubovna (North-Eastern Slovakia) in 1988 were used throughout. After steam distillation to isolate essential oil, the weight was determined. Then, the main components of essential oil were analyzed using gas chromatography /GC/.
Comparing the amounts of essential oil after each harvesting, considerable differences were observed. After the first harvesting, high percent values of essential oil content were found in both,variety "Bona" and cultivar "Kosice-2",amounting 1.33% or 1.58%,respectively. After the second and the third harvesting they decreased,in diploid "Bona" to 1.09 or 0.79%, respectively, and in tetraploid "Kosice-2" to 1.43 or 1.01%, respectively.
Evaluating the contents of effective substances of essential oil after individual harvests in variety "Bona", there was a statistical decrease in farnesene and chamazulene, and an increase in /-/-α bisabolol. In tetraploid cultivar, there was a decrease in farnesene only and an increase in /-/-α bisabololoxide A. In the other components of essential oil, it was not clear because of varying contents.
Present knowledge does not allow us to answer explicitly the question of what is the cause of changing contents of essential oil components, not only in individual periods of flower anthodia development, but also during individual harvests. The answer can be given by observing the strategy of chamomile population properties - strategy as a successful existence of plants in relation to enviromental factors, firstly, to the stress and resistance and, secondly, to the specificity of disturbance.

P41 - VOLATILE CONSTITUENTS OF FAHAM, *JUMELLEA FRAGRANS* (THOU.) SCHLTR.

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The volatiles of Faham, a flavouring and medicinal plant of the Indian Ocean, have been GC/MS analysed. Beside the main compound, coumarine, 99 minor components were identified after an enrichment with a preparative capillary chromatograph . Diterpenes (Kaurenes and Phytadienes) were confirmed, using GC/FTIR. A sensory evaluation of the leaves of Faham in rums and infusions is given.

P42 - ESSENTIAL OILS OF SEVEN *ARISTOLOCHIA* SPECIES (ARISTOLOCHIACEAE).

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The genus *Aristolochia* is widely distributed in the Brazilian territory and comprises several species reported in folk medicine as having biological activity. The genus is characterized by species possessing particularly odd looking flowers and a very unpleasant smell. The aerial parts and flowers of these plants produce essential oil in high yields but there are few reports in the literature about their chemical composition. Spectrometric scanning (I.R. and ^{1}H NMR) of the crude oils indicated their terpenoic nature. A chemical investigation by GC/MS was made on the oils obtained by steam distillation from the aerial parts and flowers of *A. gigantea*, *A. cymbifera*, *A. birostris*, *A. papillaris*, *A. tiangularis*, *A. rodriguesia* and *A. macroura*, which showed a net preponderance of the sesquiterpene fraction upon the monoterpenic one. The composition of the volatile mixtures was identified by comparison with data from literature in addition to data base of the retention times and Kovats indices.The compounds most frequently found in the studied oils are: ß-cariophyllene, α-humulene, α -copaene, the ß, γ and δ-elemene, germacrene A and D. Among the hydroxilated terpenes: α-cadinol, α-terpineol, 4-terpineol, linalol, geraniol, nerol and nerolidol.

P43 - THE ESSENTIAL OILS OF *BACHARIS LATIFOLIA* AND *B. SALICIFOLIA* FROM BOLIVIA

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The essential oils of *Bacharis latifolia* (R. & P.) Pers. and *B. salicifolia* (R. & P.) Pers. harvested in the region of Cochabamba, Bolivia, were analyzed by a combination of gas chromatography using polar and a-polar columns and a GC/MS system. 71 peaks were observed in the essential oil of *B. latifolia*. The concentration of germacrone (the main compound) is in the 20-30%- range depending on the harvesting period. A variety of monoterpenes (30%) and sesquiterpenes (15%) are also present. The most important products are α-pinene (1-5%), α-phellandrene (1-7%), and (E)-β-ocimene (3-4%).
The essential oil of *B. salicifolia* seems more complex. The monoterpene family (10%) is mainly represented by α-phellandrene (8%). Germacrene-D (6%), bicyclogermacrene (5.3%), and δ-cadinene (4.6%) are the main members of the sesquiterpene group. A dozen oxygenated sesquiterpenes have a concentration between 1 and 8%. Identification of these compounds is in progress.

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P44 - THE ESSENTIAL OIL OF *CEDRONELLA CANARIENSIS* AND *C. CANARIENSIS* VAR. *ANISATA.*

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The genus *Cedronella* (Lamiaceae) is endemic in Makaronesia. It contains one species, *Cedronella canariensis* (L.) Webb & Berth.; chemical investigation of its methanolic extract has led to pinocarvone (1). For La Gomera a variety of *C. canariensis* is described as var.*anisata* that smells like anise in contrast to *C. canariense* (2). Both varieties grow side by side on La Gomera. Our work was performed in order to clarify the differences in the composition of the essential oil of both varieties. Plant material was obtained from La Gomera, from Tenerife and by cultivation in our greenhouse. Fresh leaves were subjected to hydrodistillation. The essential oils were collected in n-hexane and fractionated on silica gel. Identification of the components was achieved by comparison of their retention indices on apolar columns (methylsilicone) and polar columns (polyethylenglycole) and by their mass spectra. A new database was developed allowing identification of volatile Lamiaceen terpenes and phenylpropanes using Kovats indices and ms data (SEKOLABMS). More than 98% of the compounds of each essential oil were identified and classified as follows:

	C.canariense	*C.c.* var.*anisata*
Monoterpene hydrocarbones	34.9% (β-pinene 19.5)	13.1% (β-pinene 10.7)
Monoterpene ketones and -aldehydes	55.2% (pinocarvone)	0.84% (mainly pinocarvone)
Monoterpene alcoholes	3.2% (myrtenol 2)	0.08% (mainly myrtenol)
Sesquiterpene hydrocarbones	2%	1.4%
Phenylpropanes	traces	83.3% (methylchavicol)

Thus, both varieties differ in that one predominantly produces monoterpenes, whereas the other accumulates phenylpropanes in its essential oil. The anise like smelling of the variety *anisata* is based on methylchavicol.

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P45 - COMPARISON OF THE ESSENTIAL OIL OF FOUR *LEPTOSPERMUM* SPECIES - MYRTACEAE

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The chemical composition of the essential oils of four species of *Leptospermum (L. scoparium, L. rupestre, L. grandiflorum, L. ericoides*) were investigated by means of GC and GC/MS. Some 70 compounds were detected in significant amount. Among the four species, three showed very similar essential oil compositions (main constituents: α- and β pinene, 1,8-cineol, α- and β- selinene, α- and β- eudesmol and (E)-methyl cinnamate) and one (*L. ericoides*) was rather different.
This is a chemotaxonomical argument that confirms the recent separation of this species from the genus *Leptospermum*

P46 - MECHANIZED SPRINKLER IRRIGATION SYSTEM MARITSA (MSIS-Maritsa)

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The effective use of sprinkler series S - Xi Part Circle (where Xi is radius in metres) is discussed. They are heavy in weight and difficult for service. It is proposed that towing be mechanized with the help of a minicultivator. A supplementary fertilizer applicator tank and a structure for water magnetizing could also be attached.
Experimental models that have technological and exploitational qualities have been constructed

P47 - INFLUENCE OF EXTRACTION PROCEDURES ON ESSENTIAL OIL COMPOSITION OF THREE VARIETIES OF *FOENICULUM VULGARE* MILL.

