28th ISEO

28th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

1-3 September 1997
Eskişehir-TURKEY

ABSTRACTS

Anadolu University
Medicinal and Aromatic Plant and Drug Research Centre
(TBAM)

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PLENARY LECTURES

PL1

SCENTS FROM RAIN FORESTS

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Since the dawn of time, the secretive and fragile kingdom of the rain forest, which covers 11-13% of all emerged land and shelters eight to nine tenths of all forms of known and unknown life, harbors extraordinary biological treasures worth to be treated with utmost care. According to a further estimation, 70-80 % of these species, meaning around 60% of the entire biodiversity, is found for ecological reasons in the canopy region of the rain forests.

Having been convinced to find this richness also reflected on the olfactory level we searched during the past years in the understory as well as in the canopy of rain forests for new attractive scents. This communication opens the discussion of chemical, biological and olfactory aspects of such scents.

PL2

THE ESSENTIAL OIL COMPOSITION OF *PIMPINELLA* SPECIES

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The genus Pimpinella, belonging to the Apiaceae (Umbelliferae) subfamily Apioideae comprises about 150 species, which occur largely in Europe and Asia extending to China. It is a slightly uniform genus among the species-rich genera of umbellifers and has no generic morphological character, which can be found in all of its species. Due to the different features which have been employed, the limitation of the genus Pimpinella, as well as its infra-generic division differ considerably. Considering those difficulties, it seemed promising to investigate the essential oils of different *Pimpinella* species and to compare the obtained results with each other and with analytical results gained from other species from the Apiaceae. Considering the large size of the family, we decided to restrict our investigations to the subfamily Apioideae neglecting the other subfamilies. Within the Apioideae, we concentrated our attention on Pimpinella and related genera.

All the essential oils which have been investigated contain considerable amounts of phenylpropane and sesquiterpene derivatives, whereas monoterpenes occur only in small amounts. Most of the phenylpropanoids found exhibit the unusual substitution pattern 2,5 of the aromatic ring, which has not been found elsewhere up till now. In addition to these phenylpropanoids, all plants of the genus Pimpinella contain trinorsesquiterpenoids. In view of the extremely restricted occurrence of these compounds in the plant kingdom, the possible taxonomic value of these substances with regard of the delimitation of the genus Pimpinella will be discussed.

PL3

AROMATHERAPY - A COMPILATION OF SCIENTIFIC FACTS IN FAVOUR OF ITS CORRECT USE

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In this review a survey of scientific methods in aromatherapy research is given. Facts obtained by serious scientific investigations are presented in order to stress the fact that aromatherapy has nothing to do with holistics, or esoterics, or magics. Data obtained by animal experiments as well as by human studies clearly show that fragrance compounds behave like pharmaca despite their psychic properties. Therefore the apt description of fragrance compounds and essential oils (as their mixtures) when used in a therapeutic manner should be AROMATHERAPEUTICS. The influence of chirality of fragrance compounds on certain pharmacokinetic parameters is discussed likewise.

PL4

RECENT DEVELOPMENTS IN PHYTOCHEMISTRY OF BRYOPHYTES

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A number of bryophytes have been used as medicinal plants in North America, China and Europe to treat burns, bruises, external wounds, *etc.* Some liverworts contain diuretic, antimicrobial, antifungal, and antitumor active substances. Some bryophytes show characteristically fragrant odors and intensely hot and bitter or saccharine-like taste. Generally, bryophytes are not damaged by insects, snails, slugs and other small animals. Furthermore, some liverworts cause intense allergenic contact dermatitis and allelopathy. We have been interested in these Bioactive substances found in bryophytes and have studied about 800 species of Hepatica with respect to their chemistry, pharmacology, and application as sources of cosmetics, and medicinal or agricultural drugs. It has been demonstrated that most of the Hepaticae contain mainly sesqui- and diterpenoids and lipophilic aromatic compounds which constitute the oil bodies. The biological activities of liverworts are due to these substances. At present more than 300 new compounds have been isolated and their structures elucidated. The present lecture shows the structures of novel terpenoids and aromatic compounds and their biological activity (fragrant odor, pungency, antimicrobial, antifungal, antifeedant, antitumor, anti-HIV-1, piscicidal, molluscicidal, cytotoxic, neuritic sprouting, muscle relaxing and 5-lipoxygenase, calmodulin, DNA polymelase, cathepsins B and L inhibitory activities).

PL5

USE OF ESSENTIAL OILS IN THE FORMULATION OF NATURAL FLAVOURS

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Outline:

Natural mixtures derived from plants: definition of an essential oil. Reglatory aspects: the FEMA/GRAS list in the USA; the case of Europe. Processing of essential oils: concentration and fractionation; natural isolates from essential oils. Purity assessment of essential oils in relation with safety regulations. Authenticity and natural character assessment: some analytical considerations. Application of essential oils in sweet flavours. The interest of using essential oils as such for implementing isolate-based formulations. The potential of essential oils in savory and fatty flavour formulations.

PL6

SYNESTHESIA

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Although fragrance is something invisible it is strongly felt, and has always excited interest and has certain magical power assigned to it. In addition to their aromachological aspects, the psychological effect of a perfume can help in differentiation of a product from its competitors and strengthen its position. A lot of work has been done in trying to understand the influence of fragrance on behavior and perception. However, the understanding of these effects is still in its infancy. At Givaudan-Roure, our research program covers **synesthesia**, odor imagery, language, odor classification and sensory mapping. It is directed to providing insight for perfumers to help them create fragrances that complement or reinforce the concept of the product, especially their sensory psychological benefits.

Synesthesia is the interrelationship of the senses. Certain people can describe music and numbers in terms of color. It is a condition in which a person experiences a sensation in one sensory modality and it triggers an involuntary and automatic sensation in a different modality. Synesthesia was investigated using the Rader and Tellengen test, which classify it into two components : experiential and translation. This methodology has enabled us to understand the cross modal correspondence between vision and olfaction. Data bases of odor-color and odor-mood associations have been developed for a large number of aroma chemicals. The basic protocol involves subject smelling an odor or fragrance and distributing five point across eleven mood words and eleven color words, the tests being carried out in custom designed sensory psychology booths.

PL7

IDENTIFICATION OF RARE SESQUITERPENE STRUCTURES IN ESSENTIAL OILS

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Sesqiterpene derivatives substantiate a major portion and may often be responsible for a specific sensoric impression of essential oils. In addition, an extraordinary structural variability, caused by numerous skeletal rearrangements during biosynthesis, is observed for sesquiterpenes. The elucidation of the structure and stereochemical properties of unknown sesquiterpenes is therefore a great challenge. The detection of a series of structurally related sesquiterpenes may also serve to recognize biogenetic correlations. The isolation of some novel sesqiterpene constituents from essential oils with the rarely found africanane, brasilane, nardosinane and asteriscane skeletens by preparative chromatographic methods and their identifcation with modern NMR techniques, mass spectrometry, enantioselective gas chromatography, and by investigating thermally and acid catalyzed rearrangements and microchemical reactions will be demonstrated.

PL8

SOME NOVEL TRICYCLIC SESQUITERPENE SKELETONS FROM THE ESSENTIAL OIL OF *ECHINOPS GIGANTEUS*

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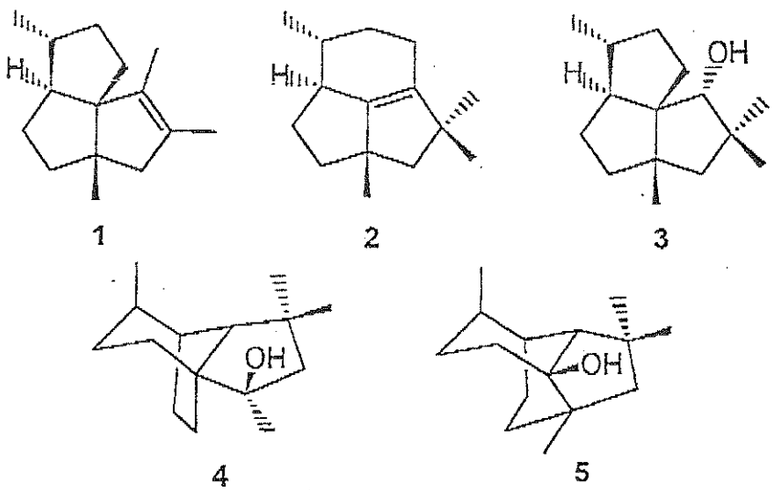
The essential oil from the roots of *Echinops giganteus var. lelyi* from Cameroon contains almost exclusively tricyclic sesquiterpenes (77%) with presilphiperfolane, silphiperfolane, isocomane and modhephane skeletons[1]. The main constituents are silphiperfol-6-ene (1, 27%) and presilphiperfol-7-ene (2, 9%). The structures of seven new sesquiterpene alcohols were elucidated by detailed NMR spectroscopy. Three alcohols with new skeletons named cameroonan-7-ol (3), nopsan-4-ol (4) and echinan-8-ol (5) were isolated. Two of them (3 and 5) contribute decisively to the strong woody, patchouli- and amber-like odor of the oil.



1 2 3 4 5

[1] C. Menut, G. Lamaty, P. Weyerstahl, H. Marschall, I. Seelmann, P. H. Amvam Zollo, *Flavour Fragr. J.*, 12, in press (1997).

ORAL PRESENTATION

01

ANTIFUNGAL PROPERTIES OF ESSENTIAL OIL COMPONENTS

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Although considerable information on the antifungal activity of spices and essential oils is available, data about the antimicrobial properties of their constituents, especially the terpenes, is very limited.

Previously we described the bioconversion of some terpenes by fungi. Nerol, geraniol and citral were completely converted by sporulated surface cultures of *P. digitatum* to methylheptenone, and by *A. niger* to linalool. Linalool on the other hand was transformed to linalool oxides by submerged liquid cultures of *A. niger*. The biotransformation of citronellol to isopulegol and α-terpineol, was carried out by sporulated surface cultures of *A. niger* and *P. digitatum*, but didn't take place when the organisms were grown as submerged liquid cultures. The bioconversion of isoeugenol to vanillin by *A. niger* was also investigated.

In this study, the inhibitory effects of the phenolic compounds ferulic acid, eugenol and isoeugenol, the terpenes geraniol, nerol, citral, citronellol and linalool and the biotransformation products vanillin, 6-methyl-5-hepten-2-one and furanoid linalool oxides towards the fungi *Aspergillus niger* and *Penicillium digitatum* were compared. It was shown that the terpene alcohol citronellol and the phenolic compounds eugenol and isoeugenol were the most inhibitory substrates, whereas the products formed through biotransformation by the fungi were the least antifungal: high concentrations of methylheptenone and linalool oxide could be tolerated by the fungi, without loss of germination and sporulation capacity. From these results, it was postulated that biotransformation of terpenes might be a detoxification mechanism of the fungi.

O2

The Effect of the Essential Oil of *Melaleuca alternifolia* on *Escherichia coli*.

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The essential oil of *Melaleuca alternifolia* (tea tree) exhibits broad-spectrum antimicrobial activity. We have further investigated this activity by examining the effect of tea tree oil on the survival, growth rate and physiology of *Escherichia coli* strain AG100. Here we report that tea tree oil-induced cell death is accompanied by an inhibition of respiration and the formation of extracellular blebs on the cell surface observable in electron micrographs.

Kill rate data indicated that 0.125 % (v/v) tea tree oil inhibited glucose-dependent respiration and was lethal to cells in the assay conditions used. Below 0.125 %, no inhibition of either respiration or the growth rate of cells was detected.

Our results also show that stationary phase cells are more tolerant to tea tree oil than cells harvested in logarithmic phase. At 0.25 %, tea tree oil completely reduced cell numbers in suspensions from a 5 hour culture within 20 minutes, whereas, cells grown for 48 hours died more slowly, with complete die-off taking 60 minutes. Similarly, tea tree oil-induced inhibition of respiration was less extensive for stationary phase cells. These findings indicate that longer exposure times were required to cause a lethal degree of physiological damage to *E.coli* AG100 cells in stationary phase.

Overall, the inhibitory effects of tea tree oil on respiration and on cell structure, as observed by electron microscopy, in *E.coli* are consistent with a disruption of membrane structure and function related to the accumulation of tea tree oil monoterpenes. The dose-dependence observed for these effects is most likely a product of the rate and extent of partitioning of monoterpenes between the aqueous phase of cell suspensions and the hydrophobic regions of the cells.

O3

BIOSYNTHESIS AND HYDROLYSIS OF MONOTERPENE GLYCOSIDES

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The objective of this work was to study the biosynthesis of ^{14}C-monoterpenol glycosides (geranyl glucoside) in the leaves and the berries of Muscat of Alexandria. This was accomplished by (a) reaction between the enzyme MUGT (isolated from the Muscat leaves) and natural geraniol in the presence of UDPG-^{14}C and (b) by reaction of glucosyl transferase and ^{14}C-labeled geraniol which was prepared by incubating the leaves of Geranium plant in ^{14}C-CO_2 containing atmosphere. The other objective was to increase the yield of β-glycosidase (which cleaves the β-glycosidic bond of the monoterpenol glycosides of wine and increase significantly the aroma (free terpenols) of wine. This was accomplished by introducing the inducer IPTGlc (Isopropyl-thio-β-glucoside) into the strain of *Aspergillus niger* B-1, grown on bran as carbon source. The enzyme (β-glycosidase) was also capable of hydrolyzing cyanogenic glycosides and thus has the potential of detoxification of foods, such as cassava.

O4

THE BIOSYNTHESIS OF ESSENTIAL OIL COMPONENTS IN AROMATIC PLANTS.

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The unique fragrant properties of essential oils are determined by the chemical structure and relative abundance of their volatile components. These volatile compounds belong to several chemical groups, such as mono- and sesquiterpenes, phenylpropanoids and others. Plants produce and store essential oils in specialized anatomical structures, such as oil cells, glands and ducts, and possess dedicated enzymatic pathways that biosynthesize the essential oil components from metabolic precursors. The presence or absence of particular biosynthetic enzymes in a given plant tissue, ultimately determines the composition of the essential oils produced and stored. A few examples demonstrating the several biosynthetic pathways to mono- and sesquiterpenes, phenolic compounds and other important essential oil components will be explained, and some of their postulated ecological roles will be discussed.

O5

SUPERCRITICAL FLUID EXTRACTION OF TERPENE HYDROCARBONS FROM CITRUS OILS

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The separation of terpene hydrocarbons from citrus oils has been studied using supercritical CO_2 as solvent. While conventional separation processes show disadvantages regarding organic solvent residues or high temperatures, supercritical fluid extraction (SFE) allows fractionation at moderate temperatures and solvent free products. Publications in the field of deterpenation by SFE are mainly about vapor-liquid phase equilibrium (VLE) data of pure components in supercritical CO_2 or about separations of model mixtures.
To study the behavior of real multicomponent mixtures orange peel oil was used for VLE measurements and multistage countercurrent extraction. This method gives the best product quality in a continuous mode depending on operating conditions and the height of the column. Experiments have been carried out in a pressure range from 8 to 13 MPa to observe the two-phase region at 323, 333, and 343 K. VLE experiments gave loadings of the CO_2 phase from 10 to 100 g extract/kg CO_2. To investigate the composition dependency of the phase behavior fractionated products from countercurrent experiments were also used for VLE measurements. Samples from both phases were analysed by GC. To evaluate the separation of (mono-)terpenes from oxygenated and other low volatile components peaks were assembled to obtain two groups, "terpene" and "aroma" group. The distribution factors of linalool and decanal were also used as separation indicators.
The best separation was observed at low temperatures and pressures far from the one-phase region of the system. Unfortunately the high operating costs at those low loading conditions make the process unfavorable from an economical point of view because of very high solvent-to-feed ratios. Therefore, higher temperatures and pressures resulting in higher loadings allow much lower operating costs. The optimal extraction temperature will be limited by the thermal stability of the feed material. Regardless of economic evaluations deterpenation by means of SFE will give high quality products of the desired composition.

O6

"The Evolution of a new Generation of Fine Natural Plant Oils"

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ABSTRACT

Over the past ten years, our successful harnessing of the remarkable properties of the solvent 1,1,1,2 - tetrafluoroethane and the perfection of economical, industrial scale equipment to exploit them, has allowed us to develop over 300 new fine plant extracts.

Many of these extracts are proving to be of major interest in the preparation of functional skin care products, fragrances, flavours and phyto-pharmaceuticals.

We initially became well known for our preparation of the first English Rose Oil for 250 years (prepared from our own roses grown in North Yorkshire, England), but now hundreds of freshly harvested herbs and flowers of all kinds have yielded up their precious, fragile natural components without loss of "volatiles", without thermal damage or mutilation with customary high vacuum stripping, without the use of hexane or alcohol and without damage to the environment.

This extraction technique may still be un-familiar to some and a demonstration of both the process (using hand-held equipment) and of some of the exquisite oils prepared by it, will be given.

This technology was recently praised as being "....the most significant advance in this field for 1000 years....".

O7

AUSTRALIAN TEA TREE OIL: EFFICACY, TOXICITY, IRRITANCY AND STABILITY

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The Australian Tea Tree Oil Research Institute (ATTORI) was created in 1996 to provide a focal point for coordinated research on all aspects of this commercially important oil. An overview of current research into plant genetics, product formulation, efficacy *in vitro* and *in vivo* and clinical trials will be discussed. The use of tea tree oil for topical applications is supported by cell-line cytotoxicity studies. Of the five cell-lines tested with tea tree oil and various pure components, epithelial cells were most robust, whilst the liver cells were the most susceptible. Recent results from a skin irritancy and sensitivity trial conducted on 151 panelists revealed an average daily score of 0.1922, thereby confirming that tea tree oil is an extremely low irritant when applied to the skin, even in neat (100%) concentrations. In various formulation bases at concentrations up to 25% the oil showed virtually zero irritancy (ranging from 0.0000 0.0060). Tea tree oil must be considered a mild allergen as 2% of the panelists showed an allergic reaction. Issues surrounding production, formulation and stability of low irritancy oil will be addressed.

O8

CHROMATOGRAPHIC STUDY OF ESSENTIAL OILS FROM *ARTEMISIA* SPECIES

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Essential oils of the plants which contain a great range of terpene compounds constitute an important group of medicines having spasmolitic, sedative, wound-healing, anti-inflammatory activities used in medical practice for the treatment of different diseases.

Asteraceae family plants are the richest ether-bearing species, especially *Artemisia* genus. Though a great number of papers regarding chemical study of *Artemisia* have been published, still, their chemical composition has not been studied properly.

This paper lists chemical studies of *Artemisia* essential oils for complex usage of medicinal material and development of wasteless technology for their processing and also in order to study the chemical composition of *Artemisia* species which are endemic in Central Kazakhstan. Four *Artemisia* species of Absinthium section were the subjects of this study: *Artemisia glabella, A.radicans, A.filatovii* and *A.austriaca*. All species were harvested in Karaganda region of Kazakhstan in 1995-96.

Composition and quantitative determination of the oils of four *Artemisia* species were studied by GC.

O9

PACKED COLUMN SFC IN THE ANALYSIS OF TERPENOIDS

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Packed column supercritical fluid chromatography (PC-SFC) has recently stimulated interest as an alternative to HPLC. This has mainly been due to the introduction of variable and programmable restrictors, on-line flow rate controllers and high pressure cells for UV detectors, which have afforded reliable quali-quantitative results. PC-SFC has now sample capacity similar to HPLC, operates with the same columns, and in general requires shorter analysis times because of the higher theoretical plate number per unit time.

A project aiming to evaluate PC-SFC/UV in the analysis of biologically active components in plant extract has been begun. This communication aims to focus on the advantages and disadvantages of PC-SFC, and the strategy adopted in the authors' laboratory to develop new SFC methods are illustrated through some examples involving terpenoids related to aromatic plants (monotepernoids, sesquiterpene lactones, diterpenoids, triterpenoids, carotenoids and related glucosides).

O10

CONTROL OF STORAGE DISEASES OF POTATO AND SPROUT INHIBITION BY PLANT VOLATILES.

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Experiments to control storage rots and skin blemishing diseases on tubers of potato (*Solanum tuberosum* L.) by using plant volatiles have been carried out during 4 storage seasons. Naturally or artificially infected tubers have been kept at +10°C in an aromatic atmosphere obtained from homogenised fresh plant material or from essential oils (EO), produced by steam distillation. The treatment period has mostly been 2-3 weeks in order to imitate the warmer wound-healing period performed after harvest in practise. The application dose of volatiles and the start of treatments relative to wounding of tubers and the time of the year have been varied. The effects on diseases as well as on sprouting ability have been recorded. In addition, *in vitro*-experiments on agar plates have been carried out to study the fungistatic/fungicidal nature of the vapours. Treatment doses were then similar to the ones of the *in vivo*-experiments.

Significant reductions of one or more of the following diseases: storage rots, caused by *Phoma foveata* Foister and *Fusarium solani* var. *coeruleum* (Sacc.) Booth, silver scurf (*Helminthosporium solani* Dur. et Mont), skin spot *Polyscytalum pustulans* (Owen & Wakef.) Ellis, and black scurf *Rhizoctonia solani* Kühn have been achieved after treatments of contaminated tubers with volatiles from plants of various families such as *Liliaceae*: garlic (*Allium sativum*) and onion (*Allium cepa*); *Lamiaceae*: sage (*Salvia officinalis*), basil (*Ocimum basilicum*) and spearmint (*Mentha spicata*); *Apiaceae*: caraway (*Carum carvi*). The far best, and consistent, control of most of the mentioned fungi, has been obtained by the vapours of garlic. A treatment period of 3 weeks has often been sufficient. Better control of silver scurf was obtained when volatiles were added immediately after harvest, compared with 2 weeks afterwards. Autumn treatment of tubers have not had any dramatic effect on the sprouting ability in spring, whereas the same aromatic compounds, applied in January and onwards, have almost completely inhibited sprouting.

O11

BIOCONVERSION OF ALKENE PHENOLS FROM AROMATIC PLANTS TO NATURAL AROMA COMPOUNDS

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Natural flavor and fragrance compounds are of great value to the food, cosmetics, and medical industries. The price for these natural chemicals can be extremely high due to their low concentration in the plant material. Many herbs and spices contain relatively high concentrations of essential oils that resemble desirable flavor and aroma compounds. By using a single or a few enzymatic steps, it is possible to convert these essential oils components into natural flavor and aroma chemicals. In this work, we describe the microbial conversion of alkene phenols from aromatic plants to natural aroma compounds.

Using enrichment culture technique, microorganisms were isolated based on their ability to degrade specific alkene phenols. These microbial strains were capable of growing on *t*-anethole, estragole, isosafrole or isoeugenol as the sole carbon source. The degradation pathway of *t*-anethole (the major component of anise seed, star anise, and sweet fennel oils) was studied by isolation of blocked mutants, identification of metabolic intermediates, and detection of specific enzymatic activities. Aroma compounds such as anisic acid, anisyl alcohol, 4-methoxybenzyl-methyl-ketone, and anisaldehyde are likely to be metabolic intermediates in the degradation pathway of *t*-anethole. Some of the blocked mutants accumulated intermediates in yields that are close to the maximal theoretical yields. To the best of our knowledge, the conversion efficiencies obtained have never been reported for these essential oil components. The results of this research may support further development of economical bioconversion processes for the production of natural aroma compounds from alkene phenols.

O12

METHYLTRANSFERASES INVOLVED IN THE BIOSYNTHESIS OF METHYL-CHAVICOL AND METHYL-EUGENOL IN SWEET BASIL.

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Sweet basil, *Ocimum basilicum* L., Lamiaceae, is an economically important crop. The major components of basil essential oils are linalol and methyl-chavicol (estragole). The biosynthetic pathway to methyl-chavicol in basil is poorly understood. Enzymatic assays using radioactively labeled SAM (*S*- of methyltransferase activity in young leaves (up to 15 mm length). Chavicol methyltransferase activity in inflorescences was only ~20% of that present in young leaves, while mature leaves, stalks and flowers had much lower levels (5 to 1%). No methyltransferase activity was detected in roots. These results indicate that young leaves are the primary site of methyl-chavicol formation in sweet basil.

Different basil varieties differ in their essential oil composition. Varieties that their oil contains high levels of methyl-chavicol, also display high *in vitro* methyltransferase activity towards chavicol but very little activity towards eugenol. Varieties containing a high methyl-eugenol content display high methyltransferase activity towards eugenol but are also able to act upon chavicol. One variety that contains mostly eugenol in its oil, lacks methyltransferase activity, towards eugenol or chavicol. Our results indicate the presence of different substrate-specific methyltransferases in basil varieties, that might partially explain the differences in their essential oil composition.

POSTER PRESENTATION

P1

COMPOSITION OF THE ESSENTIAL OILS OF *ACINOS* MILLER SPECIES IN TURKEY

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The genus *Acinos* Miller (Lamiaceae) is represented by 5 species in the Flora of Turkey. Two subspecies of *A. troodi* being endemic, as follows:

1. *Acinos troodi* (Post) Leblebici subsp. *vardaranus* Leblebici
2. *Acinos troodi* (Post) Leblebici subsp. *grandiflorus* Hartvig & Strid
3. *Acinos alpinus* (L.) Moench
4. *Acinos suaveolens* (Sm.) G. Don fil
5. *Acinos arvensis* (Lam.) Dandy
6. *Acinos rotundifolius* Pers.

Essential oils from aerial parts of *Acinos* species were obtained by hydrodistillation. The essential oils were analysed by a GC/MS system. Main components were determined as follows:

Germacrene D and hexadecanoic acid for *A.troodi*, *A. alpinus*, *A. arvensis* and *A. rotundifolius;* pulegone and isomenthone for *A. suaveolens* .

The results have shown that *A. suaveolens* is a distinct species among the others in that it has the richest oil content and the oil contains considerable amounts of pulegone and isomenthone. The compositions of the essential oils of *A. alpinus* and *A. rotundifolius* are of a complex nature.

P2

THE ESSENTIAL OILS OF *LEPECHINIA BULLATA* (BRIQ.) EPL. (LAMIACEAE) FROM VENEZUELA

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Lepechinia bullata (Briq.) Epl. (Lamiaceae) is a shrub or even a small tree with a foliage similar to *Salvia*. The species is endemic in the Andean cordillera of Venezuela and Columbia and inhabits the relatively moist upper Andean forest and subparamo from 1700 to 3900 m (1). The common plant name „Salvia negra" indicates its use similiar to sage in traditional medicine (2).

40 samples were collected from different sites in Mérida (Edo.) (Venezuela) during the flowering. The essential oil was obtained by hydrodistillation of the air-dried leaves with a yield of approximately 1 %.

GC and GC-MS proved the oils to consist of monoterpene hydrocarbons (0.3 - 50.6%; mainly α,β-pinene, camphene, limonene, β-phellandrene), oxygenated monoterpenes (0.1 - 10.1%; borneol, bornyl acetate), sesquiterpene hydrocarbons (23.2 - 84.1%; β-caryophyllene, α-farnesenes, eremophilene) and oxygenated sesquiterpenes (2.4 - 40.1%; palustrol, ledol, viridiflorol, spathulenol, T-cadinol). Two groups of plants can be distinguished. One group with a high level of monoterpene hydrocarbons and another group with a very low level. In addition the variability of the sesquiterpene alcohols indicates a considerably high chemical polymorphism of *Lepechinia bullata*.

(1) J. A. Hart: Peripheral isolation and the origin of diversity in *Lepechinia* sect. Parviflorae, Syst. Bot. 10, 134-146 (1985)

(2) H. G. Barriga: Flora Medicinal de Colombia, Tomo III, pp 17, Tercer Mundo, Bogotá (1992)

P3

CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF SOME *MENTHA* SPP., FROM IRAN

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The volatile constituents of *Mentha aquatica* (L.), *Mentha pulegium* (L.) and *Mentha X piperata* were isolated by steam distillation using a KAISER & W. LANG apparatus and analyzed by GC and GC MS. GC analyses were carried out using a Shimadzu GC-9A chromatograph provided with flame ionization detector (FID), and chromatopac C-R3A as data processor. separation was carried out on two different polarity columns (DB-1 & DB-wax, each one 60m x 0.25 mm, film thick. 0.25 micron). Composition calculated by area normalization method neglecting response factors. GC MS analyses were carried out on a Saturn II model Varian with 70 ev ionization energy on column DB-1 temp. program mode the same as GC analyses. The major constituent was menthofuran (52.6% in *M. aquatica* and 44% in *M. X piperata*) and for *M. pulegium* was pulegone (with 66.5%). Chromatograms and tables of compositions (including name of compound, its kovats retention indices and percentage) will be presented.

P4

EFFECT OF DIFFERENT PLANTING TIMES ON ESSENTIAL OIL COMPONENTS OF DIFFERENT MINT (Mentha spp.) VARIETIES.

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This study was conducted to determine the effect of different planting times on essential oil components of different mint varieties (*Mentha arvensis* var. *piperascens*, *M. piperita* Mitcham, *M. piperita* Eskişehir and *M. piperita* Prilubskaja). The field trials have been carried out at GAP Agricultural Research Station of Çukurova University Agriculture Faculty in Harran Plain condition in years of 1993 and 1994. α-Pinen, β-Pinen, 1,8-Cineol, Menthon, Menthofuran, Menthol, Pulegon, Menthylacetat and β-Caryophyllen were determined in essential oil of mint. Essential oil components were effected by planting times and mint varieties. Autumn transplants showed higher menthol's ratio than plants transplanted in spring and was appeared on *M.arvensis* var. *piperascens* mint variety.

P5

Evaluation of single plant descendants of *Origanum vulgare* ssp.

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Plants descending from open pollinated single plants of 5 different populations of *Origanum vulgare* ssp., planted in 1995 in Großenzersdorf, Austria, have been analysed in 1996.

Besides a huge variability in morphological traits like e.g. plant height (from 20 to 87cm) and the colours of different flower parts, also a huge variability in oil content and composition could be observed not only between but also within the offsprings.

The average content of essential oils was 1,24% with one offspring only containing 0,64%. Regarding the oil components 33 components could be identified allowing a classification in 6 different chemotypes:

	no. of plants per population				
chemotype	**pop 1**	**pop 2**	**pop 3**	**pop 4**	**pop 5**
carvacrol	-	5	8	1	5
thymol	2	13	10	2	-
γ-terpinen	10	1	5	-	-
linalool	-	1	3	1	4
cis-sabinene hydrate	-	2	1	3	-
mainly sesquiterpenes	10	-	3	3	-

Furthermore, 8,5% of the plants were male sterile and several partial male sterile confirming once more the frequent occurrence of male sterility in Lamiaceae.

P6

THE ESSENTIAL OILS OF *THYMUS* SPECIES GROWING THRACE

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Thrace is the name given to the European part of Turkey. Although the area covered by Thrace is only three percent of Turkey, it has a very rich flora. Eight out of sixty seven taxa of the genus Thymus recorded in Turkey grow in Thrace.

Essential oils from aerial parts of the following *Thymus* taxa have been analyzed by GC/MS.

T. atticus Celak.

T. aznavourii Velen.

T. comptus Friv.

T. longicaulis C. Presl ssp. *chaubardii* (Boiss. et Heldr. ex Reichb. fil.) Jalas var. *chaubardii*,

T. longicaulis C. Presl ssp. *longicaulis* var. *subisophyllus* (Borbas) Jalas

T. sibthorpii Bentham,

T. striatus Vahl var. *interruptus* Jalas

T. zygioides Griseb. var. *zygioides*

T. zygioides Griseb. var. *lycaonicus* (Celak.) Ronniger

References

1. Davis, P.H., Flora of Turkey and the East Aegean Islands, Vol. 7, pp 349-382, Edinburg University Press, Edinburg (1982).

P7

EFFECTS OF SOME FACTORS ON QUANTITY AND QUALITY OF THYME ESSENTIAL OIL

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The environmental factors have significant effect on the quantity an quality of plants containing essential oil. In this investigation, we have studied the effect of N-fertilizer and harvesting time on the essential oil and thymol content of *Thymus vulgaris* L. cultivated in Tehran.

Optimized conditions were:

1- 100 kg nitrogen per hectar

2- Harvesting at the fruit formation stage in july

The detail of the study will be mentioned in the paper.

P8

MULTIVARIATE STATISTICAL ANALYSIS OF ESSENTIAL OIL DATA OF *THYMUS PRAECOX* SSP. *ARCTICUS* AND *THYMUS PULEGIOIDES* FROM THE SOUTH OF ENGLAND.

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Continuing our research in the field of chemical polymorphism in the genus Thymus, studies in the South of England were undertaken. There two species occur, namely *Thymus praecox* (OPIZ) ssp. *arcticus* (E. DURAND) JALAS (syn. *Thymus drucei* RONN.) and *Thymus pulegioides* L.. 388 individual plants were collected at 45 different sampling sites. 85 of these samples belong to *Thymus pulegioides* and 303 to *Thymus praecox* ssp. *arcticus*. The essential oils were obtained from air-dried material of individual plants by hydrodistillation. The composition of the oils was determined on the basis of GC results. The essential oil data of *Thymus praecox* ssp. *arcticus* was statistically analysed using factor analysis, cluster analysis, and discriminant analysis *Thymus pulegioides* couldn't be examined by multivariate statistical analysis because there were too few cases in comparison to the number of variables. Though 5 chemotypes could be separated with the main components linalool, thymol, thymol/linalool, carvacrol resp. geraniol/geranial. These results were similar to those obtained in other European countries. In contrast *Thymus praecox* ssp. *arcticus* proved to be strongly chemically polymorph. On a high level 4 clusters could be found, but depending on the method used 11 to 20 clusters were visible. The cluster characterising components were: γ-terpinene, trans-sabinene hydrate, linalool, linalyl acetate, α-terpinyl acetate, α-terpineol, geranyl acetate, geraniol, geranial, trans-nerolidol, germacra-1(10),4-dien-6-ol, hedycaryol, τ-cadinol, thymol, α-cadinol, germacra-1(10),5-dien-4-ol, trans,trans-farnesol, one unknown component and combinations of these components. The differences between the various North European populations will be discussed.

P9

COMPOSITION OF THE ESSENTIAL OIL OF *ZATARIA MULTIFLORA* BOISS.

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GC and GC/MS analysis were carried out on the commercial oil of *Zataria multiflora* Boiss. (Lamiaceae) Iranian origin.

30 components were identified representing 97.71% of the total oil. Thymol (37.57%), carvacrol (21.19%), p-cymene (8.96%), γ-terpinene (7.52%), α-pinene (4.79%) were found as the major components.

P10

RESEARCHES ON YIELD, ESSENTIAL OIL AND ESSENTIAL OIL COMPOSITION OF SAGE (*SALVIA OFFICINALIS* L.) LINES

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Four cultivated sage lines selected according to flower color and leaf shape were used in this study. PercentAge of essential oils and their compositions were determined as follows:

Oil yields: Fresh herb (0.50-0.55%), Dried herb (1.30-1.60%)

Main components: α-thujone, β-thujone, 1,8-cineole, borneol and α-humulene

P11

THE ESSENTIAL OILS OF TWO ENDEMIC *SALVIA* SPECIES : *S. PISIDICA* AND *S. POTENTILLIFOLIA*

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The genus *Salvia* is represented in Turkey by more than 80 species of which 50 % are endemic.

Water distilled essential oils from aerial parts of two endemic *Salvia* species, *S. potentillifolia* Boiss. et Heldr. ex Bentham and *S. pisidica* Boiss. et Heldr. ex Bentham have been analyzed by GC/MS.

P12

THE ESSENTIAL OIL COMPOSITION OF *SALVIA* PECIES

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The composition of the essential oil from the aerial parts of six *Salvia* species growing wild in Iran were investigated by GC and GC/MS.

The main components of these volatile oils were as follows:

Salvia verticillata	β-caryophyllene	(24.7%)
	γ-muurolene	(22.8%)
	limonene	(8.9%)
Salvia santolinifolia	α-pinene	(70.7%)
	β-pinene	(14.8%)
	limonene	(4.5%)
Salvia officinalis	β-pinene	(16%)
	borneol	(9.4%)
	globulol	(9.3%)
Salvia nemorosa	β-caryophylene	(44%)
	germacrene B	(26.4%)
	caryophyllene oxide	(6.6%)
Salvia hypoleuca	β-caryophyllene	(45%)
	β-pinene	(11%)
	germacrene D	(10%)
Salvia reuterana	ocimene	(39.6)
	gurjunene	(12.5%)
	germacrene D	(10.7%)

P13

COMPOSITION OF ESSENTIAL OILS FROM THREE NEW *SIDERITIS* SPECIES

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The genus *Sideritis* (Lamiaceae) is represented by 40 species in the Flora of Turkey (1, 2). During recent field studies, five *Sideritis* species have been characterized and recorded as new species, increasing the number to 45. Four of them are new for science (3, 4).

We report on the essential oil compositions of these species: *Sideritis vuralii*, *S. caesarea* and *S. akmanii*. The water distilled oils were analyzed by GC/MS. Main compounds characterized in each oil were as follow:

Sideritis vuralii Duman et Başer: β-pinene (35%), β-phellandrene (14%)

S. caesarea Duman, Aytaç et Başer: β-caryophyllene (8%), caryophyllene oxide (7%)

S. akmanii Z.Aytaç, M.Ekici et A.Dönmez: *ar* -curcumene (17%), spathulenol (15%)

References:

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P14

ESSENTIAL OILS OF ANNUAL *SIDERITIS* SPECIES GROWING IN TURKEY

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Annual *Sideritis* species are represented in Anatolia by four species and altogether five taxa. These are *Sideritis lanata, S. romana* subsp. *romana*, *S. curvidens*, *S. montana* subsp. *montana* and *S. montana* subsp. *remota*. Essential oils from aerial parts of these species obtained by hydrodistillation were analysed by GC/MS. Main compounds of these essential oils were as follow:

Sideritis lanata L. : hexadecanoic acid (10%), spathulenol (9%)

S. romana L. subsp. *romana* : thymol (24%), 1-octen-3-ol (12%)

S. curvidens Stapf : bicyclogermacrene (20%), spathulenol (12%)

S. montana L. subsp. *montana* : germacrene D (25%), bicyclogermacrene (11%)

S. montana L. subsp. *remota* (d' Urv.)P.W. Ball ex Heywood: bicylogermacrene (13%), germacrene D (10%).

References:

1. P.H.Davis, Flora of Turkey and the East Aegean Islands. Vol 7, p 178- 199, University Press, Edinburgh (1972).

P15

ESSENTIAL OILS OF FOUR LABIATAE HERBS GROWING WILD IN TURKEY

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The essential oils obtained by hydro distillation of four Labiatae herbs, namely *Salvia cryptantha* Montbr. et Auch., *Satureja cuneifolia* Ten., *Thymbra spicata* L. and *Thymus cilicicus* Boiss. et Bal. growing wild in Central Anatolian provinces of Turkey, were analysed by GC an GC/MS. Fifty-six, thirty-five, thirty-three and thirty-eight compounds were identified of which major ones were camphor (18.05%), 1,8-cineole (17.79%) and bornyl acetate (11.35%); thymol (65.54%, p-cymene (9.84%) and carvacrol (7.23%); p-cymene (22.98%), γ-terpinene (21.37%), carvacrol (21.14%) and thymol (11.77%); α-terpineol (16.42%), camphor (9.66%), sesquiterpene alcohol (8.79%) and 1,8-cineole (7.78%), respectively.

P16

Morphological and phytochemical characterization of *Teucrium lucidum* L. and *T. chamaedrys* L.

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Teucrium lucidum L., an endemit from Maritimes Alps, belongs to the Sect. Chamaedrys, being rather similar to *T. chamaedrys* L. itself. However, owing possibly to the very small area of distribution, the former taxon had been scarcely studied. In this work we have compared both micromorphological and phytochemical (volatile compounds) characters of *T. lucidum* and *T. chamaedrys* growing in the same site. In both species glandular (peltate and capitate types) and non glandular trichomes are present, but in *T. lucidum* non glandular trichomes are scarce and short. In *T. chamaedrys* non glandular trichomes are numerous and long so that the whole plant appears nearly hairy. Therefore the two taxa are well distinguishable relying on the macro- and micromorphological characters.

The composition of essential oils, already known for *T. chamaedrys* because of its use in etnomedicine, do not differ notably. Particularly interesting is, in both taxa, the presence of two cycloeptenones, unusual compounds in essential oils.

P17

CHEMICAL ANALYSES OF IRANIAN AND FRENCH ESSENTIAL OILS OF *LAVANDULA* BY CAPILLARY GAS CHROMATOGRAPHY METHOD

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The essential oils were prepared by steam distillation. A Shimadzu GC-9A with chromatopac C-R3A as data processor and FID as detector were used.

Relative percentAges were calculated by area normalization method neglecting response factors. Identification was carried out by coinjection of standards, using their kovats retention indices and also BY comparison with known chromatograms.

Two columns with different polarities (DB-WAX and DB-1; each one 60 m. length, 0.25 mm. ID, 0.25 micron film thick.) were used for confirmation of the results.

Linear temperature programming modes were used.

NINETEEN compounds were identified in each essential oil among them linalool was the major one (39.5% in French and 38.9% in Iranian oils, respectively).

Four chromatograms (two for each essential oil) and two tables of compositions (including name of compound, its kovats indices and percentage) will be presented.

P18

SECOND COLUMN CONFIRMATION AS A POWERFUL METHOD FOR QUALITATIVE/QUANTITATIVE ANALYSES OF ESSENTIAL OILS

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Essential oils are constituted of various natural organic compounds with different molecular structures and many functional groups (such as hydrocarbons, aldehydes, ketones, esters, oxides, phenolic ethers) some of them are polar and others are nonpolar compounds.

In general, mass spectra (MS) and Kovats retention indices (RI) are used for identification of compounds which are separated by capillary gas chromatography. References show identity of Kovats retention indices for some pairs of compounds on nonpolar columns and identity of some other pairs of compounds on polar columns.

In this method we used two columns of different polarities (DB-1, Dimethylsiloxane 100% methyl and DB-WAX, polyethyleneglycol each one 60 mx0.25 mm, 0.25 micron film thick.).

Kovats retention indices calculated using basic Kovats equation and also from a third order equation as the following:

$$I=A+Bt(r)+Ct(r)\wedge 2+Dt(r)\wedge 3 \quad (*)$$

where, I= Kovats index t(r)= retention time

A,B,C,D= constants

Softwares written separately for each column for calculating Kovats from basic equation and determining constants in eq. (*).

Results showing good resolution of some compounds (for example trans-ocimene and γ-terpinene) on DB-1 and good resolution of some other compounds (for example, limonene and 1,8-cineole) on DB-WAX.

So in essential oil analyses it will be necessary using two columns of different polarities simultaneously to obtain desired resolution.

P19

RELATION BETWEEN LINALOL CONTENT AND FRAGRANCE OF DIFFERENT ESSENTIAL OILS

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The high or substantial concentration of linalol of essential oils contributes to the antiseptic, antiviral properties, as well as uplifting effect, however their fragrances are differring, as it will be presented by special fragrance-samples. The correlation between linalol concentration and retention time, as well as relative retention time differences has been studied by GC analysis.

The GC retention times of linalol of linalol standards, levant wormseed (*Artemisia maritima*) and lemon-scented mint (*Mentha citrata*) oils (linalol content: 90-95%) have shifted, depending on the linalol quantity. This shifting range of retention times - 0,17 minute - has been reduced in case of determination of relative retention times onto 0,02 minute (internal standard was artemisia ketone as the main component of tansy oil (*Tanacetum vulgare* in 85%). The retention time of linalol constituent of other essential oils such as coriander (*Coriandrum sativum* - 75%), basil (*Ocimun basilicum* - 65%), bergamot (*Citrus bergamia* - 20-27 %), lavender (*Lavandula officinalis* - 34-41%), petitgrain (*Citrus aurantium var. amara* - 16-24%) and clary sage (*Salvia sclarea* - 30-55%) have shown much less shifting range in retention times since the less amount of linalol.

It might be concluded, that the difference in retention time of linalol or any other component of essential oils could be a result of much or less quantity. In support of retention time shifting relative retention times should be determined before it would be supposed to discover another chemical structure, eventually an optical isomer, than it was expected.

P20

ESSENTIAL OIL COMPOSITION AT DIFFERENT BASIL (*OCIMUM BASILICUM* L.) ORIGINS FROM MEDITERRANEAN REGION

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Ocimum basilicum L. has a pleasent aromatic odour and many subspecies, varieties and chemotypes. The chemical composition of it's essential oil changes widely depending on the origin of the plant material. This study was conducted to determine the effect of different harvesting times on essential oil yields and components of basils from different localities of the Mediterranean Region. Plant materials were collected from K.Maraş, Adana and Hatay. During the experimental year, three harvests (June, August, September respectively) were done at full flowering period. The essential oil components were analysed by GC and GC/MS. Ratio of essential oil and components showed wide differences depending on origins and harvesting times. The highest essential oil ratio 37% and yields (3.09 l/da) in fresh flowers were obtained from Hatay basil and from the second harvest. Increase of temperature in the region enhanced the essential oil yield of basil. 1,8-cineole, linalool, methyl eugenol and methyl cinnamate were determined as the main components in essential oils. Although Adana, K.Maraş and Hatay basils had linalool + methyl cinnamate as the main components in all harvest, two oil samples from Hatay basil contained high amounts of 1,8-cineole (30-38%) in the first harvest.

P21

COMPOSITION OF THE ESSENTIAL OIL OF THREE *OCIMUM* SPECIES FROM S. TOMÉ AND PRÍNCIPE

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The aim of the present paper is to present some of the results already achieved in the study of the essential oil of three species of *Ocimum* from the medicinal aromatic flora of S. Tomé and Principe. This genus is widely used for the preparation of remedies in African traditional medicine. In S. Tomé the most representative species are *Ocimum gratissimum* L., *Ocimum canum* L. and *Ocimum minimum* L. All of them are used as febrifuge, for the treatment of respiratory diseases (colds and sinusitis), as well as food flavours.

The essential oils of these plants were obtained by hydrodestillation of the aerial parts. They were analysed by GC and GC-MS, using two fused silica capillary columns of different stationary phases (carbowax and methylsilicone). Identification of the compounds with a percentage higher than 1% was also performed by ^{13}C-NMR. The major compounds in the volatile oil of *Ocimum gratissimum* were thymol (48.1%) and p-cymene (11.9%). *Ocimum canum* essential oil was characterised by its high content of methyl cinnamate (80.6%), whereas the most important constituent of *Ocimum minimum* essential oil was linalool (52.9%).

P22

COMPOSITION OF THE ESSENTIAL OIL FROM TWIGS OF *JUNIPERUS NAVICULARIS* GROWN IN PORTUGAL

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Continuing our investigations on the genus *Juniperus* from Portugal we now report on the composition of the twig essential oils from three populations of *Juniperus navicularis* Gand (*Juniperus oxycedrus* subsp. *transtagana* Franco).

The essential oils were isolated by hidrodistillation (yield 0,37-0,66%) and submitted to qualitative and quantitative analysis by GC on two columns of different polarities and by GC-MS. The identity of the oil components was assigned by comparison of their R.I., relative to a homologous series of fat acid methyl esters, and MS with those of authentic samples.

More than 40 components were identified amounting to 83.8-97.3% of the total oils. Monoterpene hydrocarbons were the main components in the oil of all populations (70.3-90.2%), limonene (17.9-33.2%), α-phellandrene (12.3-15.2%) and α-pinene (10.2-25.4%) being the major constituents.

P23

COMPOSITION OF THE ESSENTIAL OIL OF THE FRUIT OF *AFRAMOMUM DANIELLI*, A PLANT USED USED IN TRADITIONAL MEDICINE IN S.TOMÉ AND PRÍNCIPE

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Continuing our study on the essential oils of the medicinal aromatic plants from S.Tomé and Príncipe we now present the chemical composition of the essential oil of the fruit of *Aframomum danielli* L. It is used by the native population for its medicinal properties (for fevers and placenta expulsion) as well as for its edible spicy flavour. To our knowledge, this is the first time that the composition of the essential oil of this taxon is studied.

The essential oil of this plant was obtained by hydrodistillation of the air-dried fruit. It was analysed by GC and GC-MS using two fused silica capillary columns of different stationary phases (carbowax and methylsilicone). Identification of the compounds with a percentage higher than 1% was also performed by ^{13}C-NMR.

The volatile oil was characterised by its high content of oxygenated monoterpenes and monoterpene hydrocarbons, being 1,8-cineol (37%), α-terpineol (11.9%) and β-pinene (13.9%) the most important ones.

P24

IN VITRO SELECTION FOR SALT TOLERANCE OF ROSEMARY (*Rosmarinus officinalis* L.)

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Leaf segments of rosemary plants were cultured on callus induction medium (CIM) supplemented with 0, 1000, 3000, 5000, 7000 ppm of salt in a form of NaCl and $CaCl_2$ at ratio of 1:1 (w/w). The induced calli were cultured on regeneration medium (RM) containing the same levels of salt. Salt levels significantly affected the callus induction and its texture. Shoot number decreased by increasing the salt levels. Shoots formed roots in a medium supplemented with indole-3-butyric acid (IBA). Similarly in both the regenerated plants under salinity stress and mother plants, ten monoterpenes were identified in the essential oil extracts.

P25

Influence of Phytohormones on Essential Oil composition of regenerated plants of *Melissa officinalis*

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From callus cultures regenerated plants of lemon balm (*Melissa officinalis*, L., Lamiaceae) have been cultivated under in-vitro-conditions (1). Thereby the growth of genetically uniform plant material was made secure under controlled conditions.
The effect of different phytohormones in various concentrations and combinations was studied on essential oil accumulation. For example some cytokinins cause a significant change of the composition of the essential leave oil (2). Alloaromadendren, a sesquiterpenhydrocarbon and trace component in regenerated control plants, accumulates up to 10% of the total amount of the essential oil. Important changes in monoterpene constitution can be established, too. So with proper phytohormones a new and unusual type of essential oil can be induced (alloaromadendren-type).
Some extremely volatile components have been detected in regenerated plants of *Melissa officinalis* for the first time, by the application of headspace-extraction with "tenax-trapping" instead of water steam distillation.

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P26

Phytohormones and processes of the second metabolism of peppermint and moldavian dragonhead

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The actions of exogenous plant growth regulators (mixture of gibberellins, chlorocholine chloride, 2-chloroethylphosphonic acid) on the balance of endogenous plant hormones (abscisic acid, 3-indolylacetic acid, zeatin, zeatinriboside and gibberellins), contents and composition of essential oils in five varities of peppermint (Menta piperita L.) and moldavian dragonhead (Dracocephalum moldavica L.) were investigated.

The treatment of aromatic plants as well the mixture of gibberellins as retardants blocked up biosynthesis of endogenous gibberellins, increased yield of essential oils, stimulated menthol formation (mint) and (or) increased the level of abscisic acid (mint, dragonhead).

Type and direction of biosynthesis of plant hormones and essential oil compounds are determined by the ability of genotype reaction, variety and species of aromatic plants.

P27

The hormonal regulation of the productivity of sklerocies spurred in biothechnological system fungus-plant.

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The influence of endogenous gibberellines and gametocydes on IV-V stage of organogenesis fall rye have led to increasing of crop capacity of sklerocies spurred and rising of contents ergoalkaloids (five times as much) in them. The gametocydes promoted the activisation of sfaciales stades fungus development of konidiogenesis. Gibberellins ingibit konidiogenesis and promote the acceleration of the approach of sklerociesal stage. This stage is a responsible for accumulation of ergoalkaloids in spurred. Exogenous gibberellins are induced the increasing of the levels of endogenous cytokinins and IAA in rye spikes and sklerocies spurred developmenting on them.

P28

COMPOSITION OF THE ESSENTIAL OIL OF THREE AROMATIC SPECIES FROM IRAN

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The essential oils of *Heracleum persicum* Desf. (dried leaf), *Bunium persicum* Boiss (seed) and *Stachys lavandulifolia* Vahl. (flower) have been analyzed by GC and GC/MS.

The oils were dominated respectively by trans-anethol (61%), β-pinene (7%) and trans-ocimene (4.7%) for *Heracleum persicum*. The components in the essential oil of *Bunium persicum* were γ-terpinene (40.2%), cuminyl aldehyde (13.8%) and terpinen-7-al (10.5%). Myrcene (20%) α-pinene (18%) and γ-muurolene (13%) were main constituents in the essential oil of *Stachys lavandulifolia*.

P29

SOME ESSENTIAL OIL PLANTS IN TURKEY

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In the present study we determined the essential oil content and composition in selected plants growing in Turkey.

Coriandrum sativum L. - annual herb. The seeds contain 2.5% essential oil.

Pimpinella anisum - annual herb. The seeds contain 4 to 5% essential oil.

Foeniculum vulgare Mill. - annual and perennial herb. The fruits contain 6% essential oil, 20% fixed oil and 22% proteins.

Mentha piperita L. - perennial herb. The leaves contain essential oil from 2 to 5.5%.

Melissa officinalis L. - perennial herb. The leaves and flowering stems contain essential oil.

Salvia officinalis L. - perennial herb. The leaves contain 2.5% (per dry weight) essential oil.

P30

HAIRY ROOT CULTURES OF *ANETHUM GRAVEOLENS* L.: ESTABLISHMENT AND COMPOSITION OF THE ESSENTIAL OIL

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Transformed root cultures of dill were induced by inoculation of 3-4 weeks-old aseptic plantlets with *Agrobacterium rhizogenes* LBA 9402 carrying plasmid pRi 1855. The plantlets were carefully wounded with the tip of a sterile hypodermic needle and injected with one drop of the bacterial suspension grown for 48h. The plantlets were transferred to an MS/2 medium [= half strength MS medium with 30g/l of sucrose] and co-cultivated with the bacteria for 48h, in a 16h light/8h dark photoperiod, at 24°C. After this period they were transferred to fresh MS/2 medium supplemented with cefotaxime and carbenicillin, 250µg/ml each. Roots developed were transferred to liquid MS/2 medium without antibiotics, maintained in darkness at 24°C on orbital shakers (80 r.p.m.), and subcultured every month.

The essential oil from the hairy roots maintained in liquid MS/2 medium, and the oils from the fruits, and from roots and aerial parts of the parent plant in the vegetative phase were analysed by GC and GC-MS and their compositions compared. In the oils from the parent plant the monoterpene fraction was always dominant, although in different amounts, whereas phenylpropanoids were the major components of the oil isolated from the hairy roots. These compounds were also present in the parent plant, except in its fruits. The main component of the oils from the fruits and from the roots of the parent plant was carvone, whereas α-phellandrene was that from the aerial parts and apiole that from the hairy roots.

Acknowledgement - We are grateful to JNICT for scholarships to P.M.L. Lourenço and P.A.G. Santos. This study was partially funded by JNICT under research contract PBIC/C/BIO/1989/95.

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THE PRODUCTION OF CUMIN SEED OIL

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Steam distillation of cumin seed oil was studied to show the effects of particle size, steam rate and batch size on oil recovery. Fractionation of the oil produced was also performed to obtain cumin seed oil high in cumin aldehyde composition of the products were analysed by GC/GC-MS. Experiments were carried out at bench and pilot plant scale.

P32

THE GERMINATION BIOLOGY OF INDIGENOUS POPULATIONS OF *FOENICULUM VULGARE* L. var. *VULGARE* IN ISRAEL- PRELIMINARY STUDIES

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Foeniculum vulgare var. *vulgare* (bitter fennel), is an aromatic hemicryptophyte. In Israel, plants occur in extremely diversified habitats: in the Central Negev Desert under 100mm of rainfall and up to the Northern regions with 1200mm. Variability of seed weight and germination biology of mericarps (so called 'seeds'), of some populations, have been studied along a transect from the Mediterranean coast at Tel Aviv, to inland areas of the Judean Mountains, ca. 60 Km from the coastal area. Data indicated that seed weight of the Nachshon population (ca 40 km from the coast), was 3.5mg/seed and significantly heavier compared to all other populations (an average increase of 15%); When planted in soil, seeds of this population exhibited also highest emergence of 73±9.3%. However, the time needed to attain 50% germination was significantly longer, as for all mountainous populations (G_{50} =11.0-13.0 days), compared to 8.5 d of the coastal population of Tel Aviv.

To enhance germination of the least germinating population (15.3% during 6.0d) of Kissalon (ca 50Km from the coast), seeds were immersed in KNO_3 solutions (100-1000mM) during 24h, prior setting to germinate. Data indicated that pre-immersion in 500mM KNO_3 had elevated final germination rate by almost 3 times (Gt=43.8%). This approach could be perhaps implied, when new chemotypes with a low germination rate are introduced into agriculture practice. At present, essential oils, amount, and composition in various populations, are determined.

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ANALYSIS OF THE ESSENTIAL OIL FROM *FERULA ASSA-FOETIDA* L.

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Ferula assa-foetida is one of the most important plant in Iran due to its several attributes is exported to many countries of the world.

The essential oil composition from the gum of *Ferula assa-foetida* growing in two regions of Iran (Kerman and Yazd) were identified by GC and GC/MS. The yield of oil were 16-17 %. The main constituents of both oils were as follows:

Kerman:

1- trans propenyl S-butyl disulfide

2- cis-ocimene

3- trans-ocimene

Yazd:

1- methyl 2-methyl-1,3-oxothiolan-2-yl ketone

2- trans-propenyls-butyl S-butyl disulfide

3- cis-propenyl S-butyl disulfide

GALBANUM

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Botanical source	*Ferula galbaniflua* Boiss & Buhse and other Ferula species
Botanical family	Umbelliferae
Foreign names	Galbanum (Fr.), Galbanum (Ger.), Galbano (Sp.), Galbano (It.)

Description: Herbaceous, tall plant that grows in northern Persia (*F.galbaniflua*) or in southern Persia (*F.rubricaulia* and *F.ceratophylla*). *F.galbaniflua* is widespread also in Turkey and Lebanon. The plant yields a resinous exudate distinguished commercially in two types: Levant galbanum (soft) and Persian galbanum (hard). The resin has a characteristic aromatic odor and bitter, warm, acrid taste. It contains approximately 16-26% essential oil.

Physical-chemical characteristics of Essential oil: The essential oil is obtained by steam distillation of the dried resinous gum. It is a pale-yellow to yellow liquid with balsamic, somewhat spicy, characteristic odor. Its main constituents include myrcene, cadinene, α-pinene, β-pinene and sesquiterpene alcohols.

Specific gravity at 20°C	:	0.867-0.916
Refractive index at 20°C	:	1.4780-1.4870
Optical rotation at 20°C	:	+3 to +20
Acid value	:	2 max
Solubility	:	1:6 in 90% ethanol

Determination of Essential Oil Content and of Main Oil Components in Umbelliferae Collections by NIRS

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For the breeding of grain-producing plants it is of special importance to investigate intact seed samples. In this context near infrared spectroscopy (NIRS) is well suitable in principle. Because collections of gene banks show a large variability in regard to concentrations of individual plant compounds they build up a suitable basis for the calibration and validation of this analytical method.

Therefore the aim of this study was to investigate whether the determination of essential oils and their main constituents can be performed by NIRS.

The calibration was set up upon solvent extracts received from the fruits of 77 coriander, 63 dill, 42 fennel and 20 caraway cultivars. The reference oil values were calculated by addition of the principal oil substances determined by GC analysis. Oil contents varied for coriander in the range of 0.1 to 1.0 %, for dill in the range of 3.0 to 9.1 %, for fennel in the range of 1.5 to 9.4 % and for caraway in the range of 2.4 to 8.1 %. Furthermore samples of running breeding projects were investigated. A partial least squares (PLS) algorithm was applied to all calibrations and the individual errror between predicted and reference values was described by the standard error of cross validation (SECV). Additionally, the coefficient of determination (r^2) was calculated to describe the relationship between reference method and NIR analysis.

In contrast to the usually applied gas chromatography measurement, the described NIRS method allows to predict the individual essential oil content as well as main oil constituents already after an analysis time of approx. 3 - 5 minutes.

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THE ESSENTIAL OIL COMPOSITION OF *ACHILLEA MILLEFOLIUM L.*

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Achillea millefolium (Asteraceae) has been used in traditional Turkish medicine as diuretic, appetizer, emmanagogue, carminative, diaphoretic. The essential oil Composition of *A. millefolium* growing in Turkey has not been reported. The essential oil was obtained by hydrodistillation from aerial parts and analyzed by using GC and GC/MS.

99 components were identified reperesenting 80.84% of the oil. Artemisia ketone (10.35%), piperitone (7.47%), chrysanthenone (7.1%), 1,8-cineole (6.61%) were found as major components.

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THE ESSENTIAL OIL COMPOSITION OF *ACHILLEA COARCTATA* POIR.

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The genus *Achillea* reperesented 40 species in Turkey is used in folk medicine as diuretic, appetizer, emmenagogue, carminative, diaphoretic. The essential oil has been obtained by hydrodistillation of *Achillea coarctata* and analyzed by using GC and GC/MS.

Two sample were collected from different localities in Ankara and Eskişehir in Turkey. 1,8-cineole and camphor were found as main components in both samples.

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ANTIMICROBIAL PROPERTIES OF THE ESSENTIAL OIL OF *ARTEMISIA ASIATICA* NAKAI

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Artemisia asiatica Nakai cultivated in the Medical Plants Garden, Institute of Biology and Botany, Medical Academy in Wrocław, Poland, yields 1.0 % of the green essential oil. Its composition differs from those observed for the oil obtained from *A. asiatica* from natural origin.

The main constituents are 1,8-cineole (40%) and selin-11-en-4α-ol (12%). The essential oil was separated by vacuum distillation into the following fractions:

1. 67 % 1,8-cineole
2. 30 % terpinen-4-ol, 21 % borneol and 11 % α-terpineol
3. residue, 75 % selin-11-en-4α-ol

From the 1st fraction 1,8-cineole and from the 3rd fraction selin-11-en-4α-ol were isolated by flash chromatography in pure forms (>98% purity).

The antimicrobial properties of the total oil, second fraction consisting of oxygenated monoterpene as well as 1,8-cineole and selin-11-en-4α-ol were studied against following fungi : *Candida albicans, Rhodotorula rubra* and *Aspergillus fumigatus*, by using well test.

C.albicans was more resistant than other fungi.

There occur signicifant differences in activity of the oil and its components. Selin-11-en-4α-ol showed the highest activity and 1,8-cineole the lowest one.

This study was supported by State Committee for Scientific Research No. 5 PO6B 103 08.

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Constituents of The Essential Oil of *Artemisia absynthium* Grown in Egypt

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Artemisia absynthium L. (Asteraceae) has been used as an anthelmintic to destroy intestinal worms, hence the name 'wormwood". Its leaves and flowering tops are used as an aromatic bitter or tonic, a diaphoretic and as a flavoring agent. It is employed in small amounts to flavor some of the aromatic alcoholic beverage and to impart a fragrance to certain liniments (1).

The hydrodistilled essential oil from fresh aerial parts of *Artemisia absynthium* L. (0.78 yield) cultivated in the Medicinal and Aromatic plants station of the Faculty of Pharmacy, Cairo University, was analysed by CGC-MS. Identification of the components of the oil was established by their retention times and mass spectra which were compared with the respective ones reported in literature. The oil was found to contain 72.3% mono- and sesquiterpene hydrocarbons and 27.7% oxygenated compounds. Monoterpene hydrocarbons (61.24%) comprised α-phellandrene (50.52%), γ-terpinene (2.99%) α-pinene (1.92%), tricyclene (1.82%), α-terpinene (1.57%) and limonene (1.12%) as the major compounds. Sesquiterpene hydrocarbons amounted to 11.05% of the oil, caryophyllene (4.12%) and *trans*-isoelemicine (3.56%) being the major compounds. Oxygenated compounds were mainly alcohols; terpinene-4-ol (11.98%) and linalool (10.48%) being the major ones. Chamazulenes and thujones known to be present in oils obtained from *A.absynthium* grown abroand, were not detected in the oil obtained from the plant grown in Egypt. Several chemotypes were recognized for the plant grown in Italy, France, Siberia and Romania (2). *A.absynthium* L. under investigation represents another chemotype.

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Composition of The Essential Oil of *Artemisia vulgaris* L. Grown in Egypt

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Several species of the genus *Artemisia* (Asteraceae) have been the subject of numerous chemical studies (1). Analysis of the essential oil from *Artemisia vulgaris* L. was reported (2,3). In this study, we report on the CGC-MS analysis of the hydrodistilled essential oil from fresh *A.vulgaris* (0.65% yield) cultivated in the Medicinal and Aromatic Plants Station of the Faculty of Pharmacy, Cairo Univ. The analysis was carried out on a BA-70 OV31-OH capillary column. Compounds were identified by their retention times and mass spectra which were compared with the respective ones reported in literature. Hydrocarbons constituted 66.12% of the oil comprising monoterpene hydrocarbons (38.42%) and sesquiterpene hydrocarbons (27.70%). The main monoterpene hydrocarbons are α-phellandrene (17.30%), $^3\Delta$ carene (4.27%) and camphene (4.19%). The main sesquiterpene hydrocarbons are: *trans*-isoelemicin (15.14%) and γ-elemene (8.82%). Oxygenated compounds amounted to 31.46% of the oil, lyratol (15.14%) being the major comound. Several compounds were found in traces and constituted together 2.56% of the oil.

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QUALITATIVE - QUANTITATIVE COMPOSITION OF CHAMOMILE OIL IN EGYPT

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Cooperation between the Egyptian herb specialists and researchers from Slovakia was started in 1993. Our mutual study of Chamomile, Chamomilla recutita (L.)Rauschert, chemotypes belongs to a very important field of a research. The purpose of this contribution is presented the qualitative - quantitative characteristics of Chamomile, which is cultivated in Egypt.

The results are presented in table:

production area	the qualitative - quantitative composition (in%) of Chamomile essential oil						
	Fa	BoB	BnA	Bo	Ch	BoA	Dc-s
El-Faiyum	18.2	4.9	9.8	5.5	2.0	40.1	9.7
El-Kanather Shalaka	5.1	2.7	8.7	2.4	1.7	50.7	8.7
El-Tahrir Sahra	3.5	2.4	7.3	11.2	2.0	51.1	5.9
Giza Cairo	2.4	1.6	2.7	3.6	2.6	68.2	7.6

Fa: Farnesene, BoB: /-/-α-Bisabololoxid B, BnA: /-/-α-Bisabolonoxide A, Bo: /-/-α-Bisabolol, Ch: Chamazulene, BoA: /-/-α-Bisabololoxide A, Dc-s: En-in-dicycloethers

In regard to these GC-results the Chamomile, which is cultivated in Egypt, belongs to the bisabololoxide chemotype. The % composition of /-/-α-Bisabolol and Chamazulene in essential oil is from 4.1 to 14.2.

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THE COMPOSITION OF ESSENTIAL OILS FROM SOME *TANACETUM* SPECIES OF TURKEY

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Water distilled essential oils from some *Tanacetum* (Compositae) species of Turkey were analyzed by GC/MS. A summary of the results are as follows:

Species	Province	Plant part	Oil yield (%)	Main components (%)
T.argyrophyllum var. *argyrophyllum*	Gümüşhane	leaf	1.03	α-thujone (51.8)
T.argyrophyllum var. *argyrophyllum*	Gümüşhane	flower	0.96	α-thujone (62.8)
T.armenum	Ankara	leaf	0.62	1,8-cineole (31.3), camphor (8.6)
T.armenum	Eskişehir	herb	0.67	camphor (26.7), 1,8-cineole (11.3), borneol (10.6)
T.balsamita	Culture	herb	0.38	carvone (52.4, α-thujone (11.7)
T.haradjani	Adana	Leaf	0.55	camphor (15.9), 1,8-cineole (10.0)
T.praeteritum subsp. *praeteritum*	Muğla	herb	1.09	borneol (28.1), 1,8-cineole (12.3), bornyl acetate (10.0%)
T.praeteritum subsp *massicyticum*	Antalya	herb	0.92	α-thujone (51.1), β-thujone (10.0)

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Compostion of the Essential Oil of *Cotinus coggygria* Scop. From Turkey

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Water distilled essential oil (0.87%) from the leaves of *Cotinus coggygria* Scop. (Anacardiaceae) was analysed by GC/MS. Fourty two components were characterized representing 99.6 % of the total components. The major constituents were identified as limonene (48.5%), (Z)-β-ocimene (27.9%) and (E)-β-ocimene (9.7%).

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CHEMICAL STUDIES ON *BOSWELLIA AND COMMIPHORA* SPECIES OF AFRICA

E.Dagne

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Boswellia and *Commiphora* species (family Burseraceae) are mainly found in Africa although a few species are known to occur in Arabia and India. These plants are sources of the culturally and economically important resins olibanum (frankincense) and myrrh respectively. The resins are used as incense, for fragrance and flavour purposes not only in the countries of origin but in many parts of the world. Somalia and Ethiopia are by far the biggest producers of these resins.

The chemistry of frankincense and myrrh has attracted the attention of several workers for many years. Limitation in the taxonomic and botanical knowledge of the genus has hindered chemical work on resins from properly authenticated species. Earlier reports that were based on these resins obtained from commerce lacked consistency and could not be reproduced. Thus it is clear that there is need to determine the composition of these resins from botanically defined species. In our laboratory chemical investigation is in progress on resins obtained from botanically authenticated *Boswellia* and *Commiphora* species.

The pressure of increasing population and accelerated urbanisation is leading to increased destruction of forests and people tend to cut trees for fuel and charcoal-making rather than tapping the resins and benefiting from these resources. The resins in the producing countries are very cheap because they are exported unprocessed to far away lands such as Far East and Europe where value adding takes place. It is therefore important to take measures to conserve these resources and at the same time increase ways and means of adding value to the resins in the countries of origin so that the producing communities derive more benefits from these species and become aware of their sustainable utilisation.

Results of our investigations on the above resins as well as on several other essential oil bearing plants of Ethiopia will be presented.

P45

SOME NEW SESQUITERPENES FROM LABDANUM OIL

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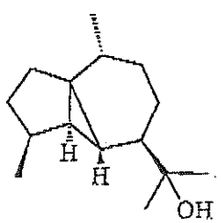
The commercial oil was separated by distillation and repeated flash chromatography. 180 components representing about 95% of the oil could be identified. The main constituents are ledene (9%), viridiflorol (4%), copaborneol (2%), ledol (1%) and the new cubeban-11-ol (1, 4%). Typical for this oil are oxidized cadinane (2) and aromadendrane (3-5) derivatives. The structures of the new compounds 1-5 were elucidated by GC-MS, ^{1}H- and ^{13}C-NMR spectra.



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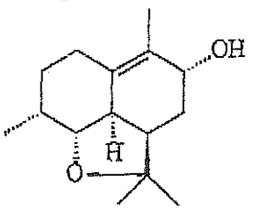

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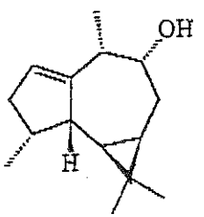

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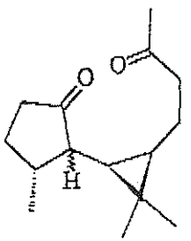

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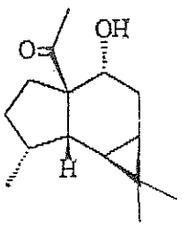

5

P46

SESQUITERPENOIC CONSTITUENTS OF THE ESSENTIAL OIL OF SOME *CYPERUS* SPECIES

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Previous studies of the essential oil of the *Cyperaceae* revealed that the major sesquiterpenoic representents in this family are those of the classes of eudesmane and patchoulane[1,2]. The first part of our work[3] was in agreement with these results. The present contribution also confirms this tendency, with the isolation and identification of new eudesmane and rotundane type sesquiterpenes. The compounds were isolated from the hydrodistillated extract of the rhizomes of the plants by preparative gas chromatography. The structure elucidation was done by spectroscopic methods and chemical transformations.

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P47

COMPOSITION OF ESSENTIAL OILS OBTAINED BY WATER-DISTILLATION AND HEAD-SPACE OF *PELARGONIUM ENDLICHERIANUM* FENZL.

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Pelargonium endlicherianum (Geraniaceae) is an endemic plant of Turkey. Water-distilled essential oil and head-space volatiles from fresh flowers of *Pelargonium endlicherianum* were subjected to GC and GC/MS analysis.

More than 100 components were identified, representing over 75% of the water-distilled oil. The main constituents of the oil were germacrene-D (15.8%), phenylethyl-2-methyl butyrate (12.5%), tricosane (5.5) and T-cadinol (4.7%), while germacrene-D (27.1%), β-caryophyllene (11.3%), α-phellandrene (9.8%, β-pinene (7.3%) and α-pinene (4.9%) were of head-space volatiles.

P48

HISTOCHEMICAL LOCALIZATION OF CITRAL ACCUMULATION IN LEMONGRASS LEAVES (*CYMBOPOGON CITRATUS* (DC.) STAPF., POACEAE).

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Lemongrasses (*Cymbopogon* spp., Poaceae) are a group of commercially important C_4 tropical grasses. Their leaves contain up to 1.5% (dry weight) essential oils with a typical lemon-like aroma, consisting mainly of citral: a mixture of the isomeric acyclic monoterpene aldehydes geranial and neral. In order to specifically locate the sites of citral accumulation in lemongrass we have employed the Schiff's reagent, that stains aldehydes. Using this technique, single oil-accumulating cells were detected in the abaxaial side of leaf mesophyll, commonly adjacent to the non-photosynthetic tissue, and between vascular bundles. The cell walls of these oil cells are lignified.

P49

Water Stress effects on aromatic plants of commercial importance growing in India.

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One of the recent trends in plant-soil-water management research is the possibility of commercial cultivation of crops under water-stress conditions. In the present study the formation of essential oil in relation to developmental physiology of three important spices of Cymbopogon viz. C. winterianus (Citronella), C. flexuosus (Lemon grass) and C. martini (Palmarosa) under water-stressed condition and its alleviation by a bio-regulator of growth, n-Triacontanol have been investigated.

Water stress condition in all the three species lowers per hectre yield of essential oil; disturbing the proper correlation between different growth entities. Stress inhibiting effects on essential oil yield are manifested through shortening the duration of particularly reproductive and post reproductive phases. n-Tria application releives the water-stress inhibiting effects on oil yield by strengthening the specific correlation between different functional entities as well as of different developmental phases.

The study claims that inhibition of essential oil synthesis by water stress is monitored through modification of durations of different developmental phases and revival of inhibition by n-Tria is manifested through suitable adjustments of different developmental phases towards normalcy. The functional parameters like linear and leaf growth, flower formation, contents of nitrogenous and CHO fractions are disturbed in a specific way by water stress condition which can be modulated by n-Tria application to the advantage of the species.

P50

Acorane-Type Sesquiterpenoids from the Liverwort *Jungermannia lanciolata*

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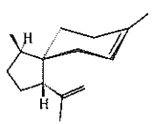
The bryophyte plant group produces a remarkable variety of rare and unknown natural compounds. In particular, liverworts (*Hepaticae*) contain a large number of structurally diverse sesquiterpenes, which are the major constituents of their essential oils[1]. Recently we studied the chemical constituent of the liverwort *Jungermannia lanciolata* and isolated compounds **1-4**. The structure of the isolated compounds was determined by GC-MS, 1D- and 2D-NMR techniques[2]. The stereochemical aspects of these compounds were investigated by two-dimensional enantioselective gas chromatography and by conversion into known compounds.



(+)-β-Acoradiene
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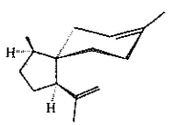
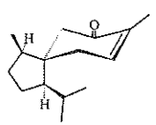


(-)-α-Neocallitropsene
2



(+)-Acorenone B
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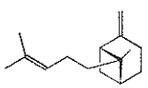

(-)-trans-β-Bergamotene
4

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2) We thank Prof. K. H. Kubeczka and Dr. D. Joulain for helpful discussions.

P51

The Composition of the Essential Oils of Some *Hypericum* Species Growing in Turkey

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Guttiferae - St. John's Wort family is represented throughout the world by 40 genera and 1300 species. 400 of them are *Hypericum* species and distributed mainly in temperate regions, tropical mountains.

The genus *Hypericum* is represented in Turkey by 77 species including 30 endemics. *Hypericum* species have a wide range of use as dye, flavouring, as medicine in wound healing, astringent, for anti-viral, anti-depressive and anti-hepatotoxic properties in Turkish folk medicine as well in the world. Anti-AIDS properties of *Hypericum* extracts are being studied in USA.

In this initial study three different species from different localities: *H. perforatum* L. (Bozdağ-Eskişehir), *H. calycinum* L. (İnegöl-Bursa), *H. cardiophyllum* Boiss. (Kilis-Diyarbakır) were investigated. Air dried flowers and leaves were water distilled to obtain essential oils which were then analysed by GC/MS. The main components were identified and reported in detail for the first time for Turkish *Hypericum* species.

P52

EUCALYPTUS SPECIES CULTIVATED AT UNIVERSITY COLLEGE DUBLIN: CUT FOLIAGE PRODUCTION AND CHARACTERISTICS OF VOLATILE OILS.

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Native to Australia, the genus *Eucalyptus* (Myrtaceae) comprises some 500 species, varying in stature from low shrubs to large trees. *Eucalyptus* is grown for timber, biomass, essential oil and cut foliage. There are many chemotypes of *Eucalyptus*, the most common being 1,8-cineole, citronellal, α- and β-pinene, limonene, eudesmol and trans-methyl cinnamate. It is a low yielding species, with oil content between 0.1-1.2%, rarely with 1.2-2.5%. The plants in this experiment were cultivated in grey-podzolic soil of pH 6.0-6.9, in temperate climate as a coppiced plantation. Marketable size of foliage is achieved after 2 years. Adult and juvenile foliage was harvested, distilled and analysed by GC and GCMS. *E. globulus, E. rubida* and *E. viminalis* belong to the same chemotaxonomical groups, producing alpha-pinene (12-46%) and 1,8-cineole (18-68%), with significant differences between juvenile and mature leaves. *E. gunii* contained *p*-cymene (15%), bicyclogermacrene 1 and 2 (8% and 14%), ent-spathulenol (27%) and viridiflorol (7%). These results compare favourably with production of oil scented species in tropical climