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AITP

25th INTERNATIONAL SYMPOSIUMON ESSENTIAL OILS

Grasse France September 5th , 6th & 7th, 1994

PROCEEDINGS

25 years ISEO, 50 years SFP (Société Française des Parfumeurs), 50 years AITP

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AITP

Association des Ingénieurs et Techniciens de la Parfumerie

25th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

September 5th , 6th & 7th, 1994
Palais des Congrès & Centre International de GRASSE 06130 - GRASSE

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GASTON ROLLES TRAITEUR	Cocktail
GERMANETTO PRESTASUD	Cocktail, dinner
MAC DONALD'S GRASSE	Coffee breaks
ROBERTET SA	Aromas and perfume materials

WELCOME

The Scientific Committee of this Symposium wishes to welcome you. We hope your travel to Nice has been without problem and that you have found your living accommodations pleasant. In the next three days we will have the opportunity to present our research and freely exchange ideas. We have attempted to facilitate this exchange by keeping the meeting informal in all respects. If there is anything that we can do to make your stay more comfortable or the Symposium more productive for you, please contact a member of the Scientific Committee.

Before starting the scientific part of this Symposium, we wish to acknowledge the host of individuals and corporations who have contributed their time, expertise and financial support to make this meeting possible. A meeting of this magnitude can not be the result of any one individual's efforts but must be the cooperative effort of many. It is through this generosity that this meeting is occurring and that the costs have been kept so modest. The Scientific Committee wishes to express their appreciation to those who given so graciously.

We sincerely hope you have the opportunity to enjoy the beauty of the surrounding area but most importantly, find the Symposium itself provocative and stimulating. Welcome to the 25th International Symposium on Essential Oils.

Dr. Ivan COSTE-MANIERE
Président de l'Association des Ingénieurs et Techniciens de la Parfumerie (A.I.T.P.)

25th International Symposium on Essential Oils

September 5-7, 1994, Grasse, France

PROGRAM

Monday, September 5, 1994 - Centre International de Grasse, Avenue Maximin Isnard

10.00 - 13.30	Registration.
13.30 - 14.00	Opening/Welcoming Ceremonies.
14.00 - 18.00	Plenary lecture and oral communications.

Evening : Free

Tuesday, September 6, 1994 - Centre International de Grasse, Avenue Maximin Isnard

8.30 - 12.20	Oral communications.
12.45 - 14.30	Buffet lunch at the Palais des Congrès, Cours Honoré Cresp.
14.30 - 18.30	Poster session, Palais des Congrès, Cours Honoré Cresp.
15.00 - 16.00	Round-table discussion organized by the Société Française des Parfumeurs, on the theme: «Future uses of essential oils in Perfumery».
19.30 - 22.30	Cocktail and ISEO Dinner. The awards for the best posters will be presented at the Symposium Dinner.

Wednesday, September 7, 1994, Centre International de Grasse, Avenue Maximin Isnard

8.3 - 12.10	Oral communications
12.10	Closing remarks
12.30	End of Symposium

25th International Symposium on Essential Oils

Monday, September 5, 1994

SESSION 1 :
Chair persons: Prof.-Dr. Baerheim Svendsen and Prof.-Dr. G. Buchbauer

2.00 pm K.-H. Kubeczka : «Twenty-five years progress in essential oils analysis - A retrospect».
2.50 E.-J. Brunke, F.-J.Hammerschmidt and G. Schmaus : «Recent results on the chemistry of sandalwood odour.»
3.10 I. Schwope., M. Schulze and P. Weyerstahl :»Synthesis of presilphiperfolan-9-ol isolated from *Artemisia laciniata*».
3.30 R. Näf and A. Velluz : «About juniper berry extracts. The role of germacra-1(10),5-dien-4β-ol».
3.50 Coffee/tea break

SESSION 2 :
Chair persons: Prof. C. Bicchi and Prof. R. Tabacchi

4.20 pm W.A. König., A. Rieck and I.H. Hardt : «Investigation of enantiomeric composition of sesquiterpenes in essential oils by enantioselective gas chromatography».
4.40 A. Mosandl, T. Köpke, P. Kreis, D. Juchelka, B. Maas and A. Dietrich : «Simultaneous stereoanalysis in the authenticity control of flavours and essential oils».
5.00 U. Ravid : «Essential oils as sources of natural aroma chemicals with high enantiomeric purity».
5.20 E. Wilhelm and E. Stahl-Biskup : «Enantiomeric composition of the chiral constituents in original and commercial lavender oils (*Lavandula angustifolia* Mill.)».
5.40 W. Schultze, W.A. König and R. Richter : «Differentiation of true lemon balm oil (*Melissa officinalis* L.) from adulterations by chirospecific GC analysis of citronellal and isotope ratio mass spectrometry».
6.00 End of the Session

Tuesday, September 6, 1994

SESSION 3 :

Chair persons: Dr. B. Lawrence and Dr. E. Stahl-Biskup

8.30 am	P. Weyerstahl, H. Marschall, C. Christiansen, A. Eckhardt, K. Schneider, L. Schwope., U. Splittgerber and H.-C. Wahlburg : «New or rare sesquiterpenes from various essential oils».
8.50	Y. Asakawa : «New terpenoids and aromatic compounds from several liverworts».
9.10	R. Kaiser and L. Tollsten : «An introduction to the scent of Cacti.»
9.30	D. Pezzuto, L. Ducci, M. Mariani, G. Speranza and P. Manitto : «Constituents of coriander leaf oil. Structure of the unsaturated aliphatic aldehydes».
9.50	J. Karlsen and A. Baerheim Svendsen : «25 years of research on the volatile constituents of *Juniperus communis* L. var. *saxatilis* (Norwegian mountain juniper)».
10.10	Coffee/tea break

SESSION 4 :

Chair persons: Prof. Y. Asakawa and Dr. Kaiser

10.40	K.R. Brain. V.J. James and A.C. Watkinson : «Determination of the dermal penetration of essential oil components used in aromatherapy».
11.00	G. Buchbauer, W. Jäger, L. Jirovetz, B. Nasel, Ch. Nasel, J. Ilmberger and H. Dietrich : «Aromatherapy research: studies on the biological effects of fragrance compounds and essential oils upon inhalation».
11.20	M. Nielsen, S. Rudong, O. Sterner and M.R. Witt : «Furanocoumarins with affinity to brain benzodiazepine receptors *in vitro*, from roots of *Angelica dahurica*».
11.40	M. Lis-Balchin, S. Deans and S. Hart : «Correlation of bioactivity with chemical composition of plant essential oils».
12.00	F. Chialva : «Benefits and limits of a new, easy and cheap vegetative reproduction system for peppermint. Three years of trials in Piedmont (Italy)».
12.20	End of the Session

14.30 - 18.30	Poster session at the Palais des Congrès and Round-table discussion with the French Society of Perfumers (SFP)

Wednesday, September 7, 1994

SESSION 5 :

Chair persons: Prof.-Dr. P. Weyerstahl and Dr. E.-J. Brunke

8.30 am	C. Bicchi, P. Rubiolo and M. Fresia : «Identification of essential oil component by GC and GC-MS techniques: some considerations».
9.00	T.A. Van Beek and D. Subrtova : «Application of silver(I) loaded HPLC ion exchangers in the separation of essential oil constituents».
9.20	Ch.R. Vuilleumier, I.A. Flament and P. Sauvegrain : «Headspace measurement of evaporation rates of perfumes applied on skin : Application to rose essential oils and their principal components».
9.40	J Casanova : «Computer-aided analysis of essential oils using carbon-13 NMR spectroscopy».
10.00	I. Hook, N. Comey and H. Sheridan : «Essential oil of edelweiss (*Leontopodium alpinum*)».
10.20	Coffee/tea break

SESSION 6

Chair persons: Prof.-Dr. K.-H. Kubeczka and Prof. Hiltunen

10.50	M.H. Boelens : «Chemical and sensory evaluation of Mediterranean lavandula oils». tem revisão da lavandula "available on request"
11.10	D. Chouchi, D. Barth and R.M. Nicoud : «Fractionation of citrus cold-pressed oils by supercritical CO_2 desorption».
11.30	Y. Gao, B. Simandi, M. Then and T. Veress : «Supercritical fluid extraction of fennel oil».
11.50	E.A. Aboutabl, H.L. De Pooter, H. Masoud, N.M. Sokkar and M.A.R. Megid : «Constituents and antimicrobial activity of the essential oil of *Otostegia fruticosa* (Forssk)».
12.10	Closing remarks
12.30	End of Symposium

NOTICE

The data, their interpretation and opinions presented by the authors participating in this symposium are their responsibility only. The Scientific Committee of this Symposium assumes no responsibility in this respect.

O-01

TWENTY-FIVE YEARS PROGRESS IN ESSENTIAL OILS ANALYSIS - A RETROSPECT.

K.-H. KUBECZKA

In the past, a great number of attempts have been made to improve the isolation of essential oils, the separation and identification of individual constituents. By means of selected examples, preferably which have been presented during the symposia on essential oils during the passed 25 years, the developments in this field will be discussed.

At first the influence of the isolation procedure on the oil composition will be discussed briefly. In addition to the commonly used hydrodistillation the advantages of combined hydrodistillation-solvent extraction, supercritical fluid extraction (SFE) and head-space techniques will be considered.

The applied separation techniques and the methods used for identification and structure elucidation of isolated compounds will be discussed according to the following classification:

1) Chromatographic separation techniques, above all improvements in gas chromatographic analysis including enantioselective separation and multi-dimensional techniques.

2) Instrumental on-line couplings of chromatographic separation devices with adapted spectrometers used in structure elucidation. Besides the commonly used GC/MS coupling, the application and improvements of additional hyphenated techniques in the field of essential oil analysis will be discussed, e.g. GC/FTIR, GC/UV and GC/AES.

3) Finally, as an alternative to the combined chromatographic/spectroscopic evaluation of a complex mixture, methods that provide direct information about the composition of a particular unprocessed sample without previous separation of components will be discussed.

In addition to this overview of different methods that have been successfully applied in the past to the analysis of essential oils and which are at present still used, some of the developments that might be expected in the near future will be mentioned.

Sauvage and Rocca, 1993
Scents of orchids – Kreis? methods

O-02

RECENT RESULTS ON THE CHEMISTRY OF SANDALWOOD ODOUR.

E.-J. BRUNKE, F.-J. HAMMERSCHMIDT and G. SCHMAUS

East Indian sandalwood oil is obtained by steam distillation of the heartwood and root of *Santalum album* L., a hemiparasitic Santalaceae growing wild in the mountaineous region of Southern India. Since ancient times, sandalwood oil is a highly appreciated and widely used perfume material, possessing a very pleasant and long-lasting, sweet-woody, animalic and slightly urinous odour which is accompanied by floral, green, buttery and nutty aspects. Due to the high price, the restricted availability and the great importance of sandalwood notes in perfumery, a lot of scientific research work on the steam distilled oil has been performed during the last decades resulting in the identification of many interesting new natural products.

During our recent work we concentrated on the sensory analysis of sandalwood oil and vacuum headspace concentrates from sandalwood by applying GC-sniffing techniques. Besides already known constituents, we additionally detected several minor compounds with interesting odours which were isolated by distillation and preparative chromatography.

The structures of these constituents, some of them being new natural substances, were elucidated by GC/MS, GC/FTIR and mainly by one- and two-dimensional NMR techniques, e.g. COSY-, HECTOR-, INADEQUATE-, and NOE-experiments. Our reinvestigations on sandalwood and sandalwood oil delivered a more clear picture of the olfactory importance of the individual oil constituents.

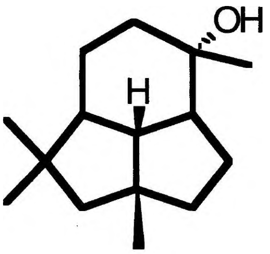
SYNTHESIS OF PRESILPHIPERFOLAN-9-OL ISOLATED FROM *ARTEMISIA LACINIATA*.

I. SCHWOPE., M. SCHULZE and P. WEYERSTAHL

As a part of an extensive screening programm of odoriferous plants from wild sources of the Kashmir region of India, we investigated some essential oils from different *Artemisia* species. One of the most interesting oils of this series is the essential oil from *Artemisia laciniata*, a plant collected at Hakinar-Gund (1800 m) and Gulmarg (2700 m). Both locations are near Srinagar, Kashmir. The odor of the essential oil is described as fresh, green, woody, herbaceous, urinous (Hakinar-Gund) and green, coumarin, hay, animal, urinous, herbaceous (Gulmarg).

We found sesquiterpene hydrocarbons and alcohols with silphiperfolane and presilphiperfolane skeleton [Weyerstahl, 1993]. Since the isolated sample of 9α-presilphiperfolanol **1** showed a very pleasant woody-sweet-spicy odor, we were interested in a synthesis of **1** to find out whether the observed odor really belongs to **1**.

We report the total synthesis of 9α-presilphiperfolanol **1** via 20 steps starting from 3,5,5-trimethylpent-2-en-1-one. Olfactive evaluation of **1** has shown that the synthetic compound possesses the interesting odor properties as strong, radiative, woody and amber.



1

O-04

ABOUT JUNIPER BERRY EXTRACTS. THE ROLE OF GERMACRA-1(10),5-DIEN-4β-OL.

R. NAEF and A. VELLUZ

The traditional essential oil of juniper berries is prepared by steam distillation. It contains complex mixtures of monoterpene hydrocarbons, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes, mainly with the cadinane or the elemane skeleton.

Significant differences in the composition of the volatile constituents are observed, when juniper berry oils are prepared by cold extraction procedures. We compared extracts from the same quality of berries prepared by traditional steam distillation or simultaneous extraction-distillation (Likens-Nickerson, L.N.), with extracts prepared with pentane, supercritical CO_2 or in a microwave oven.

These extracts present a more modest mixture of monoterpene hydrocarbons, and mainly germacrene-D and germacra-1(10),5-dien-4β-ol, which was isolated from a pentane extract and submitted to a L.N. extraction in an acidic medium. The unstable sesquiterpenic alcohol was rearranged to the same pattern of the cadinane and elemane derivatives as identified in the steam distillates. Calculations of the two most stable conformations of germa-1(10),5-dien-4β-ol by MacroModel (version 3.3, with force field MM2) confirmed the quantitative distribution of the α-cadinol, T-cadinol, T-muurolol and torreyol, the main substances formed by the cyclisation of the germacradienol.

INVESTIGATION OF ENANTIOMERIC COMPOSITION OF SESQUITERPENES IN ESSENTIAL OILS BY ENANTIOSELECTIVE GAS CHROMATOGRAPHY.

W. A. KÖNIG, A. RIECK and I.H. HARDT

After the introduction of cyclodextrin derivatives as chiral stationary phases in gas chromatography (gc) many stereochemical problems could be solved, particularly in cases of chiral compounds as constituents of complex mixtures like essential oils, pheromones or flavour compounds. We have already demonstrated the potential of enantioselective GC by separating the enantiomers of all monoterpene hydrocarbons commonly occuring as constituents of essential oils [König *et al.,* 1992]. The enantiomeric composition of these predominantly chiral plant volatiles may serve as a «fingerprint» with respect to phenotype variations, provenance and authenticity.

Sesquiterpene hydrocarbons not only display a much greater structural diversity than monoterpene hydrocarbons, but also contribute significantly to the specific fragrance character of an essential oil. Knowledge of the enantiomeric composition of these compounds should be important but has never been systematically investigated. Racemic mixtures of sesquiterpene hydrocarbons are not readily available.

We have therefore synthesized some of the less complicated structures as racemates, while others were obtained by chemical rearrangement reactions from racemic precursors. In most cases we isolated both enantiomers of sesquiterpene hydrocarbons from the essential oils of higher plants and from liverworts, which are known to contain mainly the «unusual» enantiomers, by preparative GC using packed columns with cyclodextrin derivatives as chiral stationary phases. With racemic reference compounds we were able to prove that most sesquiterpene hydrocarbons can readily be resolved into enantiomers.

For the determination of enantiomeric compositions of the sesquiterpene constituents of essential oils we applied two-dimensional capillary GC by combining conventional columns for preseparation with cyclodextrin coated capillaries for enantiomer separation in a double-oven gas chromatograph.

O-06

SIMULTANEOUS STEREOANALYSIS IN THE AUTHENTICITY CONTROL OF FLAVOURS AND ESSENTIAL OILS.

A. MOSANDL, T. KOPKE, P. KREIS, D. JUCHELKA, B. MAAS and A. DIETRICH

Authenticity control of flavours and fragrances is of considerable interest, because naturalness of food and beverages is highly demanded of the customer. Therefore, legal regulations of the European Union asks to differentiate between natural and nature-identical flavouring substances. While natural compounds are of natural origin, the nature-identical flavourings are chemically identical with their natural models, but obtained by chemical synthesis or isolated by chemical processes.

Multidimensional enantioselective capillary gas chromatography, employing heart-cutting from a non-chiral precolumn on to a chiral main column permits the simultaneous stereodifferentiation of the typical chiral constituents of mint oils, of buchu leaf oil and other essential oils. Chirality evaluation is discussed as an indicator of authenticity of flavours and essential oils.

Kreis, Dietrich, Mosandl Die Pharmazie 1994

O-07

ESSENTIAL OILS AS SOURCES OF NATURAL AROMA CHEMICALS WITH HIGH ENANTIOMERIC PURITY.

U. RAVID

The enantiomeric composition of natural chiral piperitone, menthone, isomenthone and pulegone isolated from various essential oils, was determined by chiral capillary gas chromatography on modified β- and γ-cyclodextrin phases. High enantiomeric purity of (4*S*)-(+)-piperitone was detected in the essential oils of *Mentha longifolia* (Yizre'el Valley type), *M. piperita*, *M. arvensis*, *Calamintha incana* and *Artemisia judaica.* The presence of (4*R*)-(-)-piperitone in high enantiomeric excesses in *M. longifolia* (Negev type) oil and in lower enantiomeric excesses in the other *Mentha* oils, is contrary to the postulation that only (4*S*)-(+)-piperitone occurs naturally in *Mentha* oils.

Enantiomerically pure (1*R*)-(+)-pulegone was isolated from the oils of two varieties of *M. longifolia, M. pulegium*, *M. sylvestris*, *Micromeria fruticosa* and *C. incana.* Very high enantiomeric purity of (1*R*)-(+)-pulegone (95-99 %) was detected in six oils of *M. piperita.*

Enantiomerically pure (1*R*,4*S*)-(-)-menthone and of (1*R*,4*R*)-(+)-isomenthone were detected in the oils of *M. piperita*, *M. longifolia*, *M. pulegium*, *M. sylvestris* and *M. reguienii.* Trace amounts of (1*S*,4*R*)-(+)-menthone and of (1*S*,4*S*)-(-)-isomenthone were detected in two oils of *M. piperita* and in two oils types of *M. longifolia.* In freshly distilled samples of geranium oils, (+)-menthone and (-)-isomenthone were detected in high enantiomeric purity. (-)-Menthone (trace -8 %) was detected in seven geranium Bourbon oils and in two geranium oils.

O-08

ENANTIOMERIC COMPOSITION OF THE CHIRAL CONSTITUENTS IN ORIGINAL AND COMMERCIAL LAVENDER OILS (*LAVANDULA ANGUSTIFOLIA* MILL.).

E. WILHELM and E. STAHL-BISKUP

The content of esters determines the quality of lavender oils. The German Pharmacopoeia (DAB 10) as well as the Austrian (OAB 90) and the American (USP XXI) require at least 35 % esters, 30-60 % is required by the Swiss Pharmacopoeia (Ph Helv VII). The ester content is usually represented by the ester value calculated as linalyl acetate, the main ester of the oil. This value includes other esters, such as bornyl acetate, lavandulyl acetate, α-terpinyl acetate, neryl and geranyl acetate, geranyl butyrate and aliphatic esters such as hexyl acetate, 1-octen-3-yl acetate, hexyl butyrate and hexenyl butyrate. Together they can amount to 1/3 of the esters. For this reason, the gas chromatographic method of the French Pharmacopoeia (PF X) is more appropriate, considering the actual linalyl acetate content, which must range between 25 and 40 %.

The maximum limit of 40 % seems justified, as shown by our investigations of 13 commercial oils. Three of them had linalyl acetate contents of more than 40 %. They proved to be adulterated when enantiomeric analysis of linalool and linalyl acetate was applied by means of gas chromatography of octakis(3-O-butyryl-2,6-di-O-pentyl)-—CD as described by König *et al.* (1992).

Lab-distilled lavender oils from fresh plant material harvested at two different stages of flowering were also subjected to enantiomeric analysis. For *R*-linalool enantiomeric excesses (ee) were always higher than 68 % (resp. 84 % *R*-linalool and 16 % *S*-linalool) and for *R*-linalyl acetate higher than 97.8 % (resp. 98.9 % *R*-linalyl acetate and 1.1 % *S*-linalyl acetate). These limits are in accordance with the results of Weinreich *et al.* (1992), but could not be reached by half of the investigated commercial samples. As a result a routine consultation of the enantiomeric ratios of the main chiral compounds is highly recommended in order to prove the authenticity of lavender oils.

O-09

DIFFERENTIATION OF TRUE LEMON BALM OIL (*MELISSA OFFICINALIS* L.) FROM ADULTERATIONS BY CHIROSPECIFIC GC ANALYSIS OF CITRONELLAL AND ISOTOPE RATIO MASS SPECTROMETRY.

W. SCHULTZE, W.A. KÖNIG and R. RICHTER

Essential leaf oil of balm (*Melissa officinalis* L., Lamiaceae) contains several monoterpenes among which mainly citral and citronellal account for the fresh and lemon-like odour of this oil.

Chirospecific GC analysis was performed on different chiral stationary GLC phases (heptakis[2,6-O-methyl-3-O-pentyl]-—cyclodextrin and octakis[2,6-O-methyl-3-O-pentyl]-—cyclodextrin) [König *et al.*, 1992] and showed that the enantiomeric composition of citronellal (solely or in combination with the amount of this compound) was a suitable criterion to detect adulterations of the expensive true lemon balm oil ((-)-citronellal: 1.8-2.1 %, (+)-form: 97.9-98.2 %) with other oils:

- Citronella oil (*Cymbopogon winterianus* and *C. nardus*): (-)-enantiomer 10-15 %, (+)-form: 90-85 %.

- Lemongrass oil (*C. flexuosus* and *C. citratus*): (-)-form: 30-55 %, (+)-form: 70-45 %.

- *Eucalyptus citriodora* oil: (-)-form: 40-46 %, (+)-form: 60-54 %.

- Catnip oil (*Nepeta cataria*): (-)-enantiomer: 98-99.9 %, (+)-enantiomer: 2-0.1 %.

In some types of lemon balm oils the citronellal content can be very low and the same holds for some of their adulterations (e.g. oils of certain *Cymbopogon* species and *Litsea cubeba*). Thus, it is impossible to directly analyse the enantiomeric composition of this marker compound by GC without previous time-consuming preseparation and enrichment processes. However, using GC/C/IRMS, we found that adulterations of true lemon balm oil with oils of *Cymbopogon* species can easily be detected by directly measuring $^{13}C/^{12}C$ isotope ratios of several essential oil compounds.

The δ-values range from -7.6 to -18.4 for the oil constituents of citronella oil (*C. winterianus*) and from -25.18 to -36.93 for the corresponding compounds of the *M. officinalis* oil.

O-10

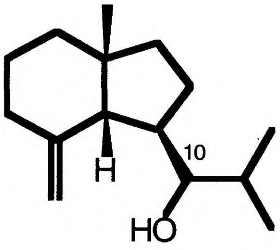
NEW OR RARE SESQUITERPENES FROM VARIOUS ESSENTIAL OILS.

P. WEYERSTAHL, H. MARSCHALL, C. CHRISTIANSEN, A. ECKHARDT,
K. SCHNEIDER, L. SCHWOPE, U. SPLITTGERBER and H.-C. WAHLBURG

Cis- (**1**) and *trans*-dracunculifoliol (**2**), sesquiterpene alcohols possessing the rare oppositane [8(7♦ 6)-*abeo*-eudesmane] skeleton, isolated from commercial Vassoura oil (*Baccharis dracunculifolia*) were synthetized in four diastereoisomers.

Porosadienone (**3**), the first member of the previously unknown porosane [8(7♦ 8)-*abeo*-eudesmane] skeleton was isolated from Phoebe oil (*Oreodaphne porosa*) and the structure was elucitated by NMR spectra.

The synthesis of **3** is just completed. Another Brazilian oil from *Lantana camara* is the source of the tricyclic aldehydes (e.g. **4**) possessing also a rare skeleton which was named before allo-cedrane or khusiane.



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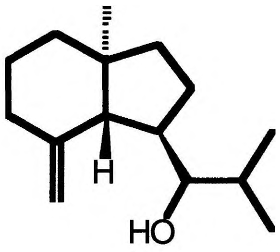

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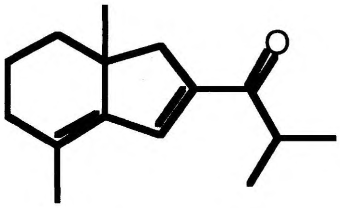

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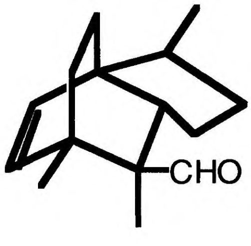


4

O-11

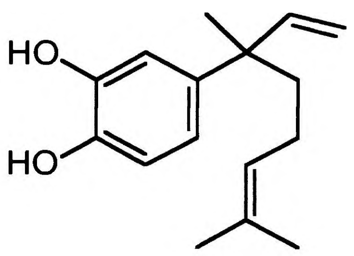
NEW TERPENOIDS AND AROMATIC COMPOUNDS FROM SEVERAL LIVERWORTS.

Y. ASAKAWA

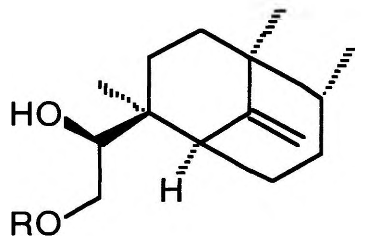
Liverworts (Hepaticae) are rich sources of terpenoids and lipophilic aromatic compounds. We are studying the biologically active substances of liverworts including characteristic scents [Asakawa,1982, 1990].

Recently we analyzed essential oils and ether extracts of 40 liverworts collected in Malaysia, New Zealand, South America and Japan and isolated 25 new compounds.

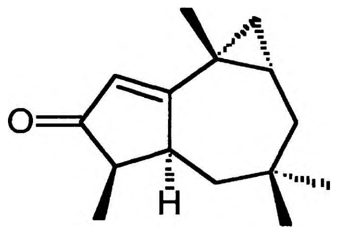
In this presentation, the isolation and structures of several novel compounds and identification of the characteristic fragrance from some liverworts will be discussed.



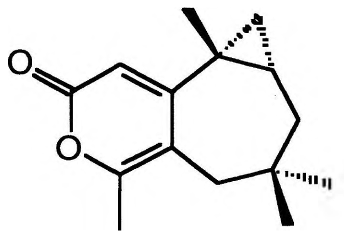
Serpentiphenol



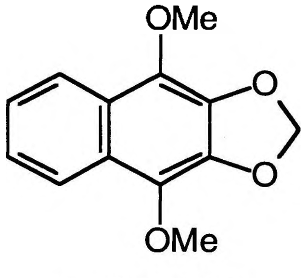
R=H Trifarienol A
R=Ac Trifarienol C



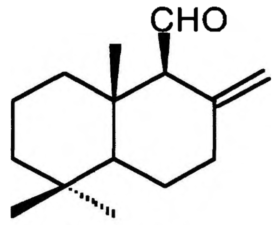
Swartzianin B



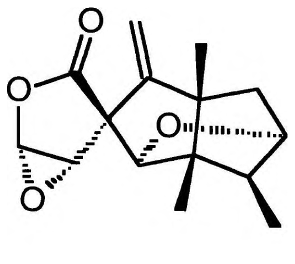
Secoswartzianin A



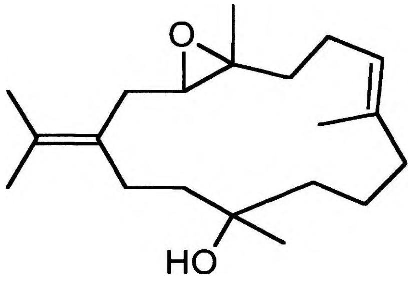
Wettstein B



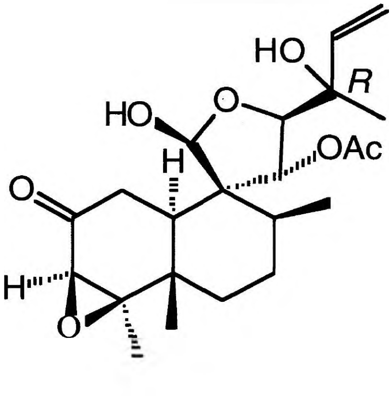
Albicanal



Spirodensifolin B



Setiformenol



Heteroscyphone A

O-12

AN INTRODUCTION TO THE SCENT OF CACTI.

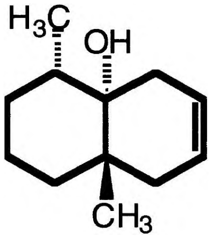
R. KAISER and L. TOLLSTEN

The Cactaceae is a large family of perennial trees, shrubs or shrublets of distinctive appearance, all more or less succulent and (excepting *Pereskia*) leafless or nearly so and all being native to semidesert regions of North, Central and South America. Up to now around 1500 species have become known which are subdivisded into ca. 100 genera. In comparison to other large families of flowering plants only a very limited number of species has been described in literature regarding their floral scent composition.

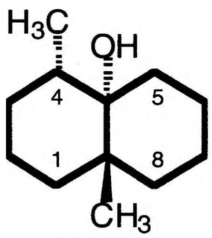
Although the flowers of Cactaceae are morphologically primitive they show adaptive traits towards different groups of pollinators, which is reflected to a certain degree in their scent composition. From the around 50 species investigated by us so far, some representatives will be discussed in more detail to illustrate the diversity in scent chemistry and in the characteristics of the individual ecological groups.

Special attention will be given to night-scented species flowering only during one night and being pollinated either by moths or by bats. The representatives of the first group show similarities to well-known moth flowers of other families and are in most cases characterized by pleasant so-called «white-floral» fragrances, while the second group is dominated by repulsive garlic- or cabbage-like odors caused by large amounts of sulfur-containing compounds like dimethyl disulfide and methyl thioacetate.

Furthermore, some structurally and/or olfactorily interesting constituents as (+)-dehydrogeosmin **1** and (-)-geosmin **2** will be discussed in more detail.



1 (+)-dehydrogeosmin



2 (-)-geosmin

O-13

CONSTITUENTS OF CORIANDER LEAF OIL : STRUCTURE OF THE UNSATURATED ALIPHATIC ALDEHYDES.

D. PEZZUTO, L. DUCCI, M. MARIANI, G. SPERANZA and P. MANITTO

Since ancient times *Coriander sativum* L. is well recognized as a medicinal and flavouring agent. Its seeds are largely utilized, while the essential oil obtained from leaves is used only to a minor extent and is also poorly known from the chemical point of view.

Two GC-MS determinations of volatile constituents of this oil have been reported in literature [Potter *et al.*, 1992] [McLeod *et al.*, 1976]. They appear to be in agreement on the majoritiy of the components but not on the structure of the most abundant ones, that is the C10-C14 unsaturated aldehydes. Unsaturation was suggested [McLeod *et al.*, 1976] to be located on the fifth carbon atom numbering from the methyl group in all aldehydes.

By contrast, Potter [Potter *et al.*, 1992] assigned the α,β-position to the double bond with respect to the carbonyl group. Such a disagreement prompted us to re-examine the essential oil from coriander leaves.

Our investigation was carried out on a Egyptian oil which was subjected to repeated fractional distillations in order to isolate the most abundant aldehydes in pure form. Unequivocal NMR evidence was then obtained for the presence of an (*E*)-configurated C=C bond conjugated to the aldehyde function in all compounds examined.

$$CH_3\text{-}(CH_2)_n\text{-}CH_2\text{-}CH{=}CH\text{-}CHO \qquad n = 5\text{-}7$$

25 YEARS OF RESEARCH ON THE VOLATILE CONSTITUENTS OF *JUNIPERUS COMMUNIS* L. VAR. *SAXATILIS* (NORWEGIAN MOUNTAIN JUNIPER).

J. KARLSEN and A. BAERHEIM SVENDSEN

Prior to the application of GC, four monoterpene hydrocarbons and one monoterpene alcohol had been identified in the needle oil of Norwegian high mountain juniper, *Juniperus communis* L. var. *Saxatilis* Pall. (1928): camphene, α-pinene, sabinene, terpinene and 1-terpinene-4-ol. GC on 8 m long, 1,5 mm ID packed columns revealed the occurrence of (1965): δ-3-carene, p-cymene, α-fenchene, β-pinene, myrcene, limonene, α-phellandrene, γ-terpinene, terpinolene, α-thujene and tricyclene. By capillary GC (1970) also *cis*—ocimene, *trans*—ocimene, α-*p*-dimethylstyrene and δ-4-carene were found.

Investigating the related species *Juniperus communis* L. and the indicated *J. communis* L. var. *saxatilis* the proportion α-pinene/sabinene/limonene was found to be 57:3:12 and 20:40:5 (1985/1992) respectively This proportion was found to be constant in plant material investigated during the years indicated.

We carried out new investigation on the *Juniperus communis* oils using coupled capillary columns, chiral stationary phase capillary GC and GC/MS and new data were obtained, that will be given in the poster to be presented. We are highlighting the advances made through new instrumental improvements but also the pitfalls in the presentation of «total analysis» of an essential oil.

O-15

DETERMINATION OF THE DERMAL PENETRATION OF ESSENTIAL OILS COMPONENTS USED IN AROMATHERAPY.

K.R. BRAIN, V.J. JAMES and A.C. WATKINSON

There has been a large increase in the use of aromatherapy over the past few years and a considerable amount of work has been carried out on the pulmonary effects of terpenes. It is therefore surprising that relatively little has been published on the percutaneous penetration of components of essential oils.

The area of skin to which products may be applied can be very large (up to 1.8 m^2) and co-application of massage and/or heat, and increased levels of skin hydration, will certainly increase the rate of permeation. In addition, a number of terpenes have been shown to dramatically increase the skin permeation of other compounds and safety questions regarding the unrestricted use of potentially toxic natural products have recently been raised. It is therefore important that the dermal absorption of terpenes is considered and quantified. Human *in vivo* studies are expensive, and the use of animal data is both ethically and scientifically questionable.

Mathematical modelling of the permeation process is therefore an attractive approach which can generate «ball-park» and rank-ordering data for particular compounds from the physicochemical properties of the permeant. However, this modelling technique does not take into account factors such as formulation effects, or modifications due to co-permeating species. These «in-use» phenomena can be more effectively and accurately assessed using *in vitro* human skin permeation techniques. A comparison of the application of these latter two approaches to the problem will be presented.

O-16

AROMATHERAPY RESEARCH: STUDIES ON THE BIOLOGICAL OF EFFECTS FRAGRANCE COMPOUNDS AND ESSENTIAL OILS UPON INHALATION.

G. BUCHBAUER, W. JAGER, L. JIROVETZ, B. NASEL, CH. NASEL, J. ILMBERGER and H. DIETRICH

In continuation of our work with animals inhaling fragrance compounds or essential oils, other fragrances were studied. A clear influence of the fragrance compounds on the motility of the test animals (mice) depending on the time of inhalation (circadian rythm) is discussed. In times of highest circadian activity e.g. the essential oil of lavender showed a marked decrease of motility.

In additional experiments with healthy volunteers, we studied the effects of some fragrances and essential oils on attentional processes (mainly alertness and vigilance). A comparative investigation as to alertness tests (defined as the general speed of information processing) was performed.

Finally new results of our studies on the cerebral blood flow in Xe-CT-experiments using linalyl acetate, one of the main constituents of the essential oil of lavender, are presented. These data are compared with those obtained upon inhalation of 1,8-cineol (one of the main constituents of the essential oil of rosemary).

O-17

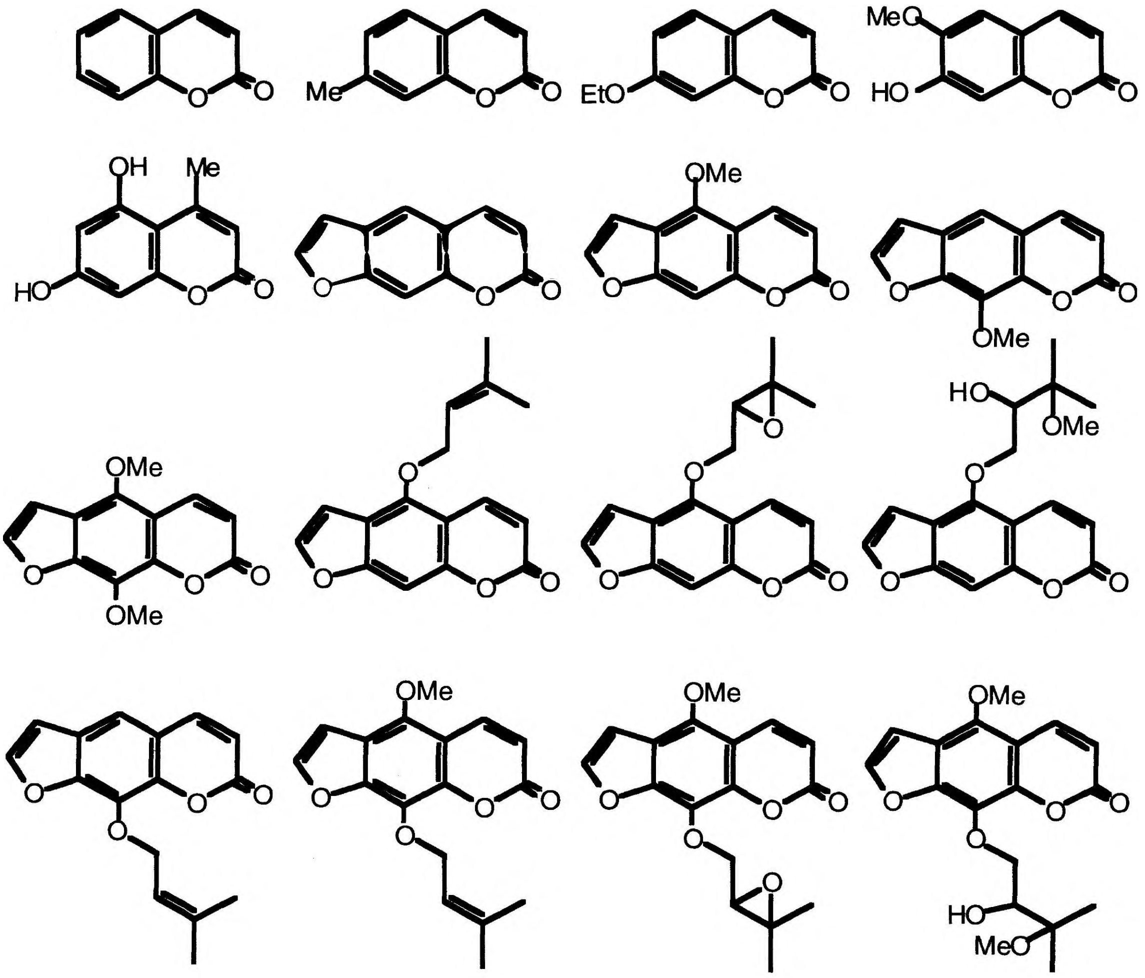
FURANOCOUMARINS WITH AFFINITY TO BRAIN BENZODIAZEPINE RECEPTORS *IN VITRO*, FROM ROOTS OF *ANGELICA DAHURICA*.

M. NIELSEN, S. RUDONG, O. STERNER and M.R. WITT

Radix Angelicae dahuricae, the dry root of *Angelica dahurica* Boiss., is listed in the Chinese Pharmacopoeia, and used as an antipyretic and analgesic for colds, headaches and toothaches. During a screening of Chinese medicinal plants for activity on central nervous system (CNS) receptors *in vitro*, methanol extracts of radix Angelicae dahuricae had a inhibitory effect on the binding of ^{3}H-diazepam to the benzodiazepine receptor.

The active compounds are furanocoumarins, and when comparing the activity of the most active (which has an IC_{50} value of less than 1 µmol/l) with similar furanocoumarins that are less active or inactive, interesting QSAR are suggested.

The poster will discuss the potency of the binding of the furanocoumarins to the benzodiazepine receptor, as well as structure-activity relationships.



O-18

CORRELATION OF BIOACTIVITY WITH CHEMICAL COMPOSITION OF PLANT ESSENTIAL OILS.

M. LIS-BALCHIN, S. DEANS and S. HART

A selection of about 50 essential oils were screened for their bioactivity against 25 bacterial species (including food, animal and plant pathogens) and three species of filamentous fungi (*Aspergillus ochraceus*, *A. niger*, and *Fusarium culmorum*). Their antioxidant values were also determined as was their pharmacological action against smooth muscle *in vitro.* The bioactivity results were correlated against the chemical composition obtained by gas-chromatography.

The results showed a very wide variation of bioactivity in different essential oils and also the same essential oil obtained from different plant and commercial sources eg. geranium, lavender, eucalyptus oil etc. The percentage of main components could not be correlated with the degree of bioactivity. The latter implies that there is a more complex correlation between bioactivity and chemical composition. Futhermore the commercial essential oils used for 'medicinal' purposes eg. aromatherapy can have variable results according to both botanical and commercial source.

During the screening an assessment was also made of the potential of different essential oils for ethnomedical application in developping countries.

O-19

BENEFITS AND LIMITS OF A NEW, EASY AND CHEAP VEGETATIVE REPRODUCTION SYSTEM FOR PEPPERMINT. THREE YEARS OF TRIALS IN PIEDMONT (ITALY).

F. CHIALVA

Three years of trials have been completed in the Piedmont (Italy) for the setting out of a new, easy and cheap plant reproductive system. The technique is based mainly on the direct transplant of vegetative epigeous parts growing in autumn after the summer's harvest. This method was found to avoid the fall earthling of stolons and/or the expensive transplantation of shoots in spring.

The main benefits obtained by this new method can be summarized in five items:

I) lower investments in mechanisation ;
II) higher operative feasibility which does not suffer of weather conditions ;
III) superior utilization, both in quality and quantity of the plant material, which is not affected by weeds ;
IV) better soil management by avoiding root and rhizome extirpation ;
V) higher control and lower diffusion of weeds.

The only limit is represented by the anatomical structure of stems and other epigeous parts which do not possess, like stolons and rhizomes, the water and drought saving systems to prevent dehydration. This problem would be particularly evident during dry and windy winters and/or when plants are cultivated on sandy soils.

However we found that easy expedients may overcome the problem.

O-20

IDENTIFICATION OF ESSENTIAL OIL COMPONENT BY GC AND GC-MS TECHNIQUES: SOME CONSIDERATIONS.

C. BICCHI, P. RUBIOLO and M. FRESIA

The analysis of an essential oil is increasingly carried out by GC or GC combined with spectroscopic techniques (mainly GC-MS, but also GC-FTIR). The development of combined techniques has greatly improved the success-rate possibility of component identification in essential oils. But the speed of computer elaboration has also increased the use of fully automated identification too far.

However, time automation has also tended to decrease the operator's critical evaluation of the results and of all that complementary but fundamental information, necessary to obtain reliable identification, which combined techniques can provide.

This communication aims to demonstrate, through some examples, how component identification is carried out as sometimes reported in the literature can be a source of serious mistakes. In particular, the identification through GC retention times on a single stationary phase, or the so-called «tentative» identification of a component through its mass spectrum, are discussed. On the other hand, the reliability of identification through retention indices (RI) on more than one stationary phase or by δ-RI on those components showing similar mass spectra is shown.

O-21

APPLICATION OF SILVER(I) LOADED HPLC ION EXCHANGERS IN THE SEPARATION OF ESSENTIAL OIL CONSTITUENTS.

T. A. VAN BEEK and D. SUBRTOVA

Apolar isomeric alkenes, like for instance essential oil constituents can be resolved by means of HPLC on cation exchange materials coated with silver(I) ion. The mechanism on which these separations are based is not fully understood of volatile alkenes, the retention of more than forty C2-C10 olefins besides a number of C14-pheromones and volatile terpenes has been determined.

The separation appears to be mainly governed by the type and number of double bonds (isolated, conjugated, aromatic), their steric accessibility (number and size of substituents) for complexation with silver(I) ions, the relief of inherent strain upon complexation and electron donating or withdrawing groups, or polar groups close to the double bond. Other functional groups, including carbonyls and overall polarity of the solute play only a minor role. Similarly most solvents have only a limited influence on the selectivity and speed of separation and function more as a transportation medium like in gas chromatography.

The comparative model gives semi-quantitative estimations of the retention time of many volatile olefins in the solvent methanol. Substituent parameters (*Ags*) which quantitatively forecast the influence of different alkyl side chains are part of this model. Some examples of the application of this HPLC system are shown: monoterpene hydrocarbons, terpene alcohols, 4 farnesol isomers, amyris oil and chamomile oil.

O-22

HEADSPACE MEASUREMENT OF EVAPORATION RATES OF PERFUMES APPLIED ON SKIN: APPLICATION TO ROSE ESSENTIAL OILS AND THEIR PRINCIPAL COMPONENTS.

CH. R. VUILLEUMIER. I.A. FLAMENT and P. SAUVEGRAIN

We report that diffusion of perfume ingredients from skin and hair can be measured by an original methodology based on dynamic headspace technology. This innovative concept, may be used for raw materials, essential oils, fine fragrances, as well as for perfumed cosmetic applications such as soaps, creams or shampoos, in order to characterise diffusion and air/skin or air/hair partitioning.

Accordingly, a special collection system, applied on the internal face of the forearm, has been developed allowing the adsorption of diffusing organic vapours from skin onto Tenax (poly-diphenyl phenylene oxide) with a controlled air flow rate. Various rose essential oils have been studied with respect to their principal components.

The diffusion rates of these components were measured by determining the concentration of each in the gas phase versus time. Conversely, the same experiment was effected by the application of an alcoholic solution of each individual component. In this manner, the relative diffusion from skin of the components alone or as a part of the essential oils was compared using the same experimental technique. It should be noted that these results were obtained on an «Average Type Skin» which had been previously characterized by measuring surface parameters including pH, hydration and lipids amount.

O-23

COMPUTER-AIDED ANALYSIS OF ESSENTIAL OILS USING CARBON-13 NMR SPECTROSCOPY.

J. CASANOVA

The structural and quantitative analysis of complex mixtures such as essential oils, is commonly realized by GC, GC-MS, GC-FTIR or a combination of these techniques. In a different way, carbon-13 NMR spectroscopy could be used for non separative, non destructive identification of the individual components of complex mixtures such as essential oils, petroleum distillates and triglycerides.

We applied this methodology to identify the main components of essential oils. We developped an experimental procedure, based on computer-aided analysis of the atom ^{13}C NMR spectrum of the mixture. We compared the chemical shift of each carbon in the experimental spectrum with spectra from pure compounds listed in our data bank. The spectral parameters (concentration, low power decoupling, temperature) were optimized to allow a good accuracy of the chemical shift values.

Each compound is identified by taking into account :

i) the number of the identified carbon,
ii) the number of superpositions of signals,
iii) the difference of chemical shift of each resonance in the mixture spectrum and in the reference.

First, we tested the experimental procedure and the software with synthetic mixtures of terpenic derivatives usualy present in essential oils. Then we analyzed a large number of essential oils from a great variety of plant material. In all cases, this method allowed a good identification of mono-, sesqui- and diterpenes present in essential oils (lower limit: 0,5-1 %), including structurally close molecules (stereoisomers) and compounds separable with difficulty by GC. This method, which allows the identification of the main components from one spectrum only, is very well suited for chemical polymorphism studies.

Further work is in progress to achieve :

i) a quantitative analysis of the main components using inverse gated decoupling mode with or without internal reference,
ii) a complete and unambiguous analysis of some complex essential oils by GC-MS and carbon-13 NMR spectroscopy,
iii) an enantiomeric differenciation using chiral shift reagents.

ESSENTIAL OIL OF EDELWEISS

I. HOOK, N. COMEY and H. SHERIDAN

Edelweiss (*Leontopodium alpinum*, Compositae) is a protected plant indigenous to the mountainous regions of Europe. Although aerial parts of the plant have some suggested medicinal properties, its flowers are more famous as the touristic emblem of alpine countries.

Our phytochemical evaluation of cultivated samples of this plant resulted in the isolation from the roots of ca. 2.0 % of an essential oil. Examination of oil yields over a 12-month growth period suggested a maximum content during the winter months, when the plants were dormant.

Gas chromatography of the oil indicated the presence of ca. 50 constituents, two of which were always present in major concentration. Seasonal changes in oil composition were apparent. Although GC-MS has indicated predominantly sesquiterpene components, separation and absolute identification of individual compounds has proved difficult, due mainly to lack of plant material.

The development of *Agrobacterium rhizogenes* - transformed «hairy» roots of edelweiss has allowed us to isolate an oil qualitatively similar to the natural root oil and is currently being subjected to chromatographic separation. Several lines of these fast-growing fransformed roots are being cultured, all producing oils in differing yields and of varying composition. Ways of influencing productivity and composition are currently being examined. The essential oils isolated from edelweiss plants developed from wild and cultivated sources are also to be evaluated.

O-25

CHEMICAL AND SENSORY EVALUATION OF MEDITERRANEAN LAVANDULA OILS.

M.H. BOELENS

The chemical composition of lavandula oils from France, Italy and Spain was studied for ecological reasons.

The oils concern isolates from:

- *Lavandula angustifolia* Mill. (lavander)
- *Lavandula hybrida* Rev. (lavandin)
- *Lavandula latifolia* Med. (spike lavender)
- *Lavandula stoechas* L. (cantueso)

More than 450 compounds have been identified in the oils. About 60 constituents were quantified in each oil, comprising over 95 % of the oil.

The variation in the concentration of the following main groups of constituents will be discussed in relation with the climatological growing environment of the plants:

- linalool, linalyl acetate and lavandulyl acetate;
- camphene, borneol (and acetate), camphor and fenchone;
- 1,8-cineole.

The French and Italian lavender and lavandin oils are featured by their relatively high contents of linalool, linalyl acetate and lavandulyl acetate, whereas the spanish spike lavender and cantueso oils possess high contents of camphor and fenchone.
The partly formation of the following compounds during isolation and storage will be mentioned: ocimenes, linalool oxides, plinols, caryophyllene oxide.

Special attention will be paid to chemical and sensory evaluation of substituted pyrazines and pyridines as trace constituents in the oils.

A sensory evaluation of the oils has been carried out with respect to the following odour aspects: green, fruity, floral, herbaceous, camphoraceous, aromatic, woody and balsamic.

O-26

FRACTIONATION OF COLD-PRESSED CITRUS OILS BY SUPERCRITICAL CO_2 DESORPTION.

D. CHOUCHI, D. BARTH and R.M. NICOUD

Cold-pressed citrus and most other related essential oils consist of mixture of terpenes, sesquiterpenes, oxygenated compounds and non-volatiles such as dyes, waxes and coumarin derivatives, some of them being phototoxic.

The oxygenated compounds are highly odoriferous and are the valuable products. The terpenes and sesquiterpenes also slightly contribute to the flavor. But these compounds are unstable to heat and light and give rise of to off-flavors ; therefore their removal improves oils stability. Moreover deterpenated oils are more soluble in alcohol: they are used as starting material in flavour industry, perfumes, cosmetics and pharmaceuticals.

The presence of coumarin derivatives in oxygenated fractions decreases the stability and the flavor quality of the samples. The common procedures for folding cold-pressed oils are vacuum distillation, which produces thermal degradations and different techniques requiring organic solvents which have to be removed : HPLC, extraction with alcohol...

Fractionation with supercritical extraction has been investigated but doesn't give good yield due to the lack of selectivity (limonene behaves like a co-solvent).

We show that a combination of supercritical fluid extraction and desorption allows the deterpenation and coumarins removal of various oils with good yields. The process is investigated on a pilot plant (column filled with Kieselgel : i.d.= 2.3 cm, L = 40 cm) with different composition in oxygenated compounds (bergamot 40%, Eurêka Lemon 3%, Coci lemon 2%, bigarade 1%, mandarin 1% and lime 15%). The average enrichment in oxygenated compounds is excellent: 2 for bergamot, 20 for Eurêka lemon, 30 for Coci lemon, 15 for mandrin, 50 for bigarade and 4.5 for lime oils.

The yield of fractionation depends on the amount of oxygenated compounds in the peel oil, the injected mass and the programming pressure.

O-26

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The yield of fractionation depends on the amount of oxygenated compounds in the peel oil, the injected mass and the programming pressure.

O-27

SUPERCRITICAL FLUID EXTRACTION OF FENNEL OIL.

Y. GAO, B. SIMANDI, M. THEN and T. VERESS

In recent years the increasing industrial production of meat products, snack foods and beverages, as one of its consequences, caused spice essential oils and extracts to become more prominent, often as replacements for natural spices.

The conventional methods used for the preparation of essential oils and extracts include steam distillation and solvent extraction. However, these methods they have some disadvantages. Steam distillation usually results in the loss of the highly volatile and heat-sensitive components. Solvent extraction makes it impossible to remove all residual solvent from final products.

Supercritical carbon dioxide extraction is a novel technology, particularly suitable to substitute traditional methods. A natural spice extract, free of solvent residues, with no changes resulting from high temperature and water, can be prepared by this method because of the favourable behaviour of carbon dioxide (non-toxic, non-explosive, readily available, easily removed from finished products).

Supercritical fluid extraction (SFE) is more selective than the extraction with hexane and ethanol, its products resemble more closely the aroma of the raw materials than the aroma of products produced by conventional methods. So it is widely applied in the food industry to extract various useful constituents from the natural foodstuffs.

Utilization of SFE for the preparation of analytical samples has been demonstrated in a number of reports over the past ten years. SFE (coupled with supercritical fluid chromatography) allows the separation and analysis of thermally labile components without resorting to sample derivatization.

Based on a previous work, in this paper fennel fruits powder was extracted with supercritical carbon dioxide, in a high pressure apparatus with 5 L extractor volume, made in Hungary. The extraction was carried out under different conditions in order to separate extracts into essential oil rich fraction and fatty oil rich fraction. This process was compared to conventional steam distillation and to Soxhlet extraction with alcohol and hexane. All the products were analyzed with TLC and GC methods.

CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *OTOSTEGIA FRUTICOSA* (FORSSK).

E.A. ABOUTABL, H.L. DE POOTER, H. MASOUD, N.M. SOKKAR and M.A.R. MEGID

Otostegia fruticosa (Forssk) is a herbaceous aromatic Lamiaceae plant which grows wildly in certain localities in Egypt [Tackholm, 1974]. Prior to this study, the plant had not been investigated for its volatile constituents.

In this work, the hydrodistilled essential oil of fresh aerial parts of the domesticated plant was analysed by capillary GC-MS. Thirty-six compounds were identified by their identical GLC Kovat's indices [Kovat, 1965] to known compounds and by comparison of their mass spectra with reference compounds or published spectra [Adams, 1989, 1981].

Quantitative determination was carried out based on peak area integration. Nearly equal amounts of oxygenated (47.6 %) and non-oxygenated (51.2 %) compounds were identified. The main oxygenated compounds are the phenols: thymol (43.7 %) and carvacrol (0.8 %), alcohols constituting only 1.4 % of the oil. Twenty-one hydrocarbons representing 32.1 % of the oil and comprising monoterpene (16.6 %) and sesquiterpene (17.6 %) hydrocarbons were identified. Para-cymene (12 %) and β-caryophyllene (9.5 %) were found to be the main hydrocarbons in the oil.

Antimicrobial screening of a dimethylsulfoxide solution of the oil was carried out according to the general qualitative assay described by Clark *et al.* [1981] using certain bacterial strains and fungi and will be discussed.

Due to its high thymol and carvacrol content, the essential oil of *O. fruticosa* can be recommended to substitute the volatile oil of thyme as a flavouring, disinfectant and antiseptic agent in pharmaceutical preparations, food products, soaps, deodorants and other cosmetics.

P-01

FIRST RESULTS IN IDENTIFICATION OF PLANT SUBSTANCES BY COMBINING GC/MS AND GC/DIRECT DEPOSITION/FTIR.

J. AUGER and S. FERARY

Until now, identification of GC components present in plant odors or extracts have been made with MS. However the complexity of many samples may require the use of more than one analytical technique to ensure adequate sample characterization.

For instances:
- MS cannot distinguish structural isomers which are encountered in virtually every GC application but IR spectra can do so clearly
- with the addition of the structural information provided by the analysis of IR spectra, identification of unknowns, such as those traditionally done by MS alone, are far more reliable.

Unfortunately the past use of GC/IR with light-pipe interface has suffered from bad sensitivity. We can use now a new interface (condensed phase technology) which has more than 100 times the sensitivity of the light-pipe methods: analytes eluting from the GC are frozen as pure substances onto an IR transparent window. Immediately after deposition, peaks are scanned and their IR spectra are displayed in real time.

Any point may be rescanned in order to achieve a higher signal to noise spectrum. To avoid interface problems, we prefered, instead of the parallel unique GC/IR/MS configuration, to use 2 distincts GC (Hewlett-Packard HP 5890 A with the same columns and the same conditions), the first linked to the MS (HP 5989 A), the other linked to the RI (BioRad FTS 45A) with the direct deposition interface (BioRad Tracer).

We applied successfully those combined hyphenated techniques to the characterization of various allelochemicals responsible of insect plants relationships (possible bio-pesticides).

For instance we identified numerous isomeric disulfides in *Allium* odors and we succeeded very easily in the identification of a new alkaloid in *Rutaceae* cell cultures by using IR and MS informations even if the compounds were not mentioned in libraries.

P-02

CHARACTERIZATION OF THE FRAGRANCE OF *THYMUS VULGARIS* BY CONVENTIONAL HEADSPACE SAMPLING AND BY MAP^{TM}.

A. BÉLANGER, L. DEXTRAZE, J. BÉLANGER and J., PARÉ

The fragrance of various specimens of *Thymus vulgaris* have been characterized by gas chromatographic analyses. Conventional one-hour static headspace sampling performed at 80°C and the newly developed gas-phase extraction applications of the patented Microwave-Assisted Process (MAP^{TM}) have been used as sample preparation methods.

The results obtained by both techniques are presented on a comparative basis and the unique characteristics of the microwave assisted process, MAP^{TM}, are discussed in greater details. A greater sensitivity level and a reduced sensibility to the state of the plant material at the time when the analysis is performed are among the advantages beside the obvious dramatic reduction in sample preparation time (less than one minute).

Furthermore, the gas-phase extract profile is more closely related to that of the essential oil of the same material, a net advantage for the purpose of assessing the value of a given lot of plant material. The reproducibility of the results clearly demonstrates that the microwave-assisted process, MAP^{TM}, offers a most valuable alternative to current static headspace technology. The rapidity and the ease of operation make the technique suitable for field work.

P-03

CYCLODEXTRIN DERIVATIVES IN GC SEPARATION OF RACEMIC MIXTURES OF VOLATILES.

C. BICCHI, A. D'AMATO, V. MANZIN, A. GALLIA and M. GALLI

This communication aims to report the results of a series of experiments carried out mainly in the field of essential oils and of perfumed compounds with different cyclodextrin (CD) derivatives. 2,6-di-O-methyl-3-O-pentyl-—CD was previously demonstrated to be successful in separating oxygenated and non-oxygenated volatiles, while avoiding the drawbacks of 2,3,6-tri-O-methyl-—CD in terms of column stability and operating temperatures.

Since a second stationary phase possibly with a polar substituent is necessary to separate several racemates (mainly oxygenated) not separated by all-alkylated CD derivatives and since the stability of the columns prepared with 2,6-dimethyl-3-trifluoroacetyl (or acetyl)-CD derivatives is very low, new CD derivatives showing comparable separation power but without the drawbacks mentioned, were synthetized.

The following CD derivatives have been synthetized: 2,6-di-O-methyl-3-O-(2-oxopentyl)-—CD, 2,6-di-O-pentyl-3-O-(2-oxo-pentyl)-β and γ-CD, 2,3-di-O-acetyl-6-O-(*t*-butyl-trimethylsilyl)-β and γ-CD. The performance of columns coated with the previous CD derivatives diluted in OV-1701 or OV-1701-OH will be shown. Each column coated with each of the newly synthetized CD derivatives was evaluated by analysing more than 150 different racemates with different structures.

DIRECT DETERMINATION OF THE ENANTIOMERIC PURITY OF MENTHOL IN MINT ESSENTIAL OIL USING CARBON-13 NMR SPECTROSCOPY.

D. RISTORCELLI, F. TOMI and J. CASANOVA

Optical purity of enantiomers is of great importance in the chemistry of natural products, especially in the fields of flavours, fragrances and pheromones. The human olfactory organ is able to discern between chiral compounds.

The enantiomteric excess is generally determined by gas chromatography using chiral columns. The ^{1}H NMR spectroscopy associated with the chiral lanthanide shift reagents (LSR) has been used for enantiomeric studies of drugs and of some isolated essential oils components.

The method developped in our laboratory allows a rapid identification of individual components of essential oils using ^{13}C NMR spectroscopy. Here, the determination of enantiomeric purity of major compounds inside essential oils directly has been achieved using chiral LSR in ^{13}C NMR spectroscopy. No fractionation of the sample has been necessary.

In this paper, we show the successful application of one optically active shift reagent, tris-(3-heptafluoropropylhydroxymethylene)-(+)-camphorato ytterbium (III) [Yb(hfc)$_3$], with the menthol in a mint essential oil.

GAS CHROMATOGRAPHY ISOTOPIC RATIO MASS SPECTROMETRY APPLICATIONS - AUTHENTICITY OF FLAVORS.

B. FAYET, C. TISSE and M. GUERERE

The European Directive 88-388 recognizes several kinds of flavors, among these it specifies the natural aroma as the mixture of only natural compounds. For characterization of these natural compounds the isotopic analysis of carbon may be used (for example vanillin in vanilla products).

With conventional Isotopic Ratio Mass Spectrometry, the analytical difficulty stays in extraction and purification of the interesting compound. The new technology Gas Chromatography Isotopic Ratio Mass Spectrometry is more convenient for this purpose because the purification step is omitted. Furthermore the quantities required for this measurement are a lot smaller. So it is possible to carry out the carbon-13 analysis on different compounds of aroma and to extend this determination to flavored foods not already examinated.

For example, natural flavorings, by direct injection after extraction with methylene chloride, have been studied by this technique. The results are submitted in chart 1.

Compound	Apricot	Blackcurrant	Hazelnut
Gamma-decalactone	-27.3	not detected	not detected
Gamma-hexalactone	-27.2	not detected	not detected
Vanillin	-28.1	-25.8	-20.1
Benzaldehyde	- 28.1	not detected	not detected
Maltol	not measured	-18.5	not detected
Raspberry ketone	not detected	-27.5	not detected

Some values show that apricot and blackcurrant aroma are not natural.

Another example is analysis of vanillin in vanilla flavored ice cream. After optimisation of the extraction, the etheral extract is analysed. The result -20.8 ‰ PDB is in good agreement with the value -21.1‰ PDB found for aroma used in the preparation. The last example is peach flavored tea. After manual pick out of encapsulated aroma, these are dissolved in ether before analysis. Results are in chart 2.

Compound	Carbon-13 deviation ‰ PDB
Linalool	-27.3
Delta-decalactone	-25.7
Gamma-undecalactone	-26.3
Delta-undecalactone	-25.7
Delta-dodecalactone	-28.3

INHERITANCE STUDIES ON SAGE (*SALVIA OFFICINALIS* L.) ESSENTIAL OILS(1).

A. EL-BELBISSY, A. BEZZI, G. CIRCELLA and CH. FRANZ

Variability studies of two sage (*Salvia officinalis* L.) populations have shown different essential oil patterns of the single plants mainly varying in their monoterpene content (α- and β-thujone, 1,8-cineole, camphor) [Bezzi *et al., 1*992]. Six distinct clones were used for crossing studies in order to clarify the inheritance of the main compounds as well as of some morphological characters. This should be the basis for breeding of chemocultivars used for different purposes.

Prerequisite of such breeding programmes is the knowledge of floral and reproduction biology: besides male fertiles one male sterile clone could also be identified, but due to environmental circumstances, also intermediate flowers with stamina and pollen more or less fertile were observed within one plant.

To get comparable results, the fifth leaf from the tip was used for HT/HS-gas chromatography [Franz, 1992]. According to the high heterozygosity of the selected clones the F1 has shown a certain tendency to medium levels of the main compounds. Similar results were reported by Putievsky *et al.* [1990] from interspecific crosses of three Salvia species. Besides it, outcrossings of chemotypes - especially α- and β-thujone - were observed in inbred lines and some crossings, leading to first assumptions concerning the inheritance control.

(1) Communication of the Austro-Italian research project Genetics and Breeding of Medicinal and Aromatic Plants.

P-07

TOTAL RECOGNITION OF CHROMATOGRAPHIC PROFILES FROM COMPLEX MATRICES.

R. FELLOUS, P. GAVINI, E. ANGELINI and L. DZIUBA

Three different approaches allow to access to the recognition and to the global identification of Complex Mixtures. From these results have developed an automatic technique of global recognition of Complex Mixtures within constituted Complex Compositions of several mixtures.

The first step consists in transforming chromatographic profiles into sticks profiles. These new profiles relate to mass spectra, in which each fragment is defined in abscissa by the retention index and in ordinate by the intensity of the peak in the analysis. This transformation facilitates the connection of the processing of data and the implementation of the PBM algorithm, as identification method, on a single system of exploitation.

A second step allows to transfer raw data under a standard exchangeformat of spectroscopic data, the JCAMP format. It becomes thus possible not only to forward data of varied sources on different systems of exploitation, but also to incorporate automatically into various apparatus, acquired mass spectra, in libraries to the PBM format. Finally we have conceived a program of automatic research of Complex Mixtures.

In the last step of our work we have built a database with the assistance of a software on a Macintosh computer. Then we have elaborated our own recognition algorithm, which articulates around the comparison of mass spectra on a variable retention index range. Each peak of the Composition selects Mixtures in which this constituent is the most intense. One searches in return in each Blend the substances that belong to the Composition.

P-08

RHYTHMIC AND NON-RHYTHMIC EMISSION OF VOLATILES FROM *BRASSICA NAPUS* IN SITU.

H.B. JAKOBSEN. P. FRIIS., J.K. NIELSEN and C.E. OLSEN

The volatiles emitted in situ from intact *Brassica napus* plants under controlled environmental conditions were analyzed qualitatively and quantitatively. Separate headspace samplings from flowers and leaves showed that six volatiles were emitted from the flowers only, twelve compounds were common to both flowers and leaves and eleven compounds were emitted from the leaves only.

Monoterpenes comprised the major part of the floral fragrance, whereas alkanes dominated the leaf fragrance. The average emission of floral volatiles was 45 mg per flower per hr.

Rhythmic emission was shown for sabinene and limonene, both emitted from flowers and leaves. In contrast, no rhythm of emission was detected for the major compounds emitted from the flowers only, i.e. α-farnesene, linalool and 3-carene. A rhythmic nature of emission could not be discerned after transfer to constant environmental conditions.

The results differ from previous investigations performed on detached plant material. The significance of floral volatiles in the plant-insect relationship is discussed.

P-09

DISTILLATION - EXTRACTION IN SPE-CARTRIDGES : A NEW PROCEDURE FOR THE SEPARATION OF ESSENTIAL OILS IN THE BREEDING OF MEDICINAL AND SPICE PLANTS.

H. KRUGER and B. ZEIGER

Breeding often requires the investigation of very small amounts of plant material. Special microtechniques in essential oil analysis have been described [Bicchi *et al.*, 1987] [Giesselmann *et al.*, 1993], our version is a simple steam distillation - extraction in SPE - cartridges (SPE: solid phase extraction).

The essential oil is transported by water steam into the solid phase, which is cooled. After the distillation time, the plant drug is removed, the oil is eluted (2x1 ml n - hexane/acetone : 9/1) and analyzed by capillary GLC.

Peppermint and savory have been taken as representive examples to demonstrate the potential of the applied technique. The main components of the SPE -eluates were compared to oils from identical samples obtained by conventional hydrodistillation. The results show a good correlation of the qualitative and quantitative compositions.

P-10

SYNTHESIS WITHIN THE 7-OXANORBORNANE SERIES.

PH. LEBADA, H. HOLBIK, H. SPREITZER and G. BUCHBAUER

β-Santalol, one of the main constituents of the East Indian Sandalwood oil, is reponsible for its unique woody fragrance [Brunke *et al.*, 1982].

In connection with our studies about structure-activity relationships of fragrance compounds exerting sandalwood odour we already showed that the variations of the β-santalol sidechain can lead to weakening and lost of odour [Buchbauer, 1977] [Zechmeister-Marchhart, 1993]. Therefore it seemed interesting and worthwhile to alter the methylene bridge of the bicyclo(2.2.1)heptane system of the β-santalol molecule to prepare new analogues [Buchbauer *et al.*, 1986]. In this paper, we wish to report on 7-oxabicyclo(2.2.1)heptane derivatives.

The access to the bicyclic ether could be realized by *Diels-Alder* reaction of furan with ethyl allenecarboxylate [Lang *et al.*, 1984]. Unfavourable reaction conditions [Ishar et al., 1990] [Landscheidt, DE 3.235.399, 1984] [Holbik, 1990] causes *Retro-Diels-Alder* reaction or polymerisation. On account of the success of the *Diels-Alder* reaction under sonification conditions we started also ultrasound experiments with *Lewis* acids [Buchbauer *et al.*, 1994]. Thus the target bicyclic system could be obtained in good yields. Methylation could be realized only by CH_3I following the procedure of *Vogel* [Holbik, 1990] [Warm *et al.*, 1985], whereas other conventional methods failed with this system. Efforts to establish the appropiate sidechain of the target molecule are described.

P-11

MULTIVARIATE CORRELATIONS OF CG/MS/FTIR DATA OF ENANTIOMERS IN COMPLEX AROMA MIXTURES (EXTRACTS AND ESSENTIAL OILS).

A. NIKIFOROV, L. JIROVETZ and A. WOIDICH

The possibility of enantiomer detection in complex aroma mixtures (eg. lactones in strawberry extracts or linalol in essential oils) was investigated using a combination of chiral/achiral GC column separations with mass spectrometer and Fourier Transform InfraRed spectrometer (FTIR) as detection device and multivariate evaluation procedure of resulting data sets.

For enantioselective gas chromatography, different column types of chiral phases on basis of peptides (Chirasil type) and cyclodextrins (O-alkyl, O-trifluoroacetyl type) were used, while the achiral separation was performed with HP-5 methylsilicon phase column. The best chiral separations were achieved with cyclodextrin columns in all cases investigated. As detectors the flame ionisation detector, mass spectrometer (MSD from Helwett-Packard) and infrared spectrometer (IRD from Hewlett-Packard) were used.

In the multivariate evaluation of the data, the strategy and the approach used involved two steps: in the first step using the achiral separation with the help of similarity profiles for spectral data all detectable isomeric components in the registered (achiral) GC/MS/FTIR data set were selected and compared in turn with the corresponding table of detectable isomers resulting from the application of the same procedure on the (chiral)-GC/MS/FTIR data set in the second step. So far, in the first step the diastereomers and simple isomeric components of a given type are found. In the second step, the procedure proves the extent of further separation of a given type when enantioselective separation is used. Thus the valuable information about the racemisation degree of separable enantiomeric components was tried to be visualised for complex systems.

For procedure development and for training purposes, a mixture of five fully racemised γ-lactones was tested first, and the procedure then applied to the aforementioned systems. All evaluations were performed on Highscreen 586-60 Pentium personal computer using programs written in TurboPascal 6.0 after transfer of original data to personal computer via Ethernet link.

(*Aknowledgment*: The support of Austrian Research Foundation (FWF), Project P9568Che is gratefully aknowledged).

P-12

EVALUATION OF ESSENTIAL OILS FROM THE FAMILIES UMBELLIFERAE, PINACEAE AND LABIATAE USING PRINCIPAL COMPONENT ANALYSIS.

R.J. OCHOCKA and H. LAMPARCZYK

Chemical analysis of essential oils is a difficult task because of their variability, complexity and wide range of possible impurities. Some of the impurities cannot be detected using chromatographic techniques. Therefore, the measurements of commonly accepted physical constants make them indispensable also in the future.

On the other hand, for effective application physical constants should be considered produce multivariate problems. Hence, this work is an attempt to resolve these problems using principal component analysis - PCA [Ochocka *et al.*, 1992] [Ochocka *et al.*, 1993].

Physical constants, such as density, refractive index and optical rotations at various wave lengths were measured for essential oil samples from different species of families Umbelliferae, Pinaceae and Labiatae. The data were processed using PCA.

The results indicate that this multivariate statistical method greatly assisted the analysts in the assessment of the identity and quality of essential oils. Some preliminary suggestions concerning selection of the physical constants are described.

P-13

INTEREST OF A MULTI GAS SENSORS APPARATUS FOR QUALITY CONTROL OF ESSENTIAL OILS.

T. TALOU and A. GASET

Qualification and quantification of odorous volatile compounds emitted by aromatic extracts are two important factors of Quality Control methodology. Actually, physico-chemical techniques (GC, GC-MS) and Sensory Analysis (olfactometric and sensorial evaluation by panels studies) are generally used for this purpose. But for aromatic industry, the main inconvenients of such «off-line» methods (not allowing an immediate decision) are their cost and the technical level they required.

For the past ten years, a lot of studies were carried out, especially in japan, in order to design gas odor sensors for environmental measurements, mainly Volatile Organic Compounds detection. The major part of these sensors are of M.O.S. type (Metal Oxide Sensors) and were composed of sintered tin oxide deposit on an alumina ceramic tube with a heating coil inside. In the presence of volatile compounds, the electrical resistance of the sensor was modified due to volatiles adsorption on its surface. The adsorption Kinetic is rapid (qq s), reversible at ambiant temperature and proportional to volatiles concentration in atmosphere.

Due to the low intrinsec selectivity of semi-conductor gas sensors, a mono-sensor apparatus could only detect and eventually measure the overall concentration of volatiles. But a multi-gas sensors device including a computerized statistical process of the results could be able to differentiate odours (complex mixtures of volatiles). With the objective to set up a rapid and on-line Quality Control methodology, a new apparatus called ODORIMETER LCA 2000 was set up on a basis of an array of 5TGS sensors and using a special Monitoring/Acquisition software including Artificial Neutral Networks technology (CYRANO).

The presented examples are related to the differentiation of essential oils (e.g. lavender, ylang-ylang, jasmine...) according to their origin. In a first step (Learning Phase), the different samples of the same essential oils were analyzed, classified according to their origin allowing the generation of the corresponding comparison algorithm. Then in a second step (Recognition Phase), unknown samples could be classified on-line (analysis: 30s, classification: 10s).

P-14

ANALYTICAL IDENTIFICATION AND CHARACTERIZATION OF SOME KETALS IN FOOD FLAVOURINGS.

F. TATEO and M. BONONI

This research focuses on the synthesis and characterization of ketals in flavorings and fragrances. These ketals may be of interest for flavor and fragrances applications.

The spontaneous formation of ketals in foods has been reported by one of these authors. The legal aspect of using synthetic ketals in food flavors is unresolved so far.

This paper will discuss the analytical problems for the instrumental characterization of some ketals, some of which are not always easily identified.

P-15

VOLATILE COMPONENTS FROM *BELLIS PERENNIS* L.

P. AVATO and A. TAVA

Bellis perennis L. (Compositae, Asteraceae), the common daisy, is a herbaceous plant widely distributed in Europe.

Traditionally used in the treatment of rheumatism and as an expectorant, it has also been employed as vulnerary and against ecchymoses in veterinary medicine [Hagers Handbuch der Pharmazeutischen Praxis, 1982]. Furthermore, it has been recently shown the plant possesses antifungal and antibacterial activity [Hiller *et al.*, 1990].

For a better knowledge of the phytochemistry of *Bellis perennis*, we have analyzed the chemical composition of the essential oils from leaves and flowers of plants grown wild in Italy. In fact, while, several reports on data concerning saponins from the roots have been published [Schöpe *et al.*, 1992], the aerial organs have been little investigated and no previous works are known on the volatile compounds from this plant.

GC and GC/MS analyses of the essential oils have revealed that the bulk of constituents is represented by monoterpenes (52 and 67 % from leaves and flowers, respectively) with β-myrcene and geranyl acetate as main volatiles in both plant parts. Discrete amounts of aldehydes and sesquiterpenes have also been found.

P-16

COMPOSITION OF THE ESSENTIAL OIL FROM THE HEAD BLOSSOMS OF *TRIPLEUROSPERMUM INODORUM* (L.) SCHULTZ-BIP.

B. BÄR and H. SCHILCHER

Tripleurospermum inodorum (L.) Schultz-bip (Asteraceae), is a middle european crop plant, which grows in company of other «weeds». It will be mistaken easily for the medicinal plants *Matricaria recutita* (L.) respectively *Chamaemelum nobile* (L.) All., however the compounds of the essential oil are completely different.

The essential oil of *T. inodorum* contains more than 90 % polyacetylenes with (2Z),(8Z)-matricariaester as the main component (80 %). Besides that we could isolate and identify all other isomeres (2E),(8Z)-matricariaester, (2Z),(8E)-matricariaester, (2E),(8E)-matricariaester), (2E)-dehydromatricariaester, (8Z),α,β-dihydromatricariaester and (5E),(9Z)-matricarialacton. (2E),(8E)-matricariaester was isolated for the first time in *Tripleurospermum inodorum* and till now (2E)-dehydromatricariaester was only found in roots.

Investigations on the ontogenetic variability showed that the yield and composition of essential oil was dependent on flowering time. The highest yield 1.2 % has been reached shortly before blossoming.

P-17

ESSENTIAL OILS FROM TWO *HESPEROZYGIS* SPECIES FROM BRAZIL.

C. MENUT, P. VERIN, A.T. HENRIQUES G. LAMATY and J.M. BESSIERE

Hesperozygis ringens and *H. rhododon* (Labiatae) are two species found in Brazil, the first one being used locally for its antiparasitic properties.

The aim of this work was to compare their essential oil chemical compositions.The volatile constituents obtained by hydrodistillation of the aerial parts of the plants with yields of 4 % and 1 % respectively were analyzed by means of capillary gas chromatography and GC-MS.

The two samples are mainly monoterpenic. The essential oil obtained from *H. ringens* is characterised by an important amount of pulegone (79 %), whereas this constituent represents only 30 % of the volatile extract of *H. rhododon* in which the major constituent is menthone (43 %).

In the first sample, there are also several oxygenated compounds derived from pulegone which are absent in the second extract, namely the different isomers of epoxypulegone whose presence has been confirmed by hemisynthese, and 8-hydroxy-p-menth-4-ene-3-one purified by silica-gel chromatography and identified by spectrometric means.

Tests carried out on lettuce seeds, using alcoholic extracts of the two species showed that the first one had interesting anti-germinating properties.

P-18

ON THE COMPOSITION OF *TAGETES LUCIDA* CAV. ESSENTIAL OIL.

C. BICCHI, P. RUBIOLO, M. FRESIA and CH. FRANZ

Tagetes lucida Cav. is a Compositae growing wild in Central South America. This plant belongs to a group of *Tagetes* species whose essential oil is characterized by estragole, methyleugenol and anethole as main components, unlike most other species of this genus, which contain tagetones, tagetenones and ocimenes as main components.

The oil was obtained by hydrodistillation of the aerial parts and was analysed by GC with two different stationary phases (OV-1 and CW-20M) and GC/MS with different ionization techniques, both as such and after fractionation. Fractionation was carried out both by distillation under vacuum and by flash-chromatography with solvents of different polarities.

Thirty five compounds were identified, the main ones being anethole (23.8 %), eugenol (24.3 %) and estragol (33.9 %).

COMPOSITION OF ESSENTIAL OILS FROM TWO AGATHOSMA SPECIES.

W. CAMPBELL

The southern African tribe Diosmeae, one of five tribes of the subfamily Rutoideae, which in turn is one of seven subfamilies of the cosmopolitan family Rutaceae, is confined to the winter rainfall regions of the Cape Province of South Africa.

With few exceptions, species of the tribe produce essential oils which in general have a pleasant smell, although some have a pungent nauseating odour. The Khoikhol and San peoples of the Cape used preparations from certain taxa, which they called «buchu», to treat rheumatism, gout, cholera, haematuria, calculus, and infections of the bladder, uretha and prostate gland [Watt et *al.*, 1962].

Buchu oil from *Agathosma betulina* and *Agathosma crenulata* is the only essential oil from these species which is marketed internationally. Our previous studies on the essential oils from the Diosmeae have resulted in the isolation of new phenolic ethers and a new class of sulphur compounds, the S-prenylthioesters [Campbell et *al.*, 1980, 1980, 1991].

This paper describes the GC and GC-MS analysis of oils from *Agathosma cerefolium* Bartl. & Wendl., and *Agathosma ciliatris* Druce. The identification of the components was achieved by comparison of their retention times with those of authentic compounds and their mass spectra with literature data. The major components from *A. cerefolium* were estragole (22 %), anisaldehyde (11 %) and *trans*-anethole (41 %), from *A. ciliatris*, limonene (10 %), linalool (29 %), 1-terpinen-4-ol (13 %) and α-terpineol (15 %). Amongst the minor components from *A. cerefolium*, isomers of 1-(4-methoxyphenyl)-1-propanone and 1-(4-methoxyphenyl)-2-propanone together with 1-(4-methoxyphenyl)-1-butanone were tentatively identified. The compounds have not previously been reported to occur in nature.

STUDY OF ENANTIOMERIC DISTRIBUTION OF LINALOOL AND LINALYL ACETATE IN ESSENTIAL OILS.

H. CASABIANCA, J.B. GRAFF and V. FAUGIER

Linalyl acetate enantiomers separation can not or hardly be achieved on commercial cyclodextrins. Despite this, the analysis of this ester gives important informations because its stereochemical configuration remains unaffected by extraction (e.g. steam distillation).

A simple way allowing chiral discrimination of linalyl acetate enantiomers was carried out by isolation and hydrogenation of the ester followed by enantiomeric separation on the commonly used heptakis tri-O-methy-—cyclodextrin.

Catalytic hydrogenation of linalyl acetate leads to 3,7-dimethyl-3-acetoxyoctane, which stereoconfiguration is inversed, that means that a (*R*) linalyl acetate gives an (*S*) acetoxyalcane.

This method avoids using helpless isotope measurements and was applied to the authentification of lavender, bergamot, thyme and sage.

Moreover, chirality of linalool was carried out on heptakis tri-O-methyl-—cyclodextrin, enabling the control of several essential oils.

P-21

CHEMICAL COMPOSITION OF ESSENTIAL OILS OF FOUR SPECIES OF PINE: *PINUS HELDREICHII* CHRIST., *PINUS NIGRA* ARNOLD SSP. *NIGRA* AND SSP. *DALMATICA*, AND *PINUS OMORIKA* PANELE. INFLUENCE OF HARVEST LOCATION, PLANT PART AND AGE OF SPECIMEN.

J.C. CHALCHAT, R.PH. GARRY and M.S. GORUNOVIC

Yields of essential oil depended mainly on the age of the specimen, being higher in older than in younger trees (0.7-1.7/04-0.6 %) irrespective of plant part, harvest location and tree species.

In *Pinus heldreichii* and *Pinus omorika* the chemical composition of essential oils from leaves differed appreciably from that of oils extracted from branches. In *Pinus heldreichii* leaves, percentages of α-pinene and limonene were lower, and those of β-caryophyllene and germacrene-D higher than in branches. In *P. omorika*, oils from leaves had higher levels of limonene, camphene and bornyl acetate, and lower levels of β-pinene and borneol than oils from branches.

In contrast, essential oils from leaves and branches of *Pinus nigra* (ssp. *nigra* or ssp. *dalmatica*) had similar compositions.

The maturity of the trees sampled did not markedly influence the chemical composition of the essential oils, except for *P. nigra* ssp. *dalmatica*, where older specimens showed high levels of borneol and *trans*-caryophyllene and low levels of α-pinene.

Comparison of the chemical compositions of the essential oils of the species studied showed that the oils of *P. heldreichii* and *P. nigra* ssp. *nigra* shared high levels of α-pinene, limonene, β-caryophyllene and germacrene-D, while *P. omorlka* Panele, *P. peuce* and *P. nigra* ssp. *dalmatica* all had high levels (30 %) of borneol and bornyl acetate.

P-22

ALLO-CEDRENE, KHUSIENE, HELIFOLENE - THREE NAMES FOR ONE RARE SESQUITERPENE SKELETON.

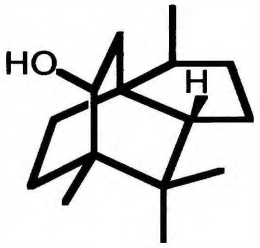
C. CHRISTIANSEN, P. WEYERSTAHL, H. MARSCHALL and A. ECKHARD

In 1973 Hirose published the structure elucidation of the tricyclic sesquiterpene alcohol **1** isolated from the wood of *Juniperus rigida* . Due to the relationship to cedrene **2** he named this skeleton *allo-cedrane.* Later [1974] Andersen isolated vetiverol b6 from vetiver oil which turned out to be *ent*-**1**. This compound also was identical with the so-called khusiol obtained from Indian vetiver oil (khus-oil) [Bhattacharyya., 1978]. Finally Weyerstahl [1986] proposed the name helifolene **3a** for a hydrocarbon identified in Helichrysum oil

Main constituents of the commercially available Brazilian lantana oil (*Lantana camara,* Verbenaceae) are the aldehydes **4a-d.** From these the hydrocarbons **3a-d** were prepared for assignment of the stereochemistry of the compounds mentioned in the literature.

Spectral data and olfactive evaluation of helifolene derivatives as well as a brief summary of the composition of lantana oil are presented.

allo-cedrol
or khusiol
or vetiverol b6



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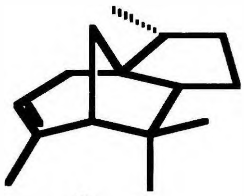
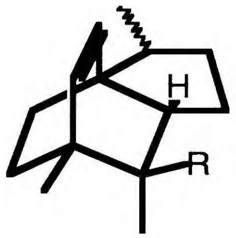
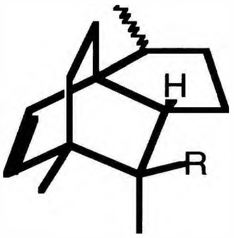


2
α-cedrene

3a
allo-cedrene
or khusiene
or helifolene





R =	H	CHO
β-Me	**3a**	**4a**
α-Me	**3b**	**4b**

R =	H	CHO
β-Me	**3c**	**4c**
α-Me	**3d**	**4d**

P-23

QUALITATIVE AND QUANTITATIVE STUDY OF MAROCCO VERBENA (*LIPPIA CITRIODORA* L.) ESSENTIAL OIL.

A. DJERRARI and J. CROUZET

The chemical composition of Marocco verbena (*Lippia citriodora* L.) essential oil, extracted from plants cultivated in Rabat area was studied by GC-MS after silica gel fractionation. Among, the volatile compounds identified, 26 are described for the first time.

The hydrocarbon content of this oil was 34 % with 63 % of terpenic derivatives. The oxygenated compounds represent about 51 % of this oil. The main constituants are neral and geranial (18 %), limonene (17 %), 1,8-cineole (9 %), α-curcumene (5 %), spathulenol (4.5 %), sabinene (2 %), α-terpineol (1.8 %) and 6-methyl-5-hepten-2-one (1.5 %).

The study of the influence of the hydrodistillation time on the verbena essential oil yield shows a two phases curves with a rapid step followed by a slow step. The results obtained indicates that the process is under the dependance of the polarity and the molecular size of the components.

The first fractions contain principally oxygenated components and more particularly monoterpene derivatives which are stripped more rapidly than the other compounds. 90 % of the oil is recovered after 4h30 of distillation, beyond 2h30 the oil is enriched in sesquiterpene hydrocarbons. The distillation time must be considered as an important factor concerning the composition and the quality of verbena essential oil.

P-24

THE ESSENTIAL OIL OF *LAVANDULA PINNATA* L. FIL. VAR. *PINNATA* GROWN ON MADEIRA.

A.C. FIGUEIREDO, J.G. BARROSO, L.G. PEDRO, I. SEVINATE-PINTO,
T. ANTUNES, S.S. FONTINHA., A. LOOMAN AND J.J.C. SCHEFFER

The genus *lavandula*, commonly known as lavender, comprises about 30 taxa of aromatic shrubs which are native to southern Europe and the Mediterranean area. *L. pinnata* L. fil. var. *pinnata* is a rare endemic species of the Madeira and Canary archipelagos.

The composition of the essential oils from different aerial parts of *Lavandula pinnata* L. fil. var. *pinnata* was analysed by GC and GC-MS. The oils were isolated separately from flowering tops, and from leaves and stems collected during the flowering period and the vegetative phase of the plant, respectively.

The three oil samples consisted mainly of monoterpene hydrocarbons (37-80 %), whereas oxygen-containing monoterpenes were hardly present (2-4 %). β-Phellandrene (12-32 %) was the main component of the oils analysed, and α–phellandrene (6-16 %) the second most important monoterpene.

The sesquiterpene fraction of the oils (13-22 %) were also characterized by hydrocarbons of which β–caryophyllene (11 % of each oil) formed the major part. Phenylacetaldehyde amounted to 6-9 % of the leaf oils. Common major components of lavender oils were not found in the oils or were present in very small amounts only.

P-25

STUDY ON THE ESSENTIAL OILS OF *O.X INTERCEDENS* RECHINGER (*ORIGANUM ONITES* X O. *VULGARE* SUBSP. *HIRTUM*).

K. PANETSOS, C. FOURNARAKI and M. SKOULA

In Crete, leaves and flowers of two Origanum species, vis. *Origanum vulgare* subsp. *hirtum* (Link) Iestwaart and *Origanum onites* L. are used as oregano spices, collected mainly from the wild during the flowering period. *Origanum vulgare* subsp. *hirtum* occurs principally in the western part of the island while *Origanum onites* is more abundant in the central-eastern part. At the intermediate area of the two species populations, several hybrid populations have been identified according to morphological criteria.

Two *O. vulgare* subsp. *hirtum* populations, one *O. onites* and one hybrid population, have been studied. The analysis of the essential oils (aprox. twenty individual plants per population have been analysed) has shown that the qualitative essential oil composition of all populations is similar.

The quantitative essential oil composition, however, has shown clear distinction between the two Origanum species. Furthermore, the values of significantly different constituents of the hybrid population fall always between the values of *O. vulgare* subsp. *hirtum* and *O. onites* populations. A strong correlation between the morphological and chemical characteristics exists, indicating that some compounds of the essential oil could be used as hybridisation markers.

P-26

ESSENTIAL OIL YIELD AND COMPOSITION IN *CORIDOTHYMUS CAPITATUS* : INTRAPOPULATION VARIATION.

K. PANETSOS, H. KARGIOLAKI and C. FOURNARAKI

Coridothymus capitatus L. Reichenb. (*Thymus capitatus* L. Hoffmanns. & Link) (thyme) is a plant species growing wild in a variety of different environments around the Mediterranean basin. Differences in the essential oil yield and composition were investigated after sampling of fifty natural populations around the island of Crete. Results showed a considerable variation between populations. Essential oil yield ranged from 0.10-6.00 % ml/g dw, revealing some very rich populations. Low altitudes showed the lowest yield, while Southern locations were more productive compared to Northern especially in Central and Eastern Crete.

α-Pinene, camphene, β-pinene, myrcene, α-terpinene, para-cymene, limonene, γ-terpinene, linalool, borneol, terpineol, thymol, carvacrol, caryophyllene, α-thujone, caryophyllene oxide have been identified in the essential oil. Its main constituents, however were para-cymene, γ-terpinene, linalool, thymol and carvacrol.

Two separate groups of populations were identified as thymol and carvacrol-rich. It was found that a high thymol concentration is related to high altitude, in contrast to carvacrol that was minimum in high altitudes. Total phenols followed the same trend as carvacrol, i.e. they were lower in high altitudes. p-cymene and γ-terpinene showed higher concentrations in high altitudes.

These findings show that the best breeding strategy for *Coridothymus capitatus* would be the selection of thymol-rich populations of high and carvacrol for low altitudes. The next step will be the investigation of variation within population and selection of the most desirable individuals.

P-27

INTER AND INTRAPOPULATION VARIATION OF THE ESSENTIAL OIL YIELD AND COMPOSITION OF *SALVIA FRUTICOSA.*

M. SKOULA, C. FOURNARAKI and H. KARGIOLAKI

Salvia fruticosa Mill. is an endemic species of the Eastern Mediterranean region. It is one of the most commercially exploited sage plants referred to as Greek or Mediterranean or wild sage. In the island of Crete (Greece) *S. fruticosa* is very abundant especially in the phryganic ecocystems and it is collected in summer by the villagers for additional income.

Study of the leaf essential oil yield and composition from 60 plants belonging to three populations of *S. fruticosa*, revealed that considerable variations exist between populations as well as within populations. Although variable, all plants examined were very rich in essential oil, ranging from 2.5 % to 6.0 % ml/g dw.

Of the 26 monoterpenes and sesquiterpenes identified, β-pinene, l-phellandrene, 1,8-cineol, α-terpineol and terpinyl acetate differ significantly amont the three populations while camphene, sabinene, α-thujone, β-thujone, camphor and borneol are higly variable within each population. All essential oils were characterised by high 1,8-cineol content (30 % to 54 %).

The results of this study show that in case of commercial exploitation of *S. fruticosa*, in Crete, selected clones (based on chemical criteria) should be cultivated in order to obtain crops with homogeneous essential oil production.

P-28

THE ESSENTIAL OIL COMPOSITION OF FRUITS FROM *ELAEOSELINUM FOETIDUM* (L.) BOISS. (APIACEAE).

K.-H. KUBECZKA, W. SCHULTZE, M. GRANDE and P. TORRES

Elaeoselinum foetidum (Apiaceae) occurring in the south of the Iberian Peninsula is one of the four Elaeoselinum species represented in Europe.

The capillary GC separation of its fruit essential oil yielded more than 80 substances from which 68 structurally were identified by GC/MS and GC-retention representing 98.5 % of the oil.

The individual constituents are nearly exclusively monoterpene derivatives, above all monoterpene hydrocarbons with α-pinene (58.15 %) and β-pinene (15.98 %) as main constituents.

Less abundant constituents are several oxygenated monoterpenoids (18.77 %), most of them with a bicyclic pinane skeleton such as *cis*- and *trans*-verbenol, verbenone, myrtenol, myrtenal, *trans*-pinocarveol, and pinocarvone ; in addition, some ubiquitous occurring monocyclic p-menthane derivatives have been found. Sesquiterpenoids and aliphatic compounds have been detected in traces only.

Comparing the essential oil composition of *E. foetidum* fruits with those of other *Elaeoselinum* species shows that a high proportion of pinane derivatives seems to be characteristic for all these species with the exception of *E. tenuifolium*. However, less abundant or trace constituents can be used as markers to distinguish all of them distinctly.

P-29

CHEMOTAXONOMIC STUDY OF JAPANESE FIR.

Y. HOLM, I. LAASKO and R. HILTUNEN

Two species of Japanese fir (*Abies sachalinensis* (Fr. Schm.) Mast., *A. mayriana* Miyabe et Kudo) originating from Hokkaido are very closely related and are thus difficult to separate by morphological characteristics. In order to find a chemotaxonomic method to separate the two species, leaf oil compositions of ten trees of both species from Ruotsinkylä (Finland) Plant Breeding Station were determined by GC-MS using a chiral β-cyclodextrin phase.

In both groups the terpene composition was almost the same. The oils are characterized by a high content of (-)-camphene (17-18 %) and bornyl acetate (26-28 %). Other significant compounds are (-)-—phellandrene (7-9 %), (-)-—pinene (8 %), (-)-—pinene (7 %), (-)-limonene (5-6 %) and (+)-—pinene (4 %). No significant differences were found between the groups.

For further classificationf of these oils of GC-MS results were submitted to a Cluster Analysis. Euclidean distance was selected as a measure of similarity between samples. For the cluster definiation the average linkage method was used. Cluster analysis had a tendency to separate the trees of *A. sachalinensis* from the trees of *A. mayriana*.

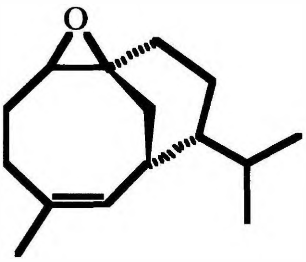
SYNTHESIS OF SALSOLENE-DERIVATIVES ISOLATED FROM *ARTEMISIA* SPECIES.

I. KAUFFMANN and P. WEYERSTAHL

Salsolene oxide **1** and salsolene ketone **2** are two new sesquiterpenes from the essential oils of *Artemisia* species. The odor of the oils is described as fresh camphoraceous and sweet. The skeleton of both compounds is an isoprenoid bicyclo[5.3.1]undecane and we named it «salsolane».

Biogenetic considerations lead to the assumption that there are only a few steps from germacrene-D to **1** and **2**. The ene-reaction with photochemically formed $^1O_2$ is the key step in the synthesis and gives the allyl alcohol germacr-10(14)-en-1-ol. Transannular electron shift in combination with *trans* protonation gives **1** in one step. Salsolene ketone **2** can be obtained by acid catalyzed rearrangement of **1**.

The key step in the total synthesis of **1** and **2** is the nickel(0)-catalyzed intramolecular [4+4]cycloaddition of a bis-diene to a bicyclo[5.3.1]undecadiene. The required bis-diene is obtained by a number of various steps but the isopropyl group at the six-membered ring complicates the synthesis. The preparation of bicyclo[5.3.1]-undecadienes by [4+4] cycloaddition is also described in connection with studies on the synthesis of other natural products (taxol, crispolide, vulgarolide).



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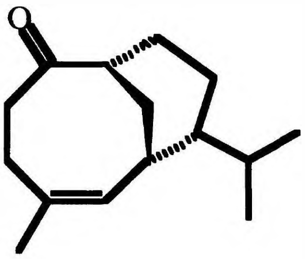


2

P-31

THE ESSENTIAL OIL COMPOSITION OF FRUITS FROM *SMYRNIUM CONNATUM* (BOISS.) KOTSCHY (APIACEAE).

K.-H. KUBECZKA, U. MOLLEKEN and W. SCHULTZE

Smyrnium connatum is a biennal umbellifer growing in the Eastern Mediterranean region, Turkey, West Syria and Libanon. In course of our investigations of essential oils from different *Smyrnium* species, the fruit oil of *S. connatum* obtained by steam distillation has been analyzed by means of GC, GC-MS, IR and NMR.

The essential oil of *S. connatum* mainly consists of oxygenated (85 %) compounds with a high proportion of furanosesquiterpenoids (77 %). Furanodiene and its Cope rearrangement product isofuranogermacrene are the main constituents (48 %).

The fraction of hydrocarbons (13.3 %) is dominated by sesquiterpenes (11.8 %), above all germacrene-D, β-selinene, β-elemene and germacrene-B.

In comparison with former studied *Smyrnium* species *S. connatum* exhibits a similar oil composition to that of S. rotundifolium, with regard to a high content of furanosesquiterpenoids. However, significant qualitative and quantitative differences within this group of constituents can be observed. The essential oils of *S. olusatrum* and *S. perfoliatum* differ completely and are characterized by a high proportion of terpene hydrocarbons.

P-32

COMPOSITION OF THE ESSENTIAL OILS FROM DIFFERENT PARTS OF *SELINUM CARVIFOLIA* (L.) L. (APIACEAE).

K.-H. KUBECZKA, J. BRANDT and W. SCHULTZE

Selinum carvifolia (L.) belonging to the Apiaceae family is a perennial plant growing in damp meadows and swamps in lowland areas of North and Middle Europe. In course of our investigations of essential oils from Apiaceae belonging to the subtribe Seselinae we analyzed the essential oils of different parts from *Selinum carvifolia* by HPLC, GC, and GC/MS.

Main constituents of the fruit essential oil are monoterpene hydrocarbons, which represent approximately 58 % of the oil with α-pinene (46 %) dominating. Besides small amounts of sesquiterpene hydrocarbons (2.7 %) two benzaldehyde derivatives (40 %) were found: 2,3,6-trimethylbenzaldehyde (38.4 %) and 2,3,4-trimethylbenzaldehyde (1.5 %). These two compounds must be considered as artifacts formed during hydrodistillation from ferulol esters by hydrolysis and rearrangement reactions [Bohlmann *et al.*, 1970] [Kubeczka *et al.*, 1981].

The herb essential oil consists of 44 % monoterpene hydrocarbons (with 23.3 % α-pinene), 32.3 % sesquiterpene hydrocarbons, and 14.3 % trimethylbenzaldehydes. The latter compounds are dominating in the root essential oil amounting to 78.5 %. In addition, 15.4 % monoterpene hydrocarbons were found with α-pinene (9.0 %) as the main constituent.

ESSENTIAL OILS OF FOUR LARCH SPECIES GROWING IN POLAND. MONOTERPENE FRACTION.

A. KUROWSKA, J. GORA and T. MAJDA

Material for study were one and two-three years old branches (without needles) of four larch (*Larix*) species :

L. decidua Mill. ssp *polonica* Domin
L. x. eurolepis Henry
L. laricina K. Koch
L. kaempferi Carr = *L. Leptolepis* Gord.

growing in central Poland, collected in winter from an experimental plantation (Rogow).

The essential oils were isolated by hydrodistillation from dry material. There were differences in the yields of essential oils. Total oil content was highest in one year old branches (varied from 1.1 to 2.1 % v/w). Two-three years old material contained 0.5-0.8 % v/w of essential oil.

At the begining of this study the components of the monoterpene fraction were investigated. The monoterpene hydrocarbons fraction which represented : 45 % of the total oil in *L. decidua*, 64 % in *L. eurolepis* and approx. 77 % in both others species, was a mixture of 14-16 identified compounds.

There were no significant differences in the qualitative composition of this fraction in the four species and the same compounds in the oils of one and two-three years old branches.

The main constituents in every sample were the same, but their contents were different e.g. α-pinene (13-49 % of total oil), β-pinene (3-25 %), camphene (2.5-7.6 %),Δ3-carene (0.25-9.5 %), limonene (1-9 %).

P-34

THE CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *LIPPIA CITRIODORA* KUNTH.

J. MASTELIC and D. KUSTRAK

Lippia citriodora Kunth. (Verbenaceae) is a small shrub with lemon-scented lanceolate leaves and spikes of white flowers. The plant is native to Argentine, Chile and Uruguay and cultivated in France, Spain, Algeria and Tunisia. The leaves are used as a source of verbena oil and also for treating stomach deseases.

The material used in the present study originates from plants cultivated in Dalmatia. The essential oil was obtained by hydrodistillation of the dried leaves in a Clevenger-type apparatus. After a three-hour distillation, the oil which was dissolved in pentane, was dried over anhydrous Na_2SO_4, the solvent evaporated and the oil weighted. The yield was 0.17 %.

To facilitate the analysis of the essential oil, 20 μl oil were fractionated by liquid-solid chromatography using silicagel. The elution was commenced with redistilled pentane yielding the hydrocarbons, and continued by redistilled diethyl ether yielding the oxygen-containing components. The oil and its fractions were analyzed by GC-MS, using two capillary columns with different polarity of the stationary phases.

The essential oil of *Lippia citriodora* contains a great number of compounds. We identified about forty compounds. The major compounds were limonene (19.6 %), *ar*-curcumene (10.4 %), 1,8-cineole (8.1 %), spathulenol (7.3 %), *Z*-citral (2.3 %), *E*-citral (2.2 %), α-terpineol (1.6 %), carvone (1.5 %), p-cymene (1.5 %), caryophyllene (1.4 %), α–pinene (1.3 %), α-copaene (1.3 %), β-bourbonene (1.3 %), *allo*-aromadendrene (1.1 %), trans-carveol (1.1 %), geranyl acetate (1.1 %), nerolidol (1.0 %).

P-35

VOLATILES OF HORSECHESTNUT BLOSSOMS, PEELS AND SEEDS.

A. NIKIFOROV, G. BUCHBAUER, L. JIROVETZ and M. WASICKY

A headspace sample, the essential oil, a petroleum ether and a methanolic extract of horsechestnut blossoms (*Aesculus hippocastanum* L.) were investigated by GC/FID, GC/FTIR/MS and GC/sniffing-technique.

In the headspace sample various monoterpenes like α-pinene (21.8 %), β-pinene (17.2 %), camphene (11.3 %), limonene (6.1 %) and 1,8-cineole (5.8 %) were identified. The occurance of decanol (7.2 %) and nonanal (4.3 %) also seems to be noteworthy. The essential oil and both extracts of *A. hippocastanum* show a similar composition of main compounds, but because of the occurence of other lower volatile compounds in distinct lower concentrations.

Additional olfactoric data of single samples are given: headspace: typical odor of the blossoms, extracts: weak odor of blossoms and essential oil: weak odor of blossoms with vegetable-soup note. Using the GC/sniffing technique no single compound was identified to be typical for the characteristic blossoms odor. The combination of main compounds including especially nonanal, decanol, limonene and benzaldehyde in combination with side (especially geraniol and methylbutenal derivatives) and some trace compounds determines the top-note of horsechestnut blossoms.

Using the aforementioned methods peels and seeds of *Aesculus hippocastanum* were also investigated. Additional results of these analyses are given.

(*Aknowledgment*: The support of Austrian Research Foundation (FWF), Project P8299Che and P9568Che is gratefully aknowledged).

P-36

ENANTIOMERIC RATIO OF TERPENIC HYDROCARBONS IN ESSENTIAL OILS FROM FAMILY RUTACEAE.

D. SYBILSKA, R.J. OCHOCKA, M. ASZTEMBORSKA and W.S. BRUD

Essential oils are complex mixtures of flavour and fragrance substances originating in plants. They have found comprehensive commercial application in the pharmaceutical, food and cosmetic industries.

Monoterpenic hydrocarbons (especially limonene) are the main fraction of essential oils from family *Rutaceae*. Many of them are chiral compounds and thus they can be present in these oils in one or two enantiomeric forms and at various proportions. The determination of enantiomeric ratio of the chiral constituents in essential oils may vary largely depending on the species, on the origin of material i.e. part of the plant and place of its cultivation. Sometimes the enantiomeric composition may indicate on the natural or artificial source of materials [Sybilska *et al.*, 1994] [Ochocka *et al.*, 1991].

Recently it has been found that a large group of chiral macrocyclic compounds including cyclodextrins present a powerful tool for enantiomeric separations of many compounds of various chemical nature: acidic, basic and neutral ones as hydrocarbons which are very resistive to diastereoisomer formation. For example α-cyclodextrin under appropriate conditions of gas-liquid chromatography permits efficient separations of chiral monoterpenic hydrocarbons [Koscielski *et al.*, 1983].

The objects under investigation by this method were citrus oils i.e. lemon oils, orange oils and mandarin oils. It has been found that (+)-limonene is the main component of investigated oils and high relationship between the optical rotation of essential oils and the content of (+)-limonene is observed.

CHARACTERIZATION OF SEVERAL ITALIAN TYPES OF WILD FEENEL (*FOENICULUM VULGARE* MILL. SUBSP. *PIPERITUM*).

R. PICCAGLIA, M. MAROTTI and V. DELLACECCA

Foeniculum vulgare Mill. (fennel) of the Apiaceae family is an important aromatic plant of the Mediterranean area. It is distiguished into two subspecies: *piperitum* and *capillaceum* (J. Verghese, 1990). The latter is the most cultivated species also as vegetable and the chemical composition of its oils has been well assessed. The subspecies *piperitum*, instead, has been less investigated. This type grows wild, is perennial and possesses an aroma with sour and sharp notes. In popular custom it is used to flavour fish and meat.

To increase the knowledge of this subspecies, in our work several types of wild fennel from different Italian localities were collected and grown in an experimental station of the University of Bologna. The aim of this research was to evaluate their agronomic performances and essential oils characteristics to verify the possible existence of different chemotypes.

The studied types showed relevant differences for the morpho-physiological characters. The essential oils were steam distilled in a Clevenger-type apparatus and were analyzed by HRGC/MS. The preliminary results showed that they have as predominant compounds methylchavicol, α-phellandrene and fenchone whereas (*E*)-anethole, the most abundant component of the subspecies capillaceum, was found only in very small amounts.

ISOLATION AND ELUCIDATION OF NEW SESQUITERPENOIDS FROM *NEOCALLITROPSIS PANCHERI* CARRIERE DE LAUBENFELS.

P.J. RAHARIVELOMANANA, J.P. BIANCHINI, M. AZZARO, A. CAMBON,
G. GEORGE and R. FAURE

Belonging to the Cupressaceae family, *Neocalliptropsis pancheri* Carrière de Laubenfels is a New Caledonian endemic tree previously botanicaly called *Eutacta pancheri* Carrière or *Callitropsis araucarioides* (Compton) Florin. Nowadays, by its status as a relict specy, *Neocallitropsis pancheri* is found only in protected and rare places in southern part of New Caledonia.

Heartwood oil of *Neocallitropsis pancheri* (wood harvested in New Caledonia), earlier considered as Araucaria oil in Perfums, was distilled in western Australia factories during the fortie's and the fiftie's years and was utilized as a fixative. This oil, having a delicately woody and seet almost floral odor, was known as a rich source of α,β,γ-eudesmol isomers.

During chemical composition investigation of *Neocallitropsis pancheri* oil, we found that it contained mainly sesquiterpenoids (more than 95 % of the oil). Isolation of major constituents from this oil was realized using argented silicagel column chromatography separation. These compounds were characterized by means of spectroscopy methods (GC/MS, GC/FTIR) and especially by the use of advanced 2D-NMR experiments.

Application of multipulse and bidimensional NMR techniques (DEPT, COSY, XHCORRD, INADEQUATE, HMBC, HMQC, SELCOSY, NEOSY) allowed to elucidate the structure of six new sesquiterpenoids from *Neocallitropsis pancheri* oil. These new compounds were found to belong to various sesquiterpene skeletons: bisabolane, acorane, guaiane and eudesmane. The eudesmane type sesquiterpenes constitue the major components beside the other ones which are biogenetically related.

STUDY OF THE COMPOSITION OF THE ESSENTIAL OIL OF CANANGA.

E. ROUBERT, L. JONCHERAY, A. CAMBON, B. MACCINI, P. BRUGEL.,
J.P. RAHARIVELOMANANA and J.P. BIANCHINI

Ylang-ylang is botanicaly called *Canangium odoratum genuina* or *Cananga odorata* Hook f. and Thompson [Guenther, 1952]. A wilder species called forma *macrophylla* [Chadefaud *et al.*, 1960] [Duve *et al.*, 1975] provides the Cananga. It has been presumed that most of the components of ylang-ylang were also detected in the essential oil of cananga, but in different ratios [Klein, 1975].

Many scientists have studied the essential oil of ylang-ylang. This oil has a typical floral sweet and strong odor. On the other hand, literature is very poor concerning the composition of cananga essential oil. This latter oil has a more ordinary evocation, that means a sweetless, dryer and balsam-like odor.

In the present work we report on a qualitative and quantitative study of an essential oil of cananga from Java. The gas-chromatography (GC) studies of the oil allowed us to calculate the Kovats indices of a variety of compounds and consecutively the GC/MS identifications.

A vacuum fractional distillation of the cananga's oil following by a silicagel and an argented silicagel column chromatography provide us several fractions that we have studied on GC/MS. During this chemical composition investigation, we identified 60 compounds among the 131 detected by GC.

We identified different classes of compounds. We characterised some of them by means of ^{13}C NMR. At last, we have established a comparison with literature studies of the essential oils of ylang-ylang and found notable differences between these two oils.

P-40

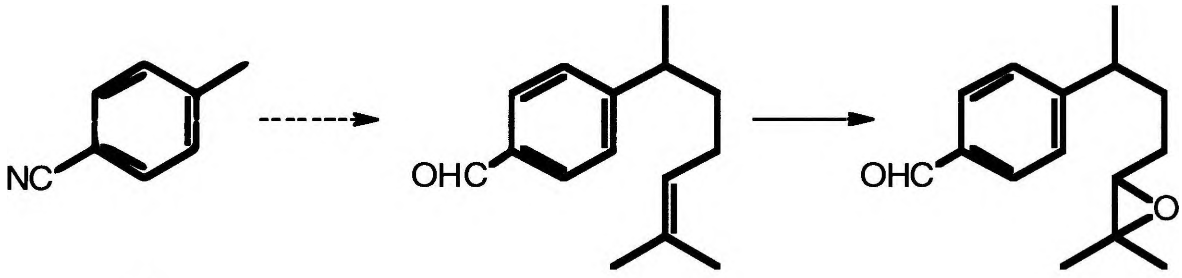
SYNTHESIS OF *AR*-CURCUMEN-15-AL - A SESQUITERPENE ISOLATED FROM *LANTANA* OIL.

V. SCHLICHT and P. WEYERSTAHL

The essential oil from the brazilian plant *Lantana camara* (Verbenaceae) was recently investigated by our group. The odor of this oil has been described as fresh, leathery, terpenic and fatty with a sweaty touch.

The oil contained mainly sesquiterpenes (~ 83 %) which were subdivided in hydrocarbones (22.1 %), ethers (5.3 %), aldehydes (32.3 %) and alcohols (23.1 %). The main compounds were elucitated as of γ- and ar-curcumene, 7-epi-—bisabolol, nuciferal, bergamotol and further oxygenated derivatives of γ-curcumen-15-al **2**. The odor of the isolated **2** was strong metallic, ozon- and waterlike.

As the isolated ar-curcumen-15-al **2** had only a purity of 90 %, we were insterested in a synthesis of **2** to evaluate the odor of the pure substance. We performed a short synthesis with only five steps starting from p-tolunitrile **1**. The key step was the alkylation of the benzylic position. This synthesis could also be applied to some derivates of **2.** Another interesting minor compound (0.4 %) of the lantana oil, the 10,11 epoxy-ar-curcumene-15-al **3**, has been prepared by epoxidation of ar-curcumen-15-al **2**.



1 **2** **3**

CONSTITUENTS OF THE MEDITERRANEAN *HELICHRYSUM* OIL.

I. SEELMANN, P. WEYERSTAHL AND H. MARSCHALL

The essential oil of *Helichrysum italicum* (Compositae) from the Mediterranean region is widely used in perfumery. Therefore it was carefully analysed by a combination of GC using Kovats-Indices, GC/MS and NMR after the constituents had been separated by means of distillative and chromatographic technics.

78 components representing more than 95 % of the oil could be identified, primarly mono- and sesquiterpenes. Underneath α-pinene (30.5 %), β-pinene (0.5 %), limonene (2.9 %), linalool (0.8 %), nerol (0.5 %) and neryl acetate (5.7 %), α-copaene (1.4 %), isoitalicene (1.5 %), italicene (2.3 %), helifolene/isohelifolene (0.4 %), caryophyllene (2.0 %), γ-curcumene (7 %) and ar-curcumene (13.5 %). A variety of diketones (9.8 %) and angelates (1.2 %) were perceived as well, which are chiefly responsible for the characteristic «sweet and spicy» odor of *Helichrysum*.

A complete table of the components of the *Helichrysum* oil and a detailed discussion of their identification will be presented.

As the italicene skeleton is scarcely known from other plant extracts, the olfactory properties of some derivatives were of great interest. The italicene and the isoitalicene isolated from the *Helichrysum* oil were oxidized to the rather unstable ally alcohols to yield the ketones and the aldehydes after further oxidation with Jones reagent and manganese(IV) oxide respectively. These structures and some odor evaluations will be exposed, as well.

P-42

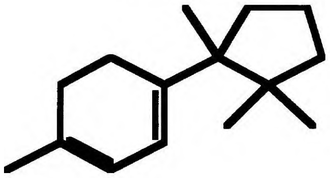
PLANTS - MASTERS OF DESIGN AND CONSTRUCTION - THE ESSENTIAL OIL OF *HYPERICUM PERFORATUM* FROM INDIA.

U. SPLITTGERBER, P. WEYERSTAHL and H. MARSCHALL

The essential oil of *Hypericum perforatum* (Hypericaceae) has previously been studied by Jacobsohn-Raposo *et al.* [1993] (plant material from Portugal). This yellow flowering weed is widely spread all over the world. We investigated an oil from plants harvested in North India at an altitude of 1200 m. The compositions of both oils are compared.

We found the main components α-pinene (67 %), nonane (4.5 %), geranyl acetate (4.7 %), β-caryophyllene (4.7 %) and a new sesquiterpene hydrocarbon which has been named γ-cuparene **1** (3.2 %).

Besides a lot of trivial monoterpenes and sesquiterpenes the oil also contains some more rare substances like the monoterpene pinol and the tetracyclic hydrocarbon ishwarane. We found two olfactorily interesting 1,3-diketones which have also been synthetized. The structure of these C_8-molecules is reflected in some bigger molecules with a formula $C_{14}H_{18}O_3$ **2**. The structure investigation of these lactones by NMR experiments will be described.



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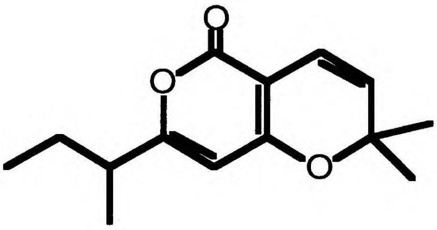


2

ESSENTIAL OIL POLYMORPHISM IN THE SCOTTISH THYMUS SPECIES *THYMUS PRAECOX* OPIZ SSP. *ARCTICUS* (E. DURAND) JALAS (SYN. *T. DRUCEI* RONN).

C. BISCHOF and E. STAHL-BISKUP

Three different Thymus species occur on the British Isles (Perring et al., 1976): *T. praecox* ssp. *arcticus* (= *T. drucei*), *T. pulegioides* L. and *T. serpyllum* L. The first, earlier described as *T. drucei* Ronn. [Piggot, 1953], is the most widespread and is common from the south to the north, including Ireland. Since the revision of the genus Thymus in the Flora Europaea by Jalas, the British population of *T. drucei* Ronn. has been handled as a synonym of *T. praecox* Opiz ssp. *arcticus* (E. Durand) Jalas [Clapham *et al.*, 1987]. Unfortunately, confusion is possible as the «Wild Thyme» of the British Isles is also described as *T. polytrichus* A. Kerner ex Bordas [Stace, 1991].

In the course of our investigations of the northern Thymus species, the Scottish population was the most interesting. More than 400 individual plants were collected at 47 different places in Scotland. The essential oil was obtained by means of steam distillation and analysed by means of GC and GC-MS.

The main components of the essential oils were found to be linalyl acetate/linalool, accompanied by some sesquiterpene alcohols, namely hedycaryol, germacradienol I and II, *trans*-nerolidol and T-cadinol. Besides those, some common monoterpene and sesquiterpene hydrocarbons were present. With this composition it closely resembles to the populations of *T. praecox* ssp. *arcticus* of Iceland, Greenland and Norway.

Concerning the chemical polymorphism, the Scottish population turned out to be more variable. The variation was found mainly in the content of linalyl acetate/linalool (LA) and the sesquiterpene alcohols (SA). The oils of one group of plants contained both LA and SA, the oils of another group contained LA but not SA, in the oils of a third group LA was missing, SA present. The fourth group contained neither LA nor SA. Those oils consisted mainly of some common monoterpene and sesquiterpene hydrocarbons, such as α- and β-pinene, myrcene, caryophyllene, germacrene and β-bisabolene. Within groups containing sesquiterpene alcohols, further subdivisions could be established. The occurrence of small amounts of thymol/carvacrol in some samples was remarkable.

MULTIVARIATE ANALYSIS OF THE ESSENTIAL OIL DATA FOR THE CLASSIFICATION OF *THYMUS* PLANTS OF THE AUSTRIAN ALPINE REGION.

J. HOLTHUIJZEN and E. STAHL-BISKUP

Previous investigations of the *Thymus praecox* subspecies *polytrichus* from the Alpine region of Austria had revealed a wide range of polymorphism concerning the essential oil chemistry. 10 different oil types existing had then been postulated [Holthuijzen *et al.*, 1992]. The same data set obtained from the gas chromatographic analyses of the essential oils of 141 samples of *Thymus praecox* ssp. *polytrichus* has now been analysed by means of Principle Component Analysis and Cluster Analysis.

Both these multivariate analysis techniques, previously applied on essential oil classification of *T. willkomi* [Adzet *et al.*, 1991] and *T. pulegioides* ssp. *chamaedrys* [Martonfi, 1992], led to the differentiation of four main types of oil:

1 Thymol type (46 samples)
2 Geraniol/geranyl acetate type (19 samples)
3 Terpinen-4-ol/trans-—sabinene hydrate type (7 samples)
4 Sesquiterpene type

The composition of the data did not allow the definition of four further subgroups of the latter (sesquiterpene hydrocarbon type, hedycaryol type, *trans*-nerolidol type, germacradienol/cadinol type) as was assumed previously.

The remaining plants (10) could not be included in either of these four types using the applied techniques. Some of them showed significant amounts of other constituents such as linalool, α-terpineol and borneol.

P-45

ESSENTIAL OIL QUANTITATIVE AND QUALITATIVE VARIABILITY IN SEMISIB PROGENIES OF *MENTHA SPICATA* (L.) HUDSON.

T. STOEVA

Seven semisib produced by sexual hybridisation after the method of free pollination of 4 representative *Mentha spicata* (L.) Hudson populations were investigated. Computer processing of the data assessed the variability in essential oil content and the presence of 14 constituents determining its quality. The following statistical values were determined mean value (x), error of the mean value (+sx), standard deviation (S), limits of variation (Limes), coefficient of variation (Cv), error of the coefficient of variation (+mCv).

Essential oil content varied considerably (Cv -26.50 % to 49.60 %). The quality composition of terpenoids showed also pronounced variability concerning their quantitative participation in the total sum of essential oils. Carvone content varied most (Cv from 49.60 % to 162.05 %) followed by linalool (Cv from 50.13 % to 152.00 %) and isomenthol (Cv from 48.44 % to 127.60 %). There were cases when secondary constituents had higher participation with respect to the mean of the sample and this led to the production of essential oils with different composition and aroma.

The semisib progenies represent a promising initial material for purposeful selection in spearmint breeding.

A NEW MONOTERPENE ACID FROM *CERATOCISTIS FIMBRIATA.*

R. TABACCHI, G. GREMAUD and N. BURKI

Ceratocistis fimbriata is a perithecial ascomycete which infests highly commercial valued crops and trees (coffee, cocoa, hevea, elm, plane, oak). Different strains have shown general specificity to their host. This pathogen enters through the wounds, into the roots and branches, and causes foliar and fruit withering accompanied by trunk cancer in adult trees. Two strains of *Ceratocistis fimbriata*, one isolated on coffee tree (*C. fimbriata* var. *coffea*) and another one on plane tree (*C. fimbriata* var. *platani*) were investigated.

Despite of quite similar synthetic growth media and extraction procedure, we observed a quite different chemical composition of the non volatile fraction. While *C. fimbriata* var. *coffea* produced a serie of isocoumarines derivatives and tetralones, *C. fimbriata* var. *platani* produced mainly tetralones.

The volatile ether fraction is also very different: *C. fimbriata* var. *coffea* contained more than 20 compounds including simple aliphatic esters that are responsible for the strong «banana like» odor of the culture medium, that are totaly absent in *C. fimbriata* var. *platani*.

The presence of monoterpenes in the genus Ceratocistis is known but in our media only β-citronellol and citronellyl acetate were present in different quantities but in both fungi.

From a polar fraction of *C. fimbriata* var. *platani* we isolated a monoterpene acid. Using modern NMR techniques, IR and MS, and the Horeau method for the determination of the absolute configuration we established the structure as (3*S*)-3-hydroxycitronellic acid.

This compound is a new natural monoterpene derivative.

P-47

DIFFERENCES ON THE EXTRACTS OF *MYRTUS COMMUNIS* L. AND *CUPRESSUS LUSITANICOS* MILL. OBTAINED BY HYDRODISTILLATION AND EXTRACTS WITH SUPERCRITICAL CO_2 (II).

F. VENANCIO, E. REIS, F. DUARTE, A. SOUSA and A. PALAVRA

Several essential oils from aromatic plants of the portuguese flora have been studied. These oils obtained by hydrodistillation were studied before and their chemical composition compared with similar oils from other countries.

Last year we have presented a work about the volatile components of *Rosmarinus officinalis* L. essential oil obtained by hydrodistillation, extraction with n-pentane and supercritical CO_2.

In this work we present the GC analytical results of the *Myrtus communis* L; and the *Cupressus lusitanicus* Mill. essential oils.

The *Myrtus communis* L. portuguese essential oil is characterized by a high quantity of myrtenyl acetate (34 %) and 1,8-cineole (30 %), with a relative low content of α-pinene (15 %) and about 3 % of linalool.

The chemical composition of the portuguese *Cupressus lusitanicus* Mill. essential oil is different from the other geographical origins. It contains monoterpene hydrocarbons (α-pinene, sabinene, myrcene, limonene), alcohols (α-terpineol, linalool), esters like linalyl, bornyl and α-terpinyl acetates, ketones (camphor, umbelullone), sesquiterpene hydrocarbons (caryophyllene, cadinene) and oxygenated constituents (1,8-cineole).

In both above-mentioned essential oils, the supercritical CO_2 technology produces extracts with a lower content of monoterpene hydrocarbons and a remarkable quantity of waxes.

P-48

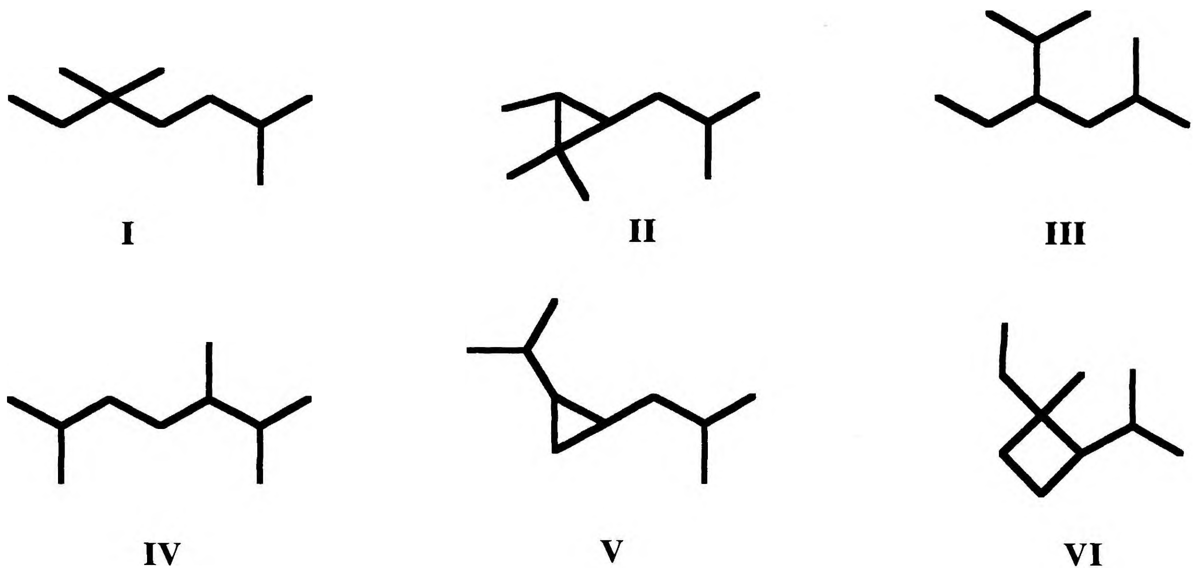
IRREGULAR MONOTERPENES IN ESSENTIAL OILS.

G. VERNIN, R.M. ZAMKOTSIAN and G.M.F. VERNIN

Non-C10 head-to-tail monoterpenes with artemisyl (I), chrysanthemyl (II), santolinyl (III), lavandulyl (IV), rothrockyl (V) and fragranyl (VI) skeletons are so far found in various Compositae plants (see Scheme 1).

They include hydrocarbons, ketones, esters and alcohols.

Their occurrence in various *Artemisia* species, *Chrysanthenum vulgare*, *Achillea wilhelsii* C. Koch and *Tanacetum vulgare* essential oils are reviewed as well as their biogenesis, synthesis and configuration. The mass spectra reconstructed from our SPECMA data bank and Kovats indices of about 40 of these irregular monoterpenes are reported.



Scheme 1. Skeletons for irregular monoterpenes

COMPOSITION AND INFRASPECIFIC VARIABILITY OF THE ESSENTIAL OIL OF *THYMUS CAMPHORATUS*, AND ENDEMIC SPECIES FROM PORTUGAL.

L. SALGUEIRO, R. VILA, F. TOMI, X. TOMAS, S. CANIGUERAL, J. CASANOVA,
A. PROENCA DA CUNHA and T. ADZET

Thymus camphoratus Hofmanns. & Link is an endemic species from Portugal, which belongs to the section *Thymus* of the genus *Thymus* (Lamiaceae).

The composition of the essential oils of seven populations of different localities was investigated by GC/FID, GC/MS and ^{13}C/NMR.

In total 92 compounds were identified, meaning a percentage of the essential oils ranging from 92 % to 98 %. Important differences on the major constituents of the populations were found, indicating the existence of infraspecific variability.

Intermedeol, an oxygenated sesquiterpene which has not been previously detected in Lamiaceae, was found in all populations, particularly in a high percentage (6.5 %) in a sample coming from Algarve.

In order to investigate the chemical polymorphism, the essential oil of individual plants of *Thymus camphoratus* was analyzed mainly by GC/FID, and, when necessary, by GC/MS. The results obtained were submitted to a multivariate analysis (Principal Component Analysis and Cluster Analysis).

The existence of a high infraspecific variability was established, six different types of essential oils being found: linalool/T-cadinol, linalool/linalyl acetate, linalool/geranyl acetate, borneol/camphene/camphor, 1,8-cineol/borneol, and 1,8-cineol.

CONSTITUENTS OF THE BRAZILIAN CABORE OIL

D. WOLF, P. WEYERSTAHL and H. MARSCHALL

On declaration of the manufacturer (Aripe S.A., Porto Alegere, Brazil) the essential oil of Cabore is prepared by hydrodistillation of the wood chips of a Brazilian tree belonging to the Leguminose family (*Micrastur ruficolis*).

The thirtyseven components of the commercial Cabore oil, representing 99.8 % of the total oil, were identified by their Kovats indices, their MS, and ^{1}H-NMR spectra which were compared with the data of reference compounds. Quantitative determination was carried out based on peak area integration.

The oil consisted mainly of sesquiterpenes (96.5 %). The hydrocarbons and the oxygenated compounds attaining 92.3 % and 4.2 %, respectively. α-copaene (22.9 %), germacrene-D (13.4 %), β-caryophyllene (8.3 %), germacrene-B (8.3 %), δ-cadinene (7.8 %), γ-elemene (5.2 %), β-elemene (4.4 %) and β-bisabolene (3.5 %) were the major constituents of the hydrocarbons, whereas the oxygenated compounds ccontained one epoxide and nine alcohols with the cubebane, eudesmane and cadinane skeleton.

In addition to a detailed discussion of the identification of the components of the Cabore oil the additive effect of odor in some fractions will be presented.

P-51

TESTING SYSTEM FOR THE EVALUATION OF ANTIMICROBIAL AND ANTIFEEDANT PROPERTIES OF ESSENTIAL OILS AND EXTRACTS OF SPICES IN STARCH BASED BIOPLASTICS.

U. BAUERMANN, J. EHRICH and R. THOMANN

Substances and building materials produced on the basis of regrowing materials by nature are subject to microbiological damage and constitute a good nutritive medium for insects and rodents. Therefore, special protective components have to be added. Traditional chemical substances, however, are an environmental impact and counteracting the ecological argumentation of products from regrowing raw materials. The application of essential oils and extracts from medicinal and aromatic herbs is an alternative.

Our research project is aimed at the search for essential oils and extracts of an antimicrobial and/or protective effect against insects and rodents, i.e. of a repellant effect against damaging pests, with the aim to increase the storage stability of biomaterials.

For analysing the effect of the essential oils and extracts, the test methods were modified to the special requirements. The test methods and first test results are presented.

P-52

ANTIOXIDATIVE PROPERTIES OF *LEVISTICUM OFFICINALIS* ESSENTIAL OIL.

E. SIMPSON, S.G. DEANS and K.P. SVOBODA

It has been known for more than 30 years, that extracts of spices show antioxidative activity. Work by Chipault *et al.*, in 1952 described the antioxidant properties of more than 30 herbs and spices. Increased awareness of the possible toxicity of the synthetic chemicals used as antioxidants has resulted in the investigation into the preparation of antioxidants from natural sources. The protective role of such antioxidants against peroxidation of polyunsaturated fatty acids (PUFAs) is now receiving considerable attention. Key PUFAs such as arachidonic and docosahexaenoic acids have been shown to decline in levels during ageing with concomitant loss of cellular membrane integrity. Diatary intake of certain plant essential oils has, however, reversed this trend to the extent that the PUFA levels are higher in the oil-fed old animals than both the age matched controls and young animals. Preliminary screening of plant volatile oils showed a number of the oils to exhibit antioxidant properties, one of which being the oil from the aromatic plant, lovage (*Levisticum officinalis*).

Lovage has been known for centuries as a spice and medicinal plant. The whole plant, including the roots, exhibit a strong celery-like odour. Lovage is a large, strong-growing, tall perennial. The plant grows wild in the hills of southern Iran and south western Europe, but was shown to grow and over-winter well in trials carried out at SAC Auchincruive. Harvesting and analysis of the oil extracted from leaf, stem and root material over a 2-year period showed both seasonal differences in antioxidant activity, and differences in activity between the various plant parts themselves.

In addition to its antioxidant activity, lovage oil was found to have both antibacterial and antifungal properties. The biological activity of the essential oil along with its ability to thrive under adverse weather conditions would suggest that lovage could prove to be a useful aromatic plant in the provision of beneficial protection to key PUFAs in mammals.

P-53

PRODUCTION OF *AGROBACTERIUM TUMEFACIENS* MEDIATED TRANSFORMED SHOOTS OF *SATUREJA HORTENSIS* L. : ESSENTIAL OIL PROFILE.

M. McEWAN, S.G. DEANS, K.P. SVOBODA, M.J.C. RHODES and N.J. WALTON

In recent years, the technology of transformed root culture has been employed for the study of plant secondary metabolite production [Hamill *et al.*, 1987, Kennedy *et al.*, 1993]. However, many interesting plant secondary metabolites are synthesised only in the aerial parts of plant [Parr *et al.*, 1988 ; Rhodes *et al.*, 1990]. Thus, the production of a similar system for shoot cultures would be equally useful.

The transformed shoot technique was originally developed by the Plant Cell Biotechnology Group at the AFRC IFR in Norwich [Spencer *et al.*, 1990] and is very similar to that of root transformation except that the bacterium used was *Agrobacterium tumefaciens*. As wild type *Agrobacterium tumefaciens* strains tend to cause crown gall tumours on most plants, development of the bacterial Ti plasmid of nopaline strain 4404 was required to help increase cytokinin production and thus produce shoots. This was achieved using a pJH10 ipt promoter construct which contains an enhanced cauliflower mosaic virus (CaMV) 35s promoter inserted to enhance the isopentenyl transferase (*ipt*) gene in the T-DNA, this gene being responsible for cytokinin production in the plant cells after transformation. This bacterial strain was successful in transforming *Satureja hortensis*.

Microscopy (both light and SEM) was used to investigate differences in the structure of the transformed shoots compared to the conventionally cultivated plant. Results revealed that the transformed cultures contained similar oil glands but structurally the plants were very different not only in stem and leaf development but also in organisation of the vascular bundles as revealed by polarised light microscopy. This disorganisation in the transformed cultures agreed with Kennedy *et al.* [1992] who showed this effect in Pokeroot (*Phytolacca americana*) ‘hairy’ root vascular bundles and may well be an indication confirming transformation.

Essential oils were extracted from the shoot cultures using the microwave distillation technique developed by Paré *et al.* [1992], this technique having been adopted due to the relatively small amount of transformed material available. These extractions were compared with steam distilled oils from conventionally cultivated plants using GC analysis. This revealed a number of similarities and differences in the peaks.

P-54

BIACTIVITY OF GERANIUM OIL FROM DIFFERENT SOURCES.

M. LIS-BALCHIN, S. DEANS and S. HART

Geranium oil is one of the most frequently used oils in Aromatherapy massage and in burners and has apparently most diverse curing potential. The diversity in the chemical composition of the different geranium oils is very high, depending partly on the country of origin, e.g., Egypt, China or Réunion, and partly on a unexplained individual variation.

Due to the variability it was probable that different geranium oils would have different bioactivity. This was shown against 25 bacteria, both Gram positive and Gram negative from animal, food and plant sources, three filamentous fungi species, 20 varieties of *Listeria monocytogenes* and in vitro pharmacological actions on smooth muscle. The number of bacteria affected varied from 7 to 17 ; the number of *Listeria* from 3 to 16.

There was a wide variation in the antifungal potency and the antioxidant value. The action on smooth muscle was always spasmolytic but varied in intensity between different geranium oils. The variations in bioactivity could not be correlated with the main chemical components, nor the country of origin.

The results suggest that Aromatherapy benefits of geranium oil may be mainly associated with the odour effects on the limbic system rather than on their overall bioactivity.

P-55

LEAF ESSENTIAL OIL COMPOSITION OF SIX STRAWBERRY CULTIVARS AND THEIR SUSCEPTIBILITY TO MITES.

A. BÉLANGER and S. KHANIZADAH

Two-spotted spider mite (TSSM) is one of the most important strawberry pest. Plant condition seems to be a major factor affecting mite population. The increasing resistance of the new strawberry lines to TSSM is one of the important characteristic in a breeding program. A great deal of work have been done on the inherent resistance of host lines. Chemical composition is among the most important factors that offert TSSM population.

The purpose of this paper was to compare the natural concentration of foliar essential oils in six strawberry cultivars with different degree of susceptibility toward TSSM.

The essential oil compositions were investigated by capillary GC and their constituents identified by GC/MS. 'Chandler', 'Fern' and 'Lester' which were highly susceptible and intermediately susceptible had lower linalool, α-terpineol, β-cyclocitral composition than 'Kent', 'Honeoye' and 'Bounty' which were classified as resistant or intermediately to resistant. Susceptible cultivars ('Chandler', 'Fern' and 'Lester') had a higher α-farnesene content in their essential oil than resistant cultivars ('Kent', 'Honeoye', and 'Bounty').

The analysis of isolated essential oil provide only a partial explaination of the total syndrome. Much more study is need to study the TSSM resistante of strawberry plants and their interaction with other plant and environmental factors.

P-56

MALE STERILE PLANTS IN *SALVIA SCLAREA* L. (LAMIACEAE LINDL.).

CITOEMBRYOLOGICAL STUDY ON THE MALE GENERATIVE ORGANS IN *SALVIA PRATENSIS* L. (LAMIACEAE LINDL.).

CV. DASKALOVA

Abstracts non available

P-57

DETERMINATION OF ORGANOCHLORINE PESTICIDES IN THE ESSENTIAL OILS OF *MENTHA X PIPERITA* L. AND *MENTHA ARVENSIS* L. VAR. *PIPERASCENS* HOLMES EX CHRISTY

M.C. HABENICHT.and H. SCHILCHER

It is well established that organochlorine pesticides are among the most persistant and toxic pollutants both in the terrestrial and the aquatic ecosystems. The use of most of these pesticides has been banned in many countries for several years, but they still have to be used in many countries of the Third World. And they are still of great importance for malaria control.

Many organochlorine pesticides can be quantitatively extracted from samples after 4-8 hours of steam distillation [Stijve *et al.*, 1973]. During the production of essential oils by using steam distillation the majority of organochlorine pesticides - which are contents of the plant material - get into the essential oil, too. Consequently the concentration of organochlorine pesticides obtained in essential oils is higher than the concentration of organochlorine pesticides in plant material. Published analysis about essential oils contaminated by organochlorine pesticides are missing because there seemed to be no scientific group which was able to develop a suitable cleanup method for determination of organochlorine pesticides.

Our aim was to develop a method to determine organochlorine pesticides in peppermint oil and mint oil.

Different cleanup methods were tested by adsorption chromatography with silica gel, Florisil and Calflo E and by gel chromatography with Bio Beads SX-3 and Sephadex LH-20.

Best of all cleanup methods was achieved by gel chromatography with system Sephadex LH-20/ethanol. The main quantity of essential oils was eluted before the organochlorine pesticides. Water and hexane were added to eluate. In a liquid-liquid partition step the organochlorine pesticides were transfered into the hexane phase.

The solution of organochlorine pesticides in hexane was evaporated. At this point the organochlorine pesticides could be determined by capillary gas chromatography with an electron capture detector. By using this method, 21 organochlorine pesticides could be analysed with high recovery rates and good reproducibility.

First results showed high residues of organochlorine pesticides in mint oil especially for the BCH-isomers.

P-58

EFFECT OF LIGNIN SUBSTANCES ON MYCELIAL GROWTH AND PRODUCTION OF *AR*-TURMERONE AND OTHER VOLATILES IN *INONOTUS OBLIQUUS, IN VITRO.*

K. KAHLOS, S. AURASAARI, I. LAAKSO and R. HILTUNEN

The living cells of trees can decisively influence the generation of the wood-rotting *Inonotus obliquus* (Pers. ex Fr.) Pilat and some constituents of bark or wood have been reported to stimulate or inhibit the fungal growth also *in vitro*.

This study investigated the effect of the media supplemented with spruce, pine, birch, rowan and alder on the mycelial growth and on the production of some volatiles in *I. obliquus*.

The mycelium samples were cultivated on solid cultures with woody sawdusts. The oils were isolated from woods, from growth media and from mycelium samples by hydrodistillation and they were then identified by GC and GC-MS analysis.

The mycelial growth was best as follows (in decreasing order): alder rowan birch spruce pine malt medium. However *I. obliquus* degraded enzymatically all lignins, producing its own metabolites in each case. Most of the mycelial volatiles were not present in the oils of woody media, except for δ-cadinol, which was often abundant in the growth media and the woody media. 1-Octen-3-ol was abundant in mycelia, whereas *ar*-turmerone, a characteristic bisabolane, in *I. obliquus,* occurred only occasionally *in vitro* .

P-59

PRODUCTION OF VOLATILE CONSTITUENTS IN *CARUM COPTICUM* CELL CULTURES.

G. ASGHARI and G.B. LOCKWOOD

The fruit of *Carum copticum* (Ajowan) is widely used as a spice and carminative in India and the Middle East due to the presence of an essential oil. Various workers have established cell cultures from a wide range of plants which produce essential oils including representatives from the Umbelliferae, but as a rule, much reduced levels of essential oil constituents are reported.

The only previous report of work with *C. copticum* cultures noted production of essential oil components to occur only in semi-organized cultures or in plantlets [Prabha *et al.*, 1991]. Fast growing suspension cultures were produced from callus which had been initiated from aseptically germinated seedlings.

Suspension cultures were grown on Murashige and Skoog's medium over more than 10 generations. Cultures were harvested at 28 days, freeze-dried, and extracted with ethyl acetate at 20°C. Although thymol (the major seed oil component) was absent, the major constituent found was a farnesene, plus also carene and cymene.

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THE EFFECT OF EMBRYOGENESIS ON PRODUCTION OF ESSENTIAL OIL CONSTITUENTS BY CELL CULTURES OF *CITRUS SINENSIS* L. OSBEK.

R. FOTOUHI, T.A. VILLIERS, G. ASGHARI and G.B. LOCKWOOD

The commercial importance of *Citrus ssp.* has generated widespread interest in *in vitro* culture and production of secondary metabolites. Much work has been carried out on the non-volatile flavonoid constituents responsible for some flavour properties [Mansell *et al.*, 1991], but little work on production of essential oil constituents has been published.

Undeveloped ovules from immature Valencia orange fruits were placed on one of five separate nutrient media. There was great variation in cell proliferation. Freeze dried ovules, embryos and callus were extracted in dichloromethane at 20°C and fractionated by capillary GC.

Constituents were quantified, and identification was carried out by GC/MS and co-chromatography. The amounts of major constituents, limonene and methyl N-methylanthranilate varied dependent upon morphological state of the plant material. Methyl N-methylanthranilate has been previously reported to occur in oils of a number of *Citrus ssp.*

It was first detected here in embryogenic callus from underdeveloped ovules, and also present in embryos and nucellar seedlings. Biosynthetically it is unrelated to limonene, which only appears later, in embryos and seedlings, the latter then becomes the major constituent of peel oil.

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INFLUENCE OF CULTURAL TREATMENT AND HARVEST TIME ON VETIVER OIL QUALITY IN BURUNDI.

M. DETHIER, Y. CORDIER, S. SAKUBU and C. MENUT

Vetiver (*Vetiveria zizanoïdes*) was introduced in Burundi for its anti-erosive properties. For a long time, vetiver essential oil has been used for preparation of high quality perfumes and valorisation by extraction of essential oil is gaining ground in Burundi.

In an agronomical station of Burundi (selected from experiments carried out in several locations) effects of adding minerals and manure (mineral fertilizer (NPK) (80-160-160), organic manure (20T/ha), fertilizer NPK + organic manure, blank block without treatment) were investigated as well as influence of harvest time (12 months, 14 months, 16 months, 20 months).

This poster presents yields of vetiver roots and corresponding cultural treatments at different harvest time. It shows essential oil yields (ml/kg dry matter) and oil quality (chromatographic analysis and olfactory tests). Essential oil yields obtained in Burundi (2.1 to 2.9 %) are higher than yields obtained in Haïti.

Highest contents of essential oil (69 lit/ha) were obtained once harvested after 20 months by means of organic manure as combined with mineral treatment. Influence of harvest time and cultural treatments on quality tests (olfactory tests) cannot be detected.

Olfactory tests have been performed by experts of Lautier-Florasynth and Charabot. A few samples are subject to trade activity.

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EFFECTS OF PLANTING AND HARVESTING TIME ON YIELDS AND ESSENTIAL OILS OF FENNEL (*FOENICULUM VULGARE* MILL.).

V. DELLACECCA, M. MAROTTI and R. PICCAGLIA

Fennel (*Foeniculum vulgare* Mill. subsp. *capillaceum*) is an important species widely cultivated in Italy as vegetable and utilized also as aromatic plant. The essential oil having a pleasant flavour is distributed in all the parts of the plant.

Three fennel «varieties» (*vulgare*, *dulce* and *azoricum*), sown in autumn and spring, were harvested at three phenological stages (full, bloom, waxy ripening and seed maturity). Among the three varieties, sweet fennel (var. *dulce*) behaved as annual plant whereas the other produced over two years. The aim of this research was to evaluate the agronomic performances (biomass and oil yield) and to compare the essential oils composition obtained from stems, umbels and seeds.

Generally, the seeds had the highest oil content and the stems the lowest one. The bitter fennel (var. *vulgare*) sown in autumn and harvested at waxy seed ripening stage, resulted in the highest oil yield. This harvesting stage is more suitable to obtain better oil yield in all compared varieties.

All the oils distilled from the different parts of the plant showed high content of (*E*)-anethole reaching the value of 92 % in the seeds of *dulce*.

During the ontogenic development (*E*)-anethole increased and limonene decreased.

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ESSENTIAL OILS EXTRACTION BY SUPERCRITICAL CO_2.

E. REVERCHON, G. DELLA PORTA and D. GORGOGLIONE

Supercritical CO_2 extraction (SFE) is in principle a simple process. Extraction can be performed by operating at supercritical fluid conditions in a pressurized vessel. Separation can be obtained by depressurizing the supercritical solution. Nevertheless, to obtain essential oils by supercritical CO_2 two main problems have to be overcome.

First, it is necessary to operate at the maximum selectivity to avoid coextraction of undesired compounds. Second, cuticular waxes have to be eliminated from the supercritical extracts. Our research group developed a SFE process that overcomes both these problems. An accurate selection of the extraction conditions; i.e., the use of supercritical CO_2 at low densities, avoids the extraction of high molecular weight compounds. A fractional separation technique has been developed to allow the precipitation of cuticular waxes before the recover of essential oil.

In this work the supercritical extraction and fractional separation of the essential oil from peppermint, chamomile and lavender was described. GC-MS analysis of extracts have been proposed. These oils have also been compared to the products obtained by hydrodistillation. Non negligible differences have been found. For example, linalyl acetate, that is one of the main compounds in lavender oil, decomposes during hydrodistillation while it is completely recovered by SFE.

The extraction process has also been tested on a pilot scale (20 dm^3) unit.

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OIL OF *MELISSA OFFICINALIS* AS AFFECTED BY STORAGE AND HERB DRYING.

A.S. SHALABY, S.E. EL-GENGAIHI and M.D. KHATTAB

The essential oil distilled from either the fresh or dried herb of *Melissa officinalis* L., grown in Egypt was subjected to GC-MS investigation. Among the 60 constituents separated, 26 were identified.

The oil consisted mainly of geranial, neral, citronellal, β-caryophyllene oxide, β-caryophyllene, geraniol and 6-methyl-5-hepten-2-ol. Drying of the herb did not change the oil spectrum, but changed the relative percentage of some constituents. Drying of melissa herb increased the content of 6-methyl-5-heptene-2-ol, linalool, citronellal, β-caryophyllene, α-terpineol and β-caryophyllene oxide, while decreased the neral, geraniol, geranyl acetate, neryl acetate and geraniol.

The freshly distilled oil was stored in glass or aluminium bottles and kept at room temperature or refrigerated for one year. The proportional content of some constituents has been changed during the storage period lasted for a year. The observed changes were due to the storage duration rather than the storage condition.

According to the fluctuation pattern of the oil constituents, they could be classified in three groups. The most noticeable change was the increase in citral content, while a decrease in caryophyllene derivatives and citronellal.

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INFLUENCE OF UV-B RADIATION AND OZONE ON ESSENTIAL OIL COMPOSITION OF NORWAY SPRUCE NEEDLES (PICEA ABIES L. (KARST.)).

R. STEINBRECHER, H. PANTEN and W. HELLER

A large number of investigations have studied the biogenic production of monoterpenes in plants. It is known that several environmental and genetic factors influence the composition and yield of monoterpenes.

Nowadays UV-B radiation and ground level ozone mixing ratios are increasing. A feedback of these factors on monoterpene synthesis in plants can not be excluded. The aim of this study was to investigate the influence of UV-B radiation in combination with ozone on the essential oil composition of Norway spruce needles. In phytotrons of the GSF Neuherberg, 4 year old spruce trees were exposed over one vegetation period to several treatments: 1x ozone/+UV-B; 2x ozone/+UV-B; 2x ozone/-UV-B.

Ozone values, air temperature, relative air humidity and light intensity were simulated on the basis of data from the Wank Mountain, Garmisch-Partenkirchen (735 m above sea level) 1990. Natural UV-B radiation was applied according to the maximum available capacity (env. 0.3 med). After an exposure to the different treatments over 18 weeks needles were harvested and ground under liquid nitrogen. After cold extraction in pentane spiked with internal standard, the extract was analysed by capillary gas chromatography with respect to terpenes.

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EFFECT OF SOME PHENYLUREA TYPE CYTOKININS ON THE PRODUCTIVITY AND QUALITY OF THE SPEARMING CULTIVAR MECHTA OIL

T. STOEVA, L. ILIEV and T. DONCHEV

The investigations concern the main physiological reactions produced by cytokinins. As it is known, they overcome apical dominance and dormancy and in this way affect plant productivity.

The preliminary investigations were conducted with the new Bulgarian Spearmint cultivar Mechta. As cytokinins were used 4PU30 in concentrations of 25 mg/l and 50 mg/l and DROPP in concentrations of 50 mg/l and 100 mg/l. Treatment of the plants took place in the autumn 4-6 weeks post Spearmint harvest and in spring, when the plants had reached the height of 10-15 cm.

Results of the field trials showed that DROPP was more active at the higher concentration. It increased the yields of overground fresh weight and of essential oil changing the ratio of its constituents. However, the final assessment of the prospects for the future extension of the two cytokinins depends on the sensory analyses of the essential oil aroma. The investigations will contribute to explain the specific mechanism of urea cytokinin action in Spearmint.

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THE EFFECT OF ORANGE OIL PROCESSING ON THE CONSUMER ACCEPTANCE OF FORTIFIED ORANGE JUICE.

R.L. SWAINE and K.A. LEAVELL

The fortification of foods and beverages is becoming increasingly more prevalent. This nutritional benefit to the consumer has presented the flavour chemist with interesting challenges as each nutrient presents a unique flavour, aroma, or mouthfeel that must be ameliorated, rendering the fortified product not only palatable or potable but pleasing to the senses. Because of the universal acceptance of citrus beverages, orange juice has been the medium for numerous nutrient supplements. The calcium fortification of orange juice is an excellent example.

The addition of calcium to fruit juices has a detrimental effect on flavor, accelerating the degradation of non-volatile precursors such as ascorbic acid, and resulting in the formation of highly unstable reactive dicarbonyl intermediates that upon further degradation give rise to potent aromatic compounds whose effects are most deleterious to the delicate flavor of orange and other citrus beverages.

Addition of large quantities of cold pressed citrus oils or traditionally processed oils required to mask untoward aromas introduce substantial amounts of volatile off flavour precursors and fatty-waxy off flavours respectively. This reports the effect of selectively processed orange oils to overcome the flavour deficiencies of calcium fortified citrus juices and beverages.

THE ESSENTIAL OIL OF *SALVIA SCLAREA* L. EXAMINED DURING THE ONTOGENESIS IN COMPARISON WITH SOME COMMERCIAL SAMPLES SEARCHING FOR THE CAUSES OF DIFFERENT COLOURS

M. THEN

The essential oils of the clary sage are extremely sensitive to environmental effects being submitted to colour changement from faint yelllow to a darkened one. In order to improve the colour characterisitics different refinery technics were applied meanwhile checking the colour of the produced oils as well as the qualitative and quantitative changements of the oil components.

According to the author's findings the applied technologies are not convenient neither for improving the colour of the oils nor for the enrichment on the components. They are inclined to think that for the quality of the oil produced from the drugs the first distillation processes are crucial. The decomposition and colour changement processes are catalyzed by Cu^{++} and Fe^{+++} ions as well as by accumulated acids.

The colour should be instrumentally measured in order to better establish the colour nuances. The colour differences can not be considered composition dependent, they could be much more related to physical parameters.

Finally, it was hoped to determine the influence exercised by the volatile oil content in the different parts of the flower (bracts, petals, carpels) on the composition, and possibily quality of the oil, and with the information thus obtained to work out a procedure to replace the extremely expensive absolute production technology currently used in Hungary.

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INTRODUCTION

The aim of this work was to determine the enantiomeric composition of the monoterpene hydrocarbons of *Abies sachalinensis* (Fr. Schm.) Mast. and *A. mayriana* Miy. et Kudo needle oils, and to study the chemotaxonomic markers by multivariate analysis in order to separate these two species.

MATERIAL AND METHODS

Plant material

Branches from *A. sachalinensis* (n=10, tree numbers 1-10) and *A. mayriana* (n=10, numbers 11-20) were obtained from an *Abies* collection at Ruotsinkylä Field Station (Forest Research Institute), Helsinki. The trees, bred from seeds of Japanese origin, were 71 years old.

Analytical conditions

GC-MS analyses were made on an HP 5890 GC coupled to an HP 5970 quadrupole MS detector operating at an ionization voltage of 70 eV and an electron multiplier voltage of 1600 V. A chiral heptakis-β-cyclodextrin column (30 m, 0.25 mm i.d., J & W Scientific, USA) was used at an oven temparature programme from 70°C to 220°C at 6°C/min.

Identification

The identification was based on GC retention times of authentic samples (Aldrich - Chemie, Germany), GC-MS spectra and retention data obtained on a β-cyclodextrin column.

RESULTS AND DISCUSSION

The needle oils of both species were characterized by high contents of (–)-camphene and bornyl acetate (see Fig. 1 and Table). Other major enantiomers included (–)-α-pinene, (–)-β-pinene, (–)-limonene and (–)-β-phellandrene.

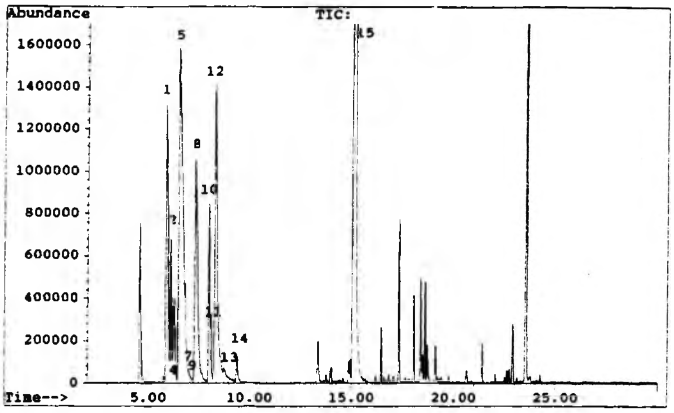

Fig. 1. GC–MS chromatogram of *A. sachalinensis* needle oil.

No significant differences between the enantiomeric compositions of the two species were found by the *t*-test, indicating a high tree-to-tree variation.

Cluster analysis

In order to study the closer relationships among the trees within species a cluster analysis was applied (see Fig. 2.).

Table. Monoterpene composition in the four cluster groups.

	Cluster I mean %	Cluster II mean %	 s.d.	Cluster III mean %	 s.d.	Cluster IV mean %	 s.d.
1. (-)-α-Pinene	11.5	8.1	1.7	7.7	1.1	8.2	2.2
2. (+)-α-Pinene	**8.3**	4.7	1.2	4.0	2.1	1.8	0.7
3. Tricyclene	2.9	2.0	0.6	2.3	0.3	1.8	0.2
4. (+)-Sabinene	0.0	0.6	0.7	0.3	0.3	0.0	0.0
5. (-)-Camphene	**27.7**	**19.9**	2.7	15.2	1.4	12.6	1.1
6. Myrcene	0.0	1.1	1.1	2.3	1.3	2.8	0.5
7. (+)-Camphene	0.0	0.1	0.2	0.1	0.2	0.4	0.7
8. (-)-β-Pinene	3.4	**6.2**	1.5	**8.9**	1.3	3.3	1.4
9. (+)-β-Pinene	0.0	0.1	0.1	0.1	0.1	0.0	0.0
10. (-)-Limonene	2.9	3.1	0.8	3.6	1.4	**25.6**	2.1
11. (+)-Limonene	1.6	1.2	0.2	0.8	0.4	0.0	0.0
12. (-)-β-Phellandrene	4.3	6.4	2.7	**11.4**	3.6	3.2	1.0
13. γ-Terpinene	0.0	0.2	0.1	0.5	0.3	0.4	0.2
14. Terpinolene	0.4	0.6	0.1	0.6	0.1	0.7	0.4
15. Bornyl acetate	26.4	**31.2**	2.8	23.9	1.5	23.6	0.5
	N = 1	N = 8		N = 9		N=2	

N = number of observations

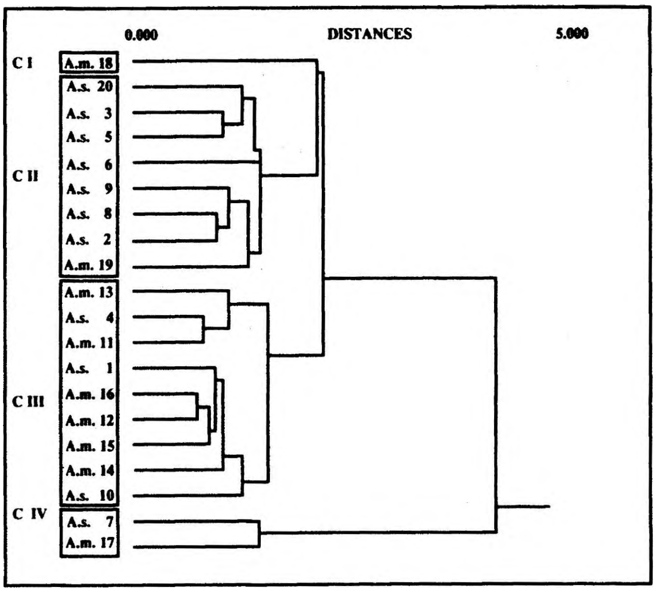

Fig. 2. Dendrogram obtained by cluster analysis. A.m. = *Abies mayriana*; A.s. = *A. sachalinensis*.

CONCLUSIONS

The cluster analysis mainly separated *A. sachalinensis* and *A. mayriana* trees into different clusters (groups).

Compositional variation of monoterpene enantiomers may provide useful information for chemotaxonomic classifications.

AITP

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Grasse France 5th, 6th & 7th 1994

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EDITION January 1995

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Grasse, January 1995

Dear Delegate,

You'll find hereafter the register of all the members who attented the 25th International Symposium on Essential Oils in GRASSE, last September.

They are classified following the alphabetic order.

We have checked the first edition, according to the wishes of some of you.

For each entry, you'll have the full address and the fax number, and for most of them, a picture ! We apologize for the absents.

See you in Hambourg
and again best wishes for 1995

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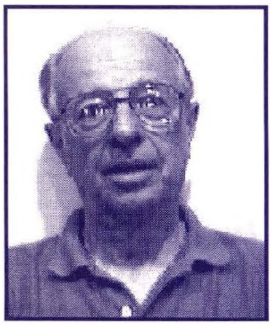

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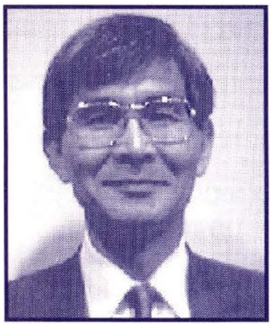

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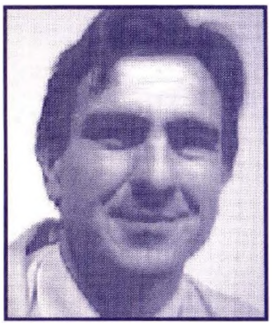

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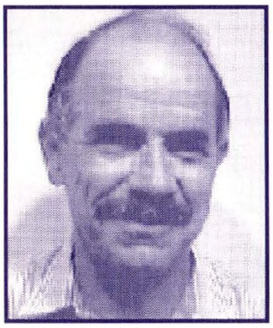

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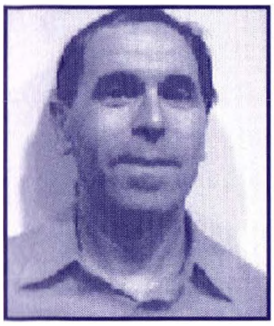

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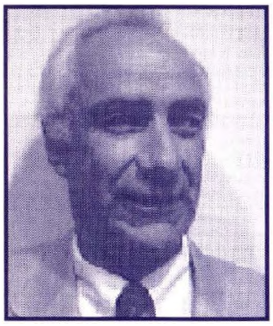

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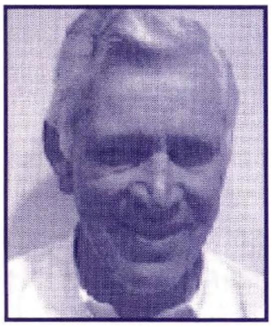

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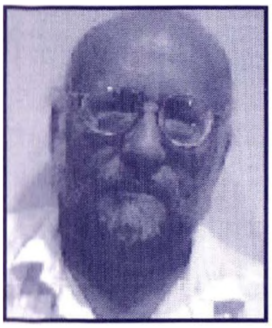

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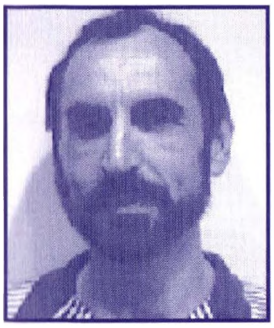

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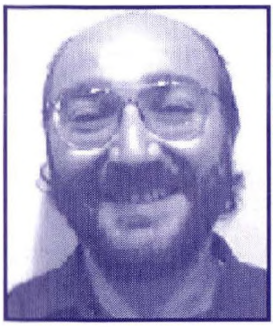

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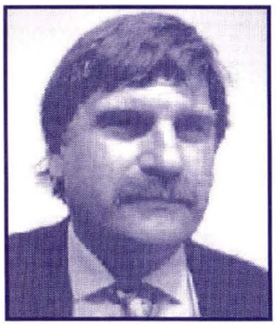

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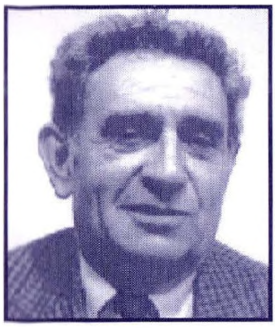

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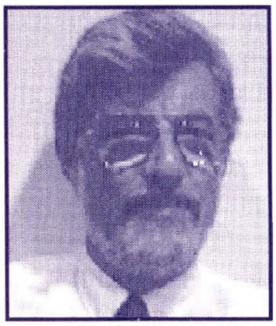

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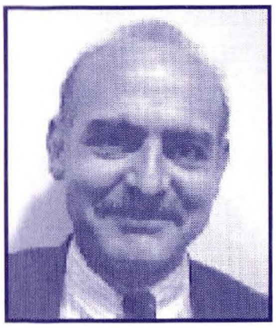

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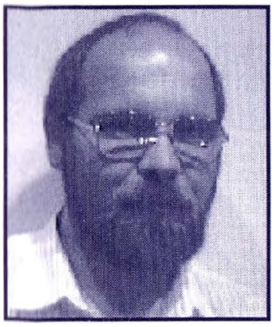

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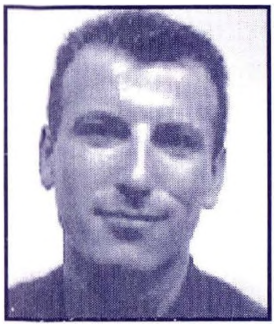

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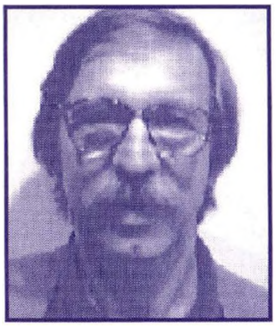

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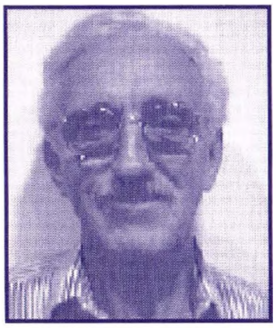

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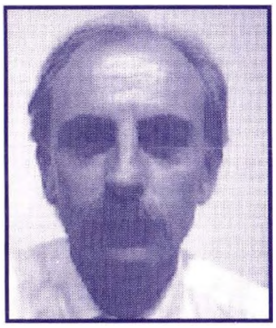

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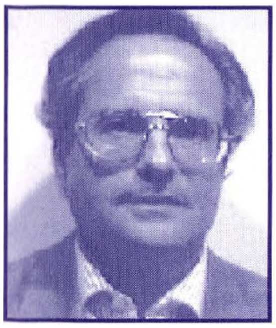

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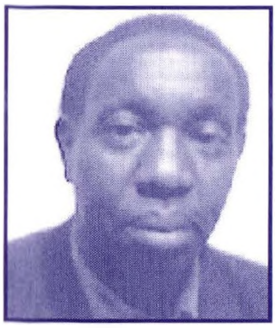

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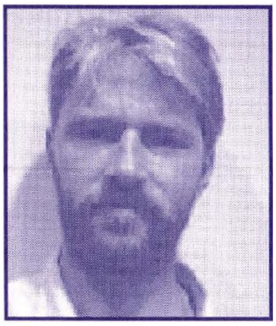

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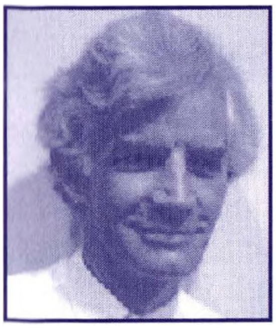

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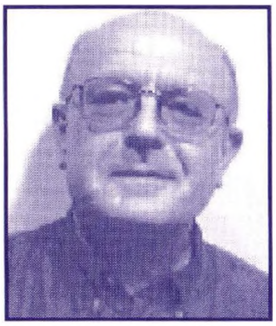

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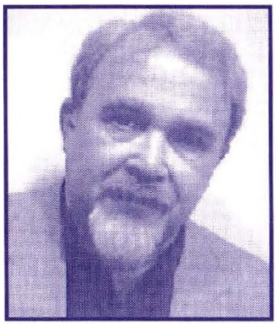

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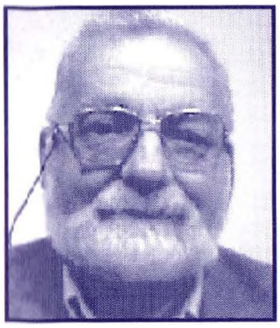

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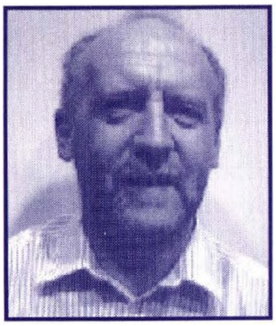

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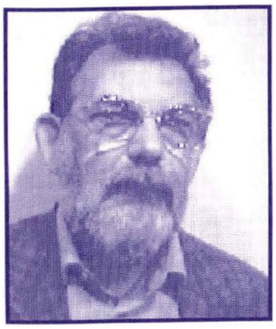

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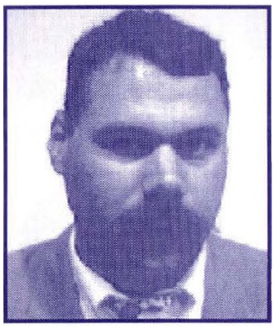

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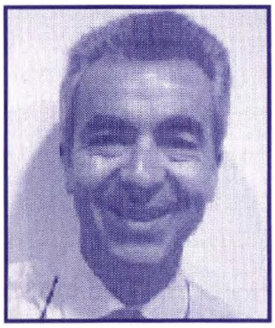

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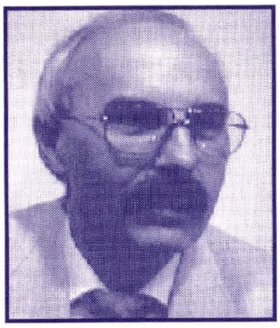

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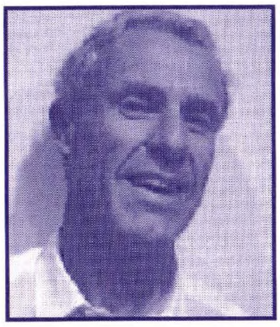

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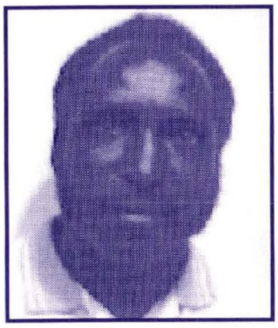

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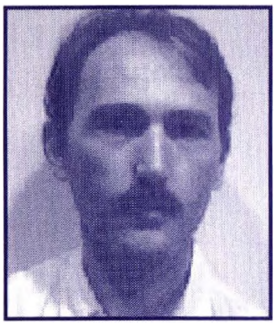

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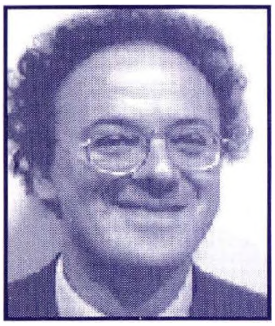

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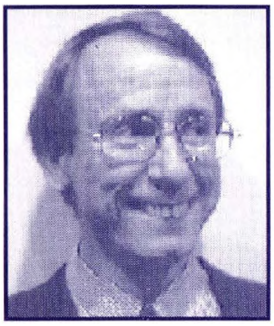

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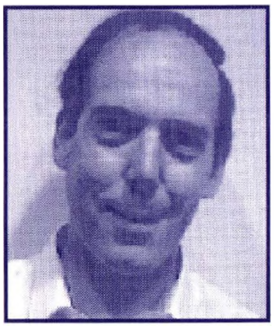

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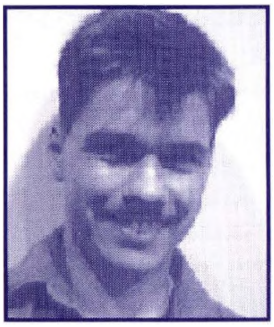

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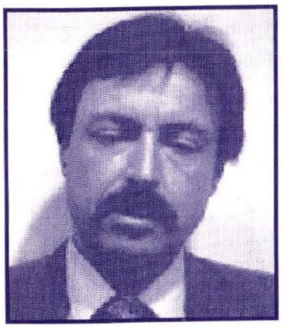

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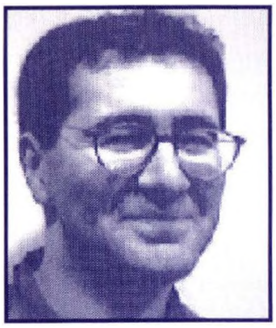

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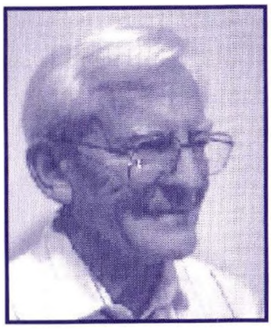

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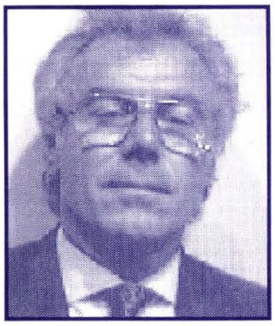

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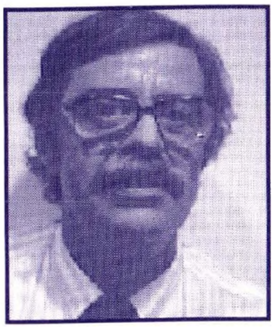

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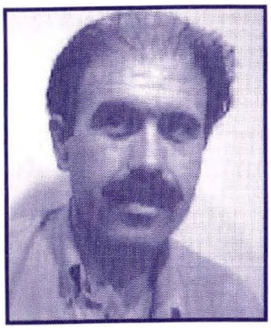

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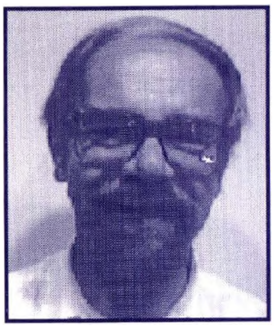

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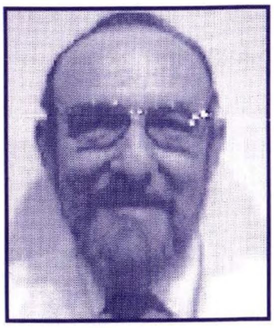

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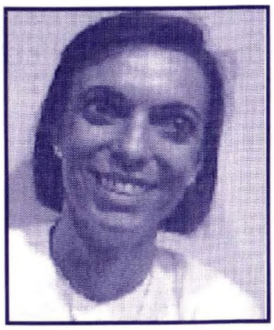

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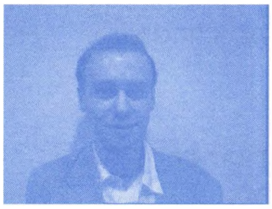

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AITP

Grasse, November 1994

Dear Delegate,

You kindly decided to attend the 25th International Symposium on Essential Oils in GRASSE, last September .

That is already two months ago .

Our friend, Kristian, cartoonist of the french TV network FRANCE 3, has been drawing his own perception of the Symposium .

We would like now have a few minutes of your time, so that we could better know what went right and wrong .

As we plan to organize a forth-coming ISEO, feel free to be cruel, if you want things to be improved...

We really enjoyed welcoming you then, and organizing the 25th ISEO, and look forward to meet you soon, at least in HAMBURG .

Yours sincerely and thankfully,

Yvan COSTE-MANIERE

ASSOCIATION DES INGENIEURS ET TECHNICIENS DE LA PARFUMERIE
48, AVENUE RIOU-BLANQUET 06130 GRASSE

AITP

Grasse, March the 24th

Ivan COSTE-MANIERE
Chairman

Dear friends,

It has already been a long time since we met, last September in Grasse, during the 25th International Symposium on essential oils.

Fortunately, we made a few mistakes then, allowing us to prepare a new - and last ! issue of the "who's who" of this edition.

Furthermore - and thanks to you who provide us with those numerous answers - you will find the results of our survey ; our feelings were right : in case of any possibility, we would like to organize and ISEO once more...

Let me, in the name of all our members, thank you for being so patient and kind to us... The marks were good and we loved to live those three wonderfull days.

Looking forward to meeting you in Hamburg.

ASSOCIATION DES INGENIEURS ET TECHNICIENS DE LA PARFUMERIE
48, AVENUE RIOU-BLANQUET 06130 GRASSE

Grasse City 1.02
Registration 0.93

Congress Hall + CIG 1.22
Post Congress 0.30
Opening session : 1
Scientific contents + quality of exchanges : 1.22
NOVARO : 1.63 (glass art works)

Gifts : 1.1

Meal : 0.69
Mac Donald's -0.02 beverages

Poster session 1.13
Proceedings 1.04
De Pass 1.20 (paintings, sculptures and ties)
Magic show 0.93

These marks are resulting from your answers (between - 2 very bad and + 2 very good).

INTERNATIONAL Citrus Symposium

A symposium on **Citrus Oils, Essences and Juices** will be held January 29 - February 1, 1996, in Orlando, Florida. This specialized symposium will provide an opportunity to present and hear the latest results of research on citrus products. Speakers from the United States, Spain, Israel and England have indicated interest in presenting reports on the citrus industry in their countries. Program chairmen are Dr. Brian Lawrence, R. J. Reynolds Tobacco Company, and Dr. John Attaway, Research Department for Citrus, State of Florida.

This symposium will be a unique opportunity to discuss all aspects of citrus products used in the preparation of flavors and fragrances. There will be papers on the international market for citrus products and on the application of citrus products to flavors and fragrances.

This will also be an opportunity to visit the citrus industry in Florida at a time of high-volume processing and production of orange juice and orange oil. It is a spectacular sight, not to be missed. Experiencing the volume production of this key food and beverage flavor will provide an understanding of the industrial processes and how they affect the quantity, quality and, ultimately, the price of citrus products and by-products.

If you have any comments or questions, or would like to present a scientific paper or poster, please contact us.

INTERNATIONAL • CITRUS • SYMPOSIUM

Who: People in academia and government involved in citrus products research and analysis. Anyone supplying citrus products and by-products to the flavor and fragrance industry, growers, processors, traders, dealers, buyers, analytical and quality control chemists.

When: January 29 - February 1, 1996

Where: Clarion Plaza Hotel
9700 International Drive, Orlando, FL - USA
Tel: 708/653-2155 • Fax: 708/653-2192

Registration Cost: $215 (before Sept. 15), $245 (after Sept. 15)

Cancellations after December 28, 1995, refund less 10%.
Cancellations after January 19, 1996, no refund, but transferrable.

Provisional Program

Monday, January 29, 1996	10:00 - 13:00 Registration 13:00 - 17:30 Lectures 17:30 - 18:30 Get-Together Party
Tuesday, January 30, 1996	09:00 - 13:00 Lectures 13:00 - 14:00 Lunch 14:00 - 18:00 Poster Session 18:30 - Cocktail Social–Sponsored by Tastemaker 19:30 - Symposium Dinner
Wednesday, January 31, 1996	09:00 - 13:00 Lectures Afternoon Free
Thursday, February 1, 1996	09:00 - Tour to Tastemaker Citrus Flavor Processing Facility, with lunch included and tour through an orange processing facility. 16:00 - Return to Hotel

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INTERNATIONAL

Citrus Symposium

Delegate Registration Fee - **$215.00** Registration Fee includes Abstracts of the presentations; attendance at the Lecture and Poster Sessions; lunch on Tuesday, January 30; Continental Buffet Breakfasts on Tuesday, January 30, and Wednesday, January 31; Coffee Breaks during Lecture Sessions; Cocktail Party and Symposium Banquet on Tuesday, January 30; Tour to the Tastemaker Citrus Flavor Processing Facility and through an orange processing facility producing juice and oils.

Accompanying Person Registration Fee - **$110.00** Registration fee includes Get-Together Party on Monday, January 29; Continental Buffet Breakfasts on Tuesday, January 30, and Wednesday, January 31; Cocktail Party and Symposium Banquet on Tuesday, January 30.

Hotel - All Symposium activities will be held at the Clarion Plaza Hotel, 9700 International Drive, Orlando, Florida. A block of rooms has been reserved at the special rate of $106.00 (plus tax) per night, either single or double. Please copy the registration form in order to send one registration form for each delegate attending. Send one night's payment with each registration.

Registration

INTERNATIONAL • CITRUS • SYMPOSIUM

☐ I have enclosed my check for full registration payment of US$ ____________

Please charge my ☐ VISA ☐ MasterCard

Name: ________________________________

Accompanying Person Name: ________________________________

Address: ________________________________

City, State, Country: ________________________________

Telephone: ____________________ Fax: ____________________

Date of Arrival: ____________________ Date of Departure: ____________________

Registration payment	**Before Sept. 15**	**After Sept. 15**
Registration fee	$215	$245
Accompanying person ($110)	$	
Hotel room(s) (☐ single; ☐ double)	$106	
Total Payment	**$**	

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