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Foeniculum vulgare Mill. of Apiaceae family is a biennial or perennial plant which grows wild in the Mediterranean area. Seeds (fruits) are used to flavour liqueurs, breads, pastries, fishes and cheeses. Essential oil is used in cosmetics, culinary articles and pharmaceutical products. In folk medicine fennel is employed for its balsamic, cardiotonic, digestive, lactogogue and tonic properties (1).

Italian consumption of fennel seeds is about 250 tonnes yearly and the material is almost all imported.

Quality of oil fennel is related to the content of anethole which is the main compound that confers flavour and aroma to the spice. Its concentration can range from 40% to 80% or more (2).

For oil extraction, several methods have been reported in literature and also Pharmacopoeias do not adopt the same procedures.

In our research we studied three varieties of *Foeniculum vulgare* Mill. var. *vulgare* (bitter fennel), var. *dulce* (sweet fennel) and var. *azoricum* (Florence fennel) in order to compare their oil yields and compositions. We also tested some different extraction conditions (degree of comminution, ratio seeds/ water and time of distillation) in order to evalutate the effects of these different conditions on quali-quantitative oil composition. HRGC/MS oil characterization and quali-quantitative results from different extractions will be reported and discussed.

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P48 - COMPOSITION OF ESSENTIAL OILS OF BIOTYPES OF GENUS *ORIGANUM* OF DIFFERENT ORIGIN (+)

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Systematic researches about varieties of genus *Origanum* have been carried out with the aim to recognize the most interesting types for the cultivation, the productivity and the quality of essential oil.

Several biotypes of *O. vulgare, O. heracleoticum, O. virens, O. majorana, O. creticum* and *O. tytthanthum*, of different origin, grown in a sperimental area placed in Policoro (Bari), have been studied.

Concerning the essential oil yield %, the biotypes of *O. heracleoticum* from greek origin show the most interesting results.

The chemical composition of essential oils obtained from different biotypes of *Origanum* has been investigated, also in relation to the different flowering bloom, to the plant parts and to the drying degree.

Thus, the essential oil of *O. heracleoticum* presents different chemical characteristics depending on the origin of biotypes. In particular:

- Greek: high content % (80-90%) of carvacrol (increasing during the flowering) and widely variable content % (3-20%) of p-cymene and γ-terpinene
- Bitonto: the % of thymol and carvacrol are almost similar, about 40% + 40%;
- Castellaneta: major constituent is thymol (52%), with 20% of p-cymene, 13% of γ-terpinene and only 2% of carvacrol;
- Zalec: high content of p-cymene + γ-terpinene (55-77%), low % of carvacrol and almost lacking the thymol.

The chemical differences, in relation to the variability factors considered for the *O. heracleoticum*, are not strictly relevant; the only significant result concerns the essential oil obtained by the distillation of the leaves, which contains minor % of carvacrol and major % of p-cymene.

Furthermore the essential oil of *O.creticum* (Zalec) is noteworthy for its high % of carvacrol (87-95%), accompaning with low amount of p-cymene (5-6%).

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P49 - THE ESSENTIAL OIL OF *SPHACELE CHAMAEDRYOIDES.*

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Sphacele chamaedryoides (Balbis) Briq., syn. *Lepechina chamaedryoides* (Balbis) Epl., *Lamiaceae*, commonly known as "salvia" or "alguelahuén", is a shrub with blue-violet flowers endemic of Chile, growing between Valparaiso and Valdivia. The leaves and young stems are used in popular medicine as emenagogue and topically in some paralysis [1,2].

Aerial parts of *S. chamaedryoides* were collected in Tomé (Concepción, Chile) in March 1989 and 1990. Plant material was identified in the Department of Botany of the University of Concepción and a voucher specimen was deposited in the BCF Herbarium (Faculty of Pharmacy, University of Barcelona) with the number 35699. The fresh aerial parts of *S. chamaedryoides* gave by hydrodistillation an essential oil yield of 0.25% (vol./wt.).

Isolation of the essential oil was made from fresh plant material by hydrodistillation using a Clevenger type apparatus. Qualitative and quantitative analysis were carried out by GC-FID and GC-MS using two different fused silica capillary columns (CW-20M and SE-30) as previously reported [3].

More than 90% (65 components) of the essential oil, which is particularly rich in sesquiterpenes, was identified: 42.6% were monoterpenes and 50.6% were sesquiterpenes. Oxygenated monoterpenes were present in a low percentage (5.1%) whereas the sesquiterpene fraction showed similar percentages of hydrocarbons and oxygenated compounds (24.2% and 26.4%, respectively).

The maior constituents were α-phellandrene (13.0%), ß-caryophyllene (10.3%), T-cadinol (10.4%), spathulenol (6.4%), limonene (6.0%) and γ-cadinene (5.9%).

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P50 - GC-MS ANALYSIS OF THE ESSENTIAL OIL OF WOOD PERICARP AND LEAVES OF *RHUS CORIARIA* L. (SUMACH)

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The essential oils obtained from wood, pericarp and leaves of *Rhus coriaria* L. by hydrodistillation, was examined by GC and GC-MS. Fiftyfive constituents in wood-oil, seventy-five constituents in pericarp-oil and sixtyfour constituents in leaf-oil were identified. Predominant compounds in wood oil were α -pinene, caryophyllene, patchulane and cembrene; whereas major components in pericarp oil were nonanal, cis-2-decenal, 2,4-decadienal and cembrene and in leaf oil were D-limonene, caryophyllene, patchulane, cembrene and farnesyl acetone though composition of the oils from two different phytogeographic regions present variations.

Anatomical studies revealed that leaves and pericarp have external glands and the wood has canals as the secretory structures. These characteristic elements were illustrated.

P51 - COMPARISON OF THE ESSENTIAL OIL COMPOSITION OF TWO POPULATIONS OF *GERANIUM ROBERTIANUM* L.

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Geraníum robertianum L. is a strong-smelling, herbaceous plant, common all over Europe, and can especially be found in moist places. This species has been used in folk medicine, for centuries, as an astringent, a hemostatic and a vulnerary.

Previous results of a histochemical study showed this species to bear glandular trichomes which secrete volatile terpenes.

GC and GC-MS analyses of the essential oil of *G. robertianum plants* collected at Leiden, The Netherlands, in April 1990, and at Lisbon, Portugal, in January 1991, showed that their quantitative composition was rather different.

Monoterpenes constituted the major part of both oils, but the relative amounts of the oil components showed great differences. In the essential oil isolated from plants collected at Leiden, linalool and γ-terpinene were the major components of the monoterpene fraction, while tricyclene, sabinene and trans-ß-ocimene were present in small amounts. In the oil obtained from plants collected at Lisbon, linalool and γ-terpinene were present in relatively small amounts, and tricyclene, sabinene and trans-ß-ocimene were the major components. Similar differences were found for the sesquiterpenes: germacrene-D was the major component of the sesquiterpene fraction, while ß-caryophyllene and α-humulene were present in small amounts in the oil from plants collected at Leiden. In the oil from plants collected at Lisbon, germacrene-D was present in small amounts, while β–caryophyllene and α-humulene were the major components of the sesquiterpene fraction. Although the time of the collection of the plant material was different, the time of the growing cycle was almost the same, as the plant starts growing much earlier in Portugal.

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P52 - IDENTIFICATION OF THE CONSTITUENTS OF SOME ESSENTIAL OILS BY THE RELIABILITY FACTOR. A SUITABLE METHOD TO IDENTIFY THE COMPONENTS OF COMPLEX MIXTURES.

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The qualitative gas-chromatographic identification of a mixture of components can be performed by means of their retention times (Rts). These value, however, couldn't be constant because of little variations of experimental conditions. The variation of the relative retention times (RRts) is more contained, but their use is correct with a mixture of few substances with a previously supposed nature, while it is less accurate when they are referred to components with a Rt far from the intemal standard.

The suggested method, already applied in the elucidation of the composition of the essential oil of *Calamintha nepeta* (L.) Savi (1), is based on the use of more internal standards, chosen in a such manner to have one of these present in each phase of the temperature program and permits to overcome the limits of the RRts. With the reliability factor method, by means of few and simple mathematical operations, is possible to identify the presence of a substance in a complex and heterogeneous mixture. As evidence of this, a gaschromatographic study of the essential oils of *Satureja montana* L., *Coriandrum sativum* L., and *Calamintha nepeta* (L.) Savi has been performed and for the peak assignation the reliability factors have been used.

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P53 - BLACK TRUFFLE ESSENCE: A NEW AROMATIC PRODUCT RECOVERED FROM PROCESSING OF INDUSTRIAL AQUEOUS EFFLUENTS.

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Black Truffles (*Tuber melanosporum Vitt.*) 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, Italy and Spain. The harvesting is exclusively carried out with animals during winter. Due to its typical flavour, black truffles are very much appreciated by gourmets and traditionnaly used by the french food industry to flavour its products.

Before to be industrially processed by heating treatment (apertization), truffles have to be whashed and brushed, and sometimes flash-heating processed. These preliminary treatments generate large volumes of flavoured aqueous effluents. In order to recover the aroma volatile compounds contained in such effluents and consequentively to minimize the losses of flavour during industrial process, adsorption chromatography is tested.

Isolations of volatiles from frozen aqueous effluents (whashing and flash-heating processes) are carried out by adsorption on apolar sorbents (Amberlite XAD-2, XAD-4 and active charcoal) followed by dichloromethane elution. 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.

Adsorption chromatography on pilot unit and ethanol elution is then carried out with selected sorbent in order to propose a new industrial aromatic base: “a truffle essence”. This extract is then sensorially evaluated by a panel of experts. The organoleptic qualities of the truffle essence could be then assessed and consequentively the efficiency of the volatiles recovery unit stated.

P54 - STUDIES ON ESSENTIAL OILS FROM NEW TRIPLOID *CITRUS* HYBRIDS AND THEIR PARENTS, DIPLOID CLEMENTINE AND TETRAPLOID GRAPEFRUIT

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Recently, induced by the grave crisis in the Sicilian *citrus* industry, we have obtained new *citrus* hybrids in the hope that they could be used both in food and in the essence and juice industry.

We would like to report the results obtained in a comparative study of the essential oils of seven hybrids and their parents.

The hybrids (P1-P7), all triploid, were obtained *in vitro* from immature embryos derived from crossing the 'Monreal' diploid clementine (*Citrus reticulata* Blanco) with the tetraploid grapefruit (*C. paradisi* Macf.) [1]. The resulting seedlings were grafted onto citrange Troyer and entered into production in 1986. All the hybrids were morphologically similar to grapefruit, and their fruit were approximately similar to the average for grapefruit, apart from those of P4, which are very similar to clementine. Peel colour ranges from dark orange (P4) to light yellow (P7). P2 seems to be the most promising hybrid as regards the taste, acidity, aroma and total solids for possible introduction into the market.

The essential oils were obtained by simultaneous distillation-extraction (SDE) using a mixture of pentane-ethyl ether as solvent, while the qualitative-quantitative analyses were carried out using GC-FID and GC-MS.

The essential oils composition is the sum of those of the parents, with greater weight to the grapefruit. Limonene is the main constituent (93-95%), the remainder accounting for the different fragrances of the various hybrids.

In conclusion, this study indicates that a hybridization program could be supported by the essential oil profiles of different species to obtain selected hybrids of commercial interest.

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P55 - CHEMICAL ANALYSIS OF ESSENTIAL OILS OF MADAGASCARIAN MEDICINAL PLANTS BY GC AND NMR SPECTROSCOPY

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In collaboration with the Institut de Recherches Appliquèes of Antananarivo, Madagascar, the composition of the essential oils of medicinal plants spontaneous or cultivated in Madagascar was determined. The aim of the study was the valorization of these plants for their technological and biological properties, as well as their possible safety use in many products of wide comsumption as foodstuffs, pharmaceuticals or cosmetics.
Analyses were performed coupling the data obtained by utilization of capillary gas chromatography and ^{1}H and ^{13}C NMR techniques. The ^{13}C NMR spectroscopy prouved to be an important tool, which can be very useful mainly in the identification of main constituents whereas only partial indications can be obtained for minor components. The results of the analyses of the essential oils either of commercial plants (i.e. *C. camphora, C. zeylanicum, E. globulus, E. citriodora, Melaleuca viridiflora, Ocimum gratissimum*) and endemic species (i.e. *Priadia goyavia, Ravensara anisata, Helychrisum gymnocephalum*) are reported. Also antimicrobial activities of analyzed essential oils of endemic plants are perfoming.

P56 - ESSENTIAL OIL OF ROSEMARY CULTIVATED IN HUNGARY

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Rosemary (*Rosmarinus officinalis*) is an extremly varying plant species morfologically as well as regarding its special product characteristics. This can be traced very well also in the publications of the professional literature (LAWRENCE, 1981., 1991.), conforming to its essential oil's quantitative and qualitative properties.

Rosemary can not stand long-lasting frost. So in Hungary it doesn't belong to the cultivated essential oils and medicinal plants. Nevertheless lately - as a results of our selection work - we got succeeded to produce a frost tolerant variety-candidate. With this variety-candidate we could start its cultivation even in Hungary on our sub-mediterranean climate like territories.

The chemical composition of the essential oil (produced by steam distillation) was determined by mass spectrometry, using a JEOL JMS-D300, electron impact ion source mass spectrometer by JEOL JGC-20K gas chromatograph, (GC/MS).

The composition of the essential oil of our new variety-candidate differs from the results examined and published so far. The characteristic chemical components we found, were: alfa-beta pinenes, octen-3-one, eucalyptol (1,8 cineole), camphor, borneol, bornyl acetate and verbenone as monoterpenoids, and a sesquiterpenehydrocarbon component like beta-caryophyllene.

These diversities have presumably genetical background, but probably it is influenced by the environmental factors as well. For settleing this latter it seems to be practical to cultivate these new selected variety-candidate at different growing sites.

P57 - VOLATILE CONSTITUENTS FROM THE INFECTED WOOD OF *AQUILARIA MALACCENSIS* BENTH.

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Upon hydrodistillation of a 500 g sample of Malayan "gaharu" (agarwood), an essential oil is obtained in 0.8% yield. The odour of this oil can be described as warm, diffusive strongly woody and characteristic. It was found to contain at least 70% sesquiterpene alcohols, with 10-epi-γ-eudesmol (13-6%) and valerianol (12-6%) predominating. The remaining part of the oil is made of sesquiterpene oxides (α-agarofurane: 4.2%, major) and a number of non-terpenic aromatic compounds (benzyl acetone: 4.1%, major). These findings and other considerations confirm the identity of the starting material as *Aquillaria malaccensis* Benth.

P58 - BUD REGENERATION IN *LAVANDULA* GENUS

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Methods for in vitro bud regeneration from various lavandin tissues have been developed. Leaves, stem nodes, shoot tips, flower parts (floral pedoncules and ovaries) and roots, from various lavandin clones have been used as in vitro callus sources. The morphogenetic potential of the different calli has been investigated. Floral tissues, irrespective of the nature of the clone, were more reactive than their vegetative counterparts. Bud regeneration from roots, was obtained with only one lavandin clone: the Abrial. A total of 250 buds has been regenerated, isolated, rooted, cloned and amplified, with all the origins (tissues and clones). Studies to determine the performance level of such material are currently underway.

P59 - BOTANICAL AND CHEMICAL STUDIES ON *TOONA* SPP.

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Toona sinensis and T. *sureni* are two aromatic trees found in montane forests in Peninsular Malaysia. To this date, there has been a dearth of information on the ecology and botany of the two species as well as the essential oils that can be obtained from the bark or wood. Some studies on the ecology and botany of the two species will be presented in this paper, including preliminary studies on the chemical properties of these trees.

P60 - LAVENDER LACTONE AND OTHER VOLATILE CONSTITUENTS OF THE OLEORESIN OF *GARCINIA KOLA* SEEDS

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Garcinia kola Heckel, Guttiferae - synonyms *G. dinklagei* Engl and *G. conrauana* Engl.

In Nigeria as well as in some other parts of Africa, the seeds of *G. kola* are added to enhance the flavour and the intoxicating properties of native liquor and palm wine. The seeds are said to be acid and to have a bitter, aromatic, astringent and resinous taste, followed by a slight sweetness. The plant is used to fix dyes and for varnishes, its resins form emulsions in water and may be used for making watercolours. Non-polar fractions and extracts of *G. kola* were reported to demonstrate significant anti-microbial activity, the observed activity was reported to be due to a benzophenone, kolanone. There is no report in the literature on the GC and GC-MS analyses of the volatiles of *G. kola* and because of the many uses of the plant we were interested in the analysis of its essential oil.

The composition of the volatiles obtained from the distilled-extracted oleoresin of a commercial sample of seeds of *Garcinia kola* growing wild in Nigeria was analysed by means of GLC, LSC and GC-MS. The volatiles consisted mainly of terpenes as, aldehydes (10%), ketones (14%), alcohols (35%); oxygen-containing monoterpenes (40%), oxygen-containing sesquiterpenes (14%) and diterpenes (4%), were also detected. Hydrocarbon compounds were hardly present. The major components of the volatiles were 6-methyl-5-hepten-2-one (13.9%), ß-farnesol (12.3%), 5-ethenyl-dihydro-5-methyl furan-2-one (=lavender lactone, 10.4%) and linalool (9.9%). Tributyl phosphate and phthalates (plasticizer contaminants) costituted about 10% of the volatiles detected in a commercial sample of *G. kola* seeds. Neither GC nor GC-MS analyses of the essential oil or volatiles of *G. kola* or any other Garcinia species have been reported previously.

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P61 - STUDIES ON THE QUALITY AND STABILITY OF GINGER OIL AND OLEORESIN FROM ETHIOPIA

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Ginger, which was introduced to Ethiopia ca 500 years ago, is presently cultivated in south and southwestern Ethiopia. Recently we undertook a R&D program to evaluate oil and oleoresin derived from indigenous ginger for export purposes. This effort has led to the introduction of Ethiopian ginger oleoresin into the world market. Subsequently a detailed investigation on ginger oil from various locations was undertaken. The oil obtained by hydrodistillation was subjected to GC analysis on a fused silica capillary column wall coated with SE-54.Several monoterpenes and sesquiterpenes, including α–pinene, camphene, limonene, cineole, linalool, borneol, citronellyl acetate, geraniol, citral, ar-curcumene, zingiberene, ß-bisabolene and ß-sesquiphellandrene, were identified. The last three sesquiterpenes are the major constituents (ca 60%) of the oil. The composition of the oil is more like other African ginger oils (1) and differs from others (2) in having low levels of citral. Stability studies were conducted to determine the rate of deterioration of ginger oil due to exposure to heat, light and air. Previous studies on stability of oils have indicated that air and light cause considerable deterioration of ginger oil. In this study, freshly distilled samples were kept: 1) in a sealed dark bottle in a cold room, 2) in a tightly closed dark bottle at room temperature, 3) in a tightly closed transparent bottle exposed to diffuse light and 4) in an open dark bottle exposed to air. Samples were periodically removed during a period of 6 months for GC analyses and optical rotation measurements. GC measurements were useful in determining the particular constituents that were undergoing changes during deterioration. The deterioration of the oil could most easily be followed by monitoring the optical rotations of the oil. The sample that was exposed to air (sample 4) showed the most dramatic changes with the rotation values changing from -45.95 to +17.02. The levels of ar-curcumene increased from 6.96% to 29.52% while those of zingiberene and sesquiphellandrene decreased from 37% to 6.68% and from 13.10 to 7.72% respectively (3). No significant changes were observed in the other samples. Our results show that diffuse light does not bring about noticeable changes over a period of six months.

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P62 - A STUDY ON EVAPOTRANSPIRATION OF ROSE OIL UNDER DRIP IRRIGATION

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A study on evapotranspiration (ET) of Rose oil was made in 1989-1990 in eleven treatments. The researches were made at optimal and shortage of water supply irrigation. ET at optimal treatments were maintained at 75 and 85% of field capacity at 6,663 m^3/ha and 8,255 m^3/ha respectively during the experimenting period.
Biophysical coefficients R,Z and K were calculated at the above stated soil moisture contents. These coefficients could be applied in designing irrigation systems.

P63 - A STUDY ON INFLUENCE OF IRRIGATION AND FERTILIZING ON PRODUCTIVITY OF OLEAGI - NOUS ROSE

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During the period of 1987 - 1990 an agrarian experiment with oleaginous rose at two intensive factors, irrigation and fertilizing was made in the experimental field of The Scientific Research Institute of Roses, Essential oils and Medicinal Plants. That experiment embraced 10 versions of fertilizing at maintaining the same irrigation conditions for all versions within 75-85-70 %, of field capacity. The irrigation conditions were differentiated, i.e. different soil moisture was kept during oleaginous rose vegetation. The highest yield of rose blossom and oil was obtained at version 6-7,761 kg/ha average for the experimenting period.

P64 - VOLATILE DITERPENOIDS FROM *AMMI* SP. (APIACEAE)

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Ammi majus L. and *Ammi visnaga* (L.) Lam., both indigenous in the Mediterranian area, are of pharmaceutical interest. The furanocoumarin content (xanthotoxin and imperatorin) in *Ammi majus* causes its use in dermatology whereas the furanochromones khellin and visnagin isolated from *Ammi visnaga* fruits are important antispasmodic substances. Due to this fact, *A. visnaga* fruits are admitted to the Egyptian and German pharmacopeia. Concerning the essential oils only few results have been published (1,2). In our previous communication we reported on the presence of a remarkable diterpenoid acetyl ester in the essential oil of *A. visnaga* fruits (3). Further diterpenoids have been found in other organs of *A. visnaga* and *A.majus.* Their structure, distribution in the plants and seasonal variation will be presented in this paper.

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P65 - 5-(1- METHYL) BICYCLO-[3.1.0]-HEX-2-ENE-2-CARBOXALDEHYDE, A NEW NATURAL MONOTERPENOID FROM *LEDUM PALUSTRE* VAR. *DILATATUM*

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Ledum palustre L. var. *dilatatum* Wahl. (Ericaceae) is an essential oil containing plant growing in the northeastern part of China and the inner Mongolia. From the aerial green parts of this plant an essential oil is commercially obtained* called "Duxiang Oil" which has been investigated by chromatographic and spectroscopic methods.
The pale yellow oil was subjected to dry column chromatography using Si gel and n-pentane as a eluent. The obtained fraction containing the hydrocarbons revealed after capillary GLC the presence of several monoterpene hydrocarbons (57% of the oil), above all sabinene (20,5%) and p-cymene (18,1%).
The oxygenated compounds were obtained after elution with an increasing polar mixture of pentane/diethyl ether. Main constituent of this fraction was in addition to terpinen-4-ol(6,1% of the oil) an unknown compound (25,2%) with a characteristic intensive odour which was purified by preparative GLC. Spectroscopic investigations of this compound furnished the following data:

IRυ_{max}: 3055, 2955, 2870, 2805, 2710, 1685, 1598, 1173, 980, 737, cm^{-1}
UVλ_{max} (ethanol): 254 nm
MS 70 eV, m/e (rel.int.): 150 (M$^+$, 1), 79 (100), 77 (42), 107 (24), 39 (19), 105 (19), 41(16), 91 (14)
^{13}C NMR (125 MHz, C_6D_6); δ 187,55 (d), 152,40 (s), 147,48 (d), 37,63 (t), 33,61 (s), 32,43 (d), 24,66 (d), 21,29 (t), 19,90 (q), 19,68 (q).

From these data the presence of a bicyclic monoterpene aldehyde with a conjugated double bond and the molecular formula $C_{10}H_{14}O$ could be deduced. After the application of various NMR spectroscopic methods including 2-dimensional techniques the structure of the unknown compound was determined to be 5-(1-methyl)bicyclo[3.1.0]hex-2-ene-2-carboxaldehyde, a monoterpenoid which has not been found up till now as a natural compound. However, this compound has been obtained synthetically by oxidation of α - thujene with selenium dioxide by Catalan and Retamar (1). We therefore performed a synthesis of the aldehyde according to the prescription of the before mentioned authors and confirmed our result by comparison of the spectral data of the natural and synthetic compound.

* We thank Christian Oswald, Import-Export GmbH for providing samples of Duxiang-Oil

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P66 - VOLATILE CONSTITUENTS OF THE LEAVES OF *PERSEA GRATISSIMA* GAERTNER NEW SOURCE OF METHYLCHAVICOL

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The essential oil of the leaves of *Persea gratissima* Gaertner cultived in Turkey was investigated. Using HRGC and HRGC/ EIMS about 36 compounds were found and 29 identified. The bulk of this oil is represented by methylchavicol (78%). This percentage is higher than that normally found in the tarragon oil. No previous works on this subject are reported. Investigations to recognize some other variously substituted alkylbenzene, that seem to be present in this oil, are in progress.

P67 - ANALYSIS OF SULPHURATED COMPOUNDS IN THE ESSENTIAL OILS OF SOME *TAGETES* SPECIES BY GC/MS AND GC/FT-IR

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This communication reports the results of the analysis of some essential oils of different *Tagetes* species by GC/MS with different ionisation techniques (EI, PCI and NCI), GC/FT-IR and S-GC/AED.
These essential oils were chosen because they contain a series of acetylenic thiophenes in variable amounts; the most abundant of them have already been described in the literature, while the structure of some others, above all trace components, is still unknown. These compounds are known to be strongly repellent to insects. The contribution to the identification and structure elucidation of acetylenic thiophene compounds by GC/MS with different ionisation techniques in some essential oils of different varieties of *Tagetes patula* and *Tagetes erecta* with coupled techniques will be shown.

P68 - VOLATILE COMPONENTS OF VIOLET LEAVES

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Volatile components of violet leaves were extracted with 1,1,2- trichloro-1,2,2-trifluoroethane as well as hexane and analysed using GC, GC/MS, GC/FTIR. Quantitatively, the yields of violet leaves concrete vary much according to the experimental parameters. Qualitatively, the chemical composition differs with respect of the solvent used. More than 100 compounds were separated, of which 22 compounds have been identified representing 95% of isolate, 6 are reported as violet leaves volatiles for the first time, they are dodecanol, pentadeca-5,10-dien-1-ol, penta-5-decen-1-ol, octadecene-1, eicosene-1 and octadeca-9,12-dienoic acid.
In addition, the extract obtained with hexane was compared, the considerable variations have been observed in both quality and quantity .

P69 - EXTRACTION OF YARROW (*ACHILLEA MILLEFOLIUM* L.) CULTIVATED IN QUÉBEC

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Achillea millefolium L., also known as yarrow, milfoil, herbe à dinde or achillée millefeuille, is a widely distributed specie in Québec that grow in arid soil and take over any ground available. Field trials had demonstrated that the culture of that wild plant is well adapted in cold climate region. Yarrow was cultivated to give under steam distillation a blue essential oil rich in chamazulene. Plants were collected at different blooming stages during the season: beginning of flowering, at full bloom, and beginning of the seed formation.

The average yield of cultivated plant material were respectively 21, 24 and 18 t/ha for the first, second and third harvest. Essential oil yield on a dry basis was 0,33%, 0,29% and 0,20% for the first, second and third harvest respectively.

Fresh plants yielded more essential oil compared to dry ones but chamazulene was higher in essential oils from dried plants. The highest amount of chamazulene in plants was obtained when harvested at flowering time giving a yield of 3,97 kg/ha for fresh plant and 4,43 kg/ha for dried plant.

The main components of yarrow oil are α-pinene (1.8%), camphene (2.7%), sabinene (8.4%), ß-pinene (7.6%), myrcene (4.0%), limonene (2.1%), 1 ,8-cineole (3.1%), α-thujone (0.4%), ß-thujone (3.2%), camphor (6.2%), bornyl acetate (1.7%), caryophyllene (3.3%), germacrene D (12.9%), farnesene (1.1%) and chamazulene (19.0%).

Essential oil was higher in the flower heads (0,82%) than in the leaves (0,22%) and none was detected in the stem. Also the chamazulene content in the essential oil from flower was twice as much than the leaves.

P70 - COMPOSITION OF GENUINE MARJORAM OIL OF DIFFERENT CLONES AND STRAINS USING HT-HS TECHNIQUE AND SNIFFING GAS CHROMATOGRAPHY

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The composition of marjoram oil recovered by steam distillation is well known. Cis-sabinene hydrate is supposed to be the key compound for typical marjoram flavour and terpinene-4-ol on the other hand, counts for off flavour (Lossner 1968, Fischer 1968). Artifacts could arise from high temperatures during the distillation process, as consequence of rearrangement reactions of cis-sabinene hydrate and its acetate, which should be the genuine synthetized monoterpenes in marjoram oil glands (Fischer *et al* 1987).

By means of HT-HS (High Temperature Head Space) direct solid sampling technique in gas chromatography the genuine oil composition has been determined from dried leaf and flower fragments as well as isolated oil glands. The equipment used was described by Franz (1990). In addition to GC a sniffing analysis is carried out by comparing with pure test substances. This technique allows to define the smelling peculiarity of single different components and to determine which compounds besides cis-sabinene hydrate, also present in small quantities, contribute to typical marjoram flavour and which are unpleasant. Different clones and breeding lines of marjoram are presented with the respective results.

P71 - CHARACTERIZATION OF SEVERAL AROMATIC PLANTS GROWN IN SARDINIA.

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In view of a more extensive study of Sardinian aromatic plants, some species as *Nepetella, Teucrium, Foeniculum vulgare*, *Rosmarinus officinalis* and *Stachys glutinosa* were studied in order to determine a relation between their environment and essential oil composition.

An evaluation about influence of different climatic conditions on quali - quantitative oil compositions will be showed.

All analyses were performed involving capillary gaschromatographic MS-FTIR technique. A F.I.D. was used to obtain quantitative data.

P72 - GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC STUDIES OF THE VOLATILE CONSTITUENTS OF THE FRESH AND HEAT TREATED TOMATO (*LYCOPERSICON ESCULENTUM*)

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Volatile fractions of some tomato lines cultivated in Campania (Southern Italy) have been studied by gas chromatography and mass spectrometry and the presence of ca. ninenty compounds has been detected. On the whole, 66 different components of volatile fraction have been identified. The components of the carbonilic fraction are considered the most relevant aromatic compounds. Among volatile compounds found in fresh tomato there are some halides such as: hexanal, cis-2-hexenal, trans-2-hexenal amounts of which depend on the ripening stage of the tomato. All the investigated tomato lines in addition to the halides enumerated above, contain also: 6-methyl-5-hepten-2-one, 2-isobutylthiazole, neral, geranial, methylsalicylate, geranylacetone, 2-phenylethanol, dihydroactinidiolide. Differences in the compositions found between the aromatic fractions of fresh and heat treated tomato both in serum and in solid part are shown. Particularly in the serum, thermal generation of new volatile compounds (linalol, ß-damascenone and α-terpineol) is observed; their presence is probably due to the tomato glycosides hydrolysis. The occurence of ß-carotene and lycopene degradation products are also discussed.

P73 - VOLATILE FLAVOUR COMPONENTS FROM GARLIC (*ALLIUM SATIVUM* L.)

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The volatile components of crushed fresh garlic of mediterranean origin were analyzed and identified by both capillary gas chromatography (GC) and combined gas chromatography-mass spectrometry (GC-MS). About seventy volatile compounds were detected and 21 of them were identified in methanolic and water extracts. Three new sulfur compounds, cis-methyl-1-propenyl disulfide, cis,cis(?)-dipropenyl disulfide and trans,trans-dipropenyl disulfide, were tentatively identified for the first time as components of garlic .

Differences between fresh garlic bulblet in an early stage of development and fully mature garlic bulb were pointed out. The main sulfur compounds in both methanolic and water extracts were diallyl disulfide, 3-vinyl-4H-1,2-dithiin, 2-vinyl-4H-1,3-dithiin, trans,trans-dipropenyl disulfide, methylallyl disulfide, methylallyl trisulfide and diallyl trisulfide. In particular, methanolic extract shows higher amounts of 3-vinyl-4H- 1 ,2-dithiin and 2-vinyl-4-H- 1,3 -dithiin, two important compounds with antithrombotic activity, in comparison with the water extract. An hypothetic synthetic pathway of the identified sulfur compounds and of the artifacts formed during the extraction and analysis is shown and discussed.

P74 - SEASONAL VARIATION OF THE ESSENTIAL OIL OF *CRITHMUM MARITIMUM* L.

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Seafennel (*Crithmum maritimum* L.) is a species of the Umbelliferae that grows wild along the Portuguese coast. The plant has been used in folk medicine as an antiscorbutic and a diuretic.
In the literature, some data have been given about the influence of climatological and soil conditions on the essential oil composition of various plants.
In our previous studies on samples of seafennel collected at two different times of the year, differences in essential oil composition were detected. Therefore it was decided to study the seasonal variation of the composition of this oil. For this purpose, samples of plant material were collected every two weeks, during a period of one year.
Throughout this study, monoterpenes were shown to be the major components of the oil, amounting up to 86%. The sesquiterpenes were present in a relatively small amount, constituting up to 2% of the total oil.
Although all monoterpenes showed fluctuations in their relative amounts, the most conspicuous ones were recorded for sabinene (7-42%) and γ-terpinene (26-55%). For sesquiterpenes and other components, e. g. methylthymol and dillapiole, no relevant fluctuations were detected in their concentrations.

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P75 - CHARACTERIZATION OF THE ESSENTIAL OIL OF *SIDERITIS DICHOTOMA*

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A tea prepared from the dried flowering herb of *Sideritis dichotoma* Huter, an endemic species of Turkey, is widely used for common cold in Balikesir region. There is no previous report on the constituents of this plant. This study comprises the results of GC and GC-MS analysis of its essential oil obtained by water distillation and comparison with those of other *Sideritis* oils already reported.

α pinene	19.51
β "	18.01
limonen	3.6
Cis.2 decanal	7.2
β bisabolene + γ elem	7.19
α bisabolol	3.94
β felandrene	2.16
δ-3-carene	2.09
Sabinene	1.28
β-mircene	1.15
α-felandre	1.36
α-terpin	1.08
Nonanal	1.37
β-cariofilene	3.01
Spatulenol	1.79
nonacoic ac.	1.50

P76 - *SALVIA POMIFERA* L. SUBSP. *POMIFERA*: SEASONAL VARIATION OF ESSENTIAL OIL YIELD AND COMPOSITION

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The study of the seasonal variation of essential oil yield and composition of *Salvia pomifera* L. subsp. *pomifera*, a Cretan endemic taxon, revealed that remarkable changes occur during the year as well as that there are noticeable differences between the different plant parts collected at the same time.

The essential oil content of the plants is higher during the summer and it reaches the lowest quantity in March - April. The pattem of the yield variation follows the changes of the seasonal temperature. Leaves produce always the highest amount of essential oil (ranging from 2.2 - 5.5%), whereas the contribution of stems in the yield of the total plant is very low (0.1- 0.4%).

The qualitative composition is stable, always characterized by the high amount of thujyl ketones (63.4% in May - 81.3% in November). However, differences occur in the quantitative composition and the most important is the opposite trend in the participation of thujone and isothujone. Furthermore, though sabinene is always present in low amounts (1.4 - 3.3%) in the leaves, it is a major constituent of the flower essential oil (reaching c. 25%).

P77 - COMPOSITION OF THE ESSENTIAL OILS FROM LEAVES AND FLOWERS OF *ACHILLEA MILLEFOLIUM* L. ssp. *MILLEFOLIUM*

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Owing to pharmacological and taxonomic interests, the composition of the essential oils of different *Achillea* species has been studied by several authors (Brunke *et al.*, 1986; Lemberkovics *et al.*, 1988). Although it is difficult to define a chemotype within the *A. millefolium* aggregate, Eglesser et al. (1988) suggested that the concentrations of α- and β-pinene, caryophyllene, sabinene, 1,8-cineole, camphor and chamazulene in the oils may serve as distinguishing criteria. According to Motl *et al.* (1990), the amount of germacrene-D could also be used as a differentiating feature.
In this study we analysed the composition of the oil from leaves of *A. millefolium* ssp. *millefolium* during its vegetative phase as well as that of the oils from flowers and leaves during the flowering period of the plant.
During the flowering period both oils consisted mainly of monoterpenes (about 80%). 1,8-Cineole was the most dominant component in the oils from the flowers (27%) and leaves (26%), while sabinene was the second most important component in the oil from the flowers (14%) and trans-sabinene hydrate in that from the leaves (10%). The sesquiterpene fraction was in both cases dominated by germacrene-D (2% in flower oil and 8% in leaf oil).
In the essential oil which was isolated from leaves collected during the vegetative phase, the monoterpene fraction was small (2%), whereas sesquiterpenes amounted to 92%, germacrene-D being the major component (65%) of the oil.
The results obtained for the oil samples analysed clearly show that there are striking differences between the oils from the leaves collected during the vegetative phase or the flowering period of the plant studied, since the former oil is characterized by sesquiterpenes hydrocarbons and the latter by monoterpenes.

The oil isolated from flowers is qualitatively similar to the oil from leaves collected during the flowering period, although some quantitative differences exist, particularly in the amount of germacrene-D. The differences observed should be kept in mind when essential oils are used in differentiating taxa among the *A. millefolium* aggregate.

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P78 - SYSTEMATIC CORRELATION OF STRUCTURE AND ODOR OF CYCLOPROPANE DERIVATIVES

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In extension of our investigations of cyclopropane derivatives we present some new results. The syntheses and olfactive properties of alkylcyclopropanes with several functional groups are shown. Some of the compounds prepared are known to be useful as fragrance material. Depending on the functional group, stereochemistry and length of the alkyl side chain, the structure-odor correlation will be discussed.

P79 - CONSTITUENTS OF THE ESSENTIAL OIL OF *ARTEMISIA SIEBERI* FROM IRAN

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The essential oil of the Iranian *Artemisia Sieberi* was investigated by means of GLC, NMR and MS. The oil contains about 90 % of monoterpenes. Main constituents are camphor (48 %), 1 ,8-cineole (19 %), camphene (5 %), terpinen-4-ol(3%), borneol (2%) and α-terpineol (2%).
From the sesquiterpene fraction (6 % of the oil) several oxygenated bisabolanes could be isolated such as the new compounds dehydrosesquiscineole (1) and the acetal 3 as well as some esters 2.

P80 - DETERIORATION OF SOLUBLE FLAVOURS OF LEMON, SWEET ORANGE AND MANDARIN IN AQUEOUS ACID SOLUTIONS.

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Soluble flavours are widely employed in food industry mostly for soft drinks. In order to ascertain the behaviour, in aqueous acid solutions by citric acid, of soluble citrus flavours, to detect the products responsible for off-flavour, to correlate the transformated products to the original flavours, to compare the stability in time of different citrus soluble flavours, to find out mixtures of natural soluble flavours and/or of single components that are stable for long time, we have compared the behaviour of acyclic, rnonocyclic, bicyclic monoterpenes and citral with that of the soluble flavours of lemon, sweet orange and mandarin for a period of ninety days.

For the lemon flavour an increase in almost all alcohols and p-cymene and the formation of new alcohols and diols has been observed. Primary hydration products show the highest value at the 14th day and then decrease. After that there is an increase of diols. Short-term modifications can be attributable to the sabinene and citral; variation of the content of terpinen-4-ol, terpinolene, α- and γ-terpinene, 1.4-cineol and cis-p-menthan-1.4-diol are derived from the former; the degradation of neral and geranial is responsible for the increase of α-terpineol and p-cymene and formation of cis-p-menthan-1.5-dien-8-ol. Other modifications are mostly due to α- and ß-pinene which diminish costantly during the course of the experiment giving origin to 1.8-cineol, terpinolene, fenchyl alcohol, borneol, α-terpineol, cis- and trans-p-menthan-1.8-diol.

- The soluble flavour of sweet orange, in the same conditions, undergo lesser changes than those in lemon flavour and are limited to a decrease of sabinene and an increase of terpinen-4-ol and -terpineol.
- The transformations in soluble flavour of mandarin are more evident than those in orange flavour but definitely less important than those in lemon flavours. The behaviour of soluble flavours of lemon, sweet orange and mandarin agrees with what has been previously observed for the single components.

P81 - ROOTING OF TREE-PROPS OF *ROSMARINUS OFFICINALIS* L., *LAVANDULA ANGUSTIFOLIA* L. AND *THYMUS VULGARIS* L., INFLUENCE OF DISTINCT SUBSTRACTS AND HORMONAL LEVELS.

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The need of competition in quality, quantity and uniformity of the original product inside the E.E.C. made us carry out some experiences of vegetative propagation (tree-props) with 3 species that show a higher strength and size, using two substracts: 1 - combination of carth, turf and sand, 2- perlit; and two hormonal levels.

These experiences have been made on a factorial bases of 2x3x3 with 4 repetitions.

The final purpose of these works is the acquisition of new plants with good production of activ principles and by the other side to be able to dispose them to the farmers at an accessible price.

P82 - ESSENTIAL OIL OF THE LEAVES OF *EUCALYPTUS DEALBATA*.

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Several *Eucalyptus* species have been introduced in Morocco due to its easy adaptability and rapid growth [1]. Between them, there is *Eucalyptus dealbata* A. Cunn. ex Schau. (*Myrtaceae*), commonly known as "tumbledown red gum".

Plant material was collected from cultivated trees in Oued Cherrat (South of Rabat, Morocco) and a voucher specimen was deposited in the BCF Herbarium (Faculty of Pharmacy, University of Barcelona) with the n° 36378.

The air-dried leaves were submitted to steam distillation and the volatile oil was extracted with ethyl acetate from the distillate. The essential oil yield was 0.8% (wt/wt). The investigation of the composition of the volatile oil was made by GC-FID and GC-MS according to the methods previously described [2].

Ca. 80% of the essential oil was identified. It showed high content in oxygenated compounds, the main constituents being 1,8-cineole (22.46%), caryophyllene epoxide (16.37%), spathulenol (11.96%) and cryptone (8.47%).

Among the monoterpene hydrocarbons (8.3%), p-cymene (5.38%) and α-pinene (2.48%) were the more abundant. α-Phellandrene, known for its irritant properties, and present in elevated percentages in some other *Eucalyptus* sp., was hardly detected.

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P83 - THE ESSENTIAL OILS OF SOME *EUCALYPTUS* SPECIES GROWN IN MOROCCO: PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION.

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The essential oils of 31 *Eucalyptus* species of Moroccan origin have been examined using GC and GC/MS. More than sixty constituents were identified in the oils achieved. 23 of the 31 species investigated were found to possess an oil rich in 1,8-cineole (> 40%). In eight species: *E.propinqua*, *E.stuartiana*, *E.baneriana*, *E.cinerea*, *E.foecunda*, *E.coolabah*, *E.saithii*, *E.pulverulenta*, the 1,8-cineole content exceeded 70%. *E.kirtoniana*, *E.maculata* and *E. megacarpa* were found to possess oils rich in α-pinene (50.0%, 66.6% and 79.1% respectively), while *E.gardneri* and *E.dwyeri* possessed an oil rich in p-cymene (38.8% and 55.8% respectively).

P84 - ANATOMIC STUDIES OF *VETIVERIA ZIZANIOIDES* (L.) STAPF (GRAMINEAE)

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Vetiveria zizanioides (L.) Stapf commonly called Vetiver Bourbon belonging to *Gramineae* family, originating in India and now cultivated on the Reunion island, has been in recent years the focus of several interdisciplinar investigations. Following chemical studies on the essential oils, we have extended our field of investigations to include the problems of root secretary cells.

Electron and optical microscopy have been used to locate the position of the secretary cells in the roots and to determine their cellular structure. Various types of roots (different age and diameter) were collected in different Stations on the Reunion island (Petite France and Sainte Clotilde).

From optical spectroscopy, we have localized the different root tissues and observed their evolution during growth of the plant.

From the transmission electron microscope investigations, we reached the following conclusions: only cells of the youngest roots preserved their contents. Cells of the other roots were senescing or contained autophagic vacuoles full of endoplasmic reticulum fragments. In particular, lysigen lacunae in cortical parenchyma (internal cortex) were optically empty.

Also, from the preceding investigations, numerous bacteria were observed inside the cells of the lysigen lacunae and more especially in the cortical parenchym adjacent to the endodermis. It seems that these bacteria coexist with essential oils crystals detected in the inner bark, within the cortical layer. These bacteria were present in specimens for all the different stations of the Reunion island and also from roots of all thickness.

P85 - CHEMICAL COMPOSITION OF TERPENE ALCOHOLS AND PHENOLS FROM ESSENTIAL OIL AND TERPENEGLYCOSIDES ISOLATED FROM SOME *THYMUS* SPECIES GROWN WILD IN DALMATIA

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Thin layer and gas chromatography of the oil composition, as well as aglycones of the terpeneglycosides isolated from two wild *Thymus* species differ in chemical composition significantly.
According to the results, it can be suggested that one of *Thymus* species is thymol - carvacrol chemotype (*Thymus vulgaris*), because of large content of phenols (cca 20 %) and the second with large content of geraniol, which may be classificated as a *Thymus serpillum* var.

P86 - FORMATION OF ESSENTIAL OIL CONTENT AND COMPOSITION OF *OCIMUM BASILICUM* L. DURING FLOWERING

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The aim of our work was to study the change of the essential oil content and composition in *Ocimum basilicum* - cultivated in our country - during the different stages of flowering.

We obtained the essential oil with water steam distillation from the plant and the total oil content was measured with method described in the 7th Hungarian Pharmacopoea. For the identification of each oil components we used analytical and preparative GC and GC-MS techniques with packed and also with capillary column system.

In the essential oil we identified the linalool as the main component, then α-, ß-pinenes, camphene, myrcene, α-terpineol, terpineol-4, geraniol, bornyl acetate, carvone monoterpenes, some aromatic components as follows: estragol, carvacrol, p-cymene, eugenol (in traces) and sesquiterpenes: γ-cadinene, α-bergamotene, α-cedrene, γ-muurolene, α-biaabolol, ß-caryophyllene and 3 sesquiterpene hydrocarbons of unknown structure.

We established that the total essential oil content is the highest in the total flowering stadium. The oil composition changes during flowering but its characteristics stays the same. Linalool is the main component (40-60%) of oil during the whole flowering period.

It is interesting that in the second period of flowering-productive stadium the linalool content decreases suddenly (25-40%), and the same time the sesquiterpene content reached own maximum.

P87 - DETERMINATION OF THE ENANTIOMERIC COMPOSITION OF THE CONSTITUENTS OF ESSENTIAL OILS BY ENANTIOSELECTIVE CAPILLARY GAS CHROMATOGRAPHY

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Essential oils are known to contain numerous chiral constituents. In many cases these compounds are responsible for the characteristic flavour or fragrance of an essential oil. The absolute configuration of many of the chiral components has been correlated in the past after their isolation by polarimetric measurements. Methods for the precise determination of the enantiomeric composition, however, became available only recently after the development of hydrophobic cyclodextrin derivatives as chiral stationary phases for enantioselective capillary gas chromatography. The enantiomers of many naturally occurring terpenoid hydrocarbons (α-pinene, ß-pinene, limonene, sabinene, camphene, 3-carene, α-phellandrene, β-phellandrene), alcohols (without derivatization: linalool, hotrienol, cis- and trans-nerolidol, bomeol, isoborneol, fenchol, cis- and trans-carveol, menthol, neomenthol, α-terpineol, cis- and trans-verbenol; as trifluoroacetylated derivatives: citronellol, myrtenol), ketones (menthone, isomenthone, verbenone, piperitone, carvone, α-damascone, α–ionone, α–dihydroionone, cis-α-irone, cis-γ-irone, fenchone, camphor, isopinocamphone), and esters (linalyl acetate, menthyl acetate, α-terpinyl acetate) could be resolved using selectively substituted cyclodextrins [1 - 4].

In most cases certain enantiomeric compositions but only in rare cases pure enantiomers were found. This makes it difficult to prove if an essential oil is natural or if it is adulterated by the addition of synthetic material. One should also be cautious when "pure enantiomers" of natural terpenoid compounds are to be used as chiral building blocks or auxiliaries in organic synthesis.

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P88 - VOLATILE OILS FROM PLANTS GROWING IN TURKEY

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Turkey has the flora rich in volatile oil containing plants. Some of the volatile oil containing families such as Labiatae, Umbelliferae, Compositae, Myrtaceae, Lauraceae are represented by many genera, for example the family Labiatae is represented by 546 species and 730 taxa. The ratio of volatile oil containing genera in Labiatae is approximately 40 percent. There are many studies carried out and others on the wild growing plants which contain volatile oils. The chemical composition of the volatile oils obtained from wild growing plants e.g. *Thymus, Origanum, Thymbra, Mentha, Melissa, Ocimum, Salvia, Stachys, Ziziphora, Ferula, Foeniculum, Pimpinella, Achillea, Anthemis, Myrtus, Eucalyptus, Laurus, Geum, Orthorus* species are summarized in this study. In addition to these wild growing volatile oil containing plants, many other volatile oil plants are also cultivated, and four principle obtaining methods such as distillation, extraction, pressing and traditional distillation are used. The chemical compositions of volatile oils thus produced in large scale such as Rose Oil, Lemon Oil, etc. are also reviewed.

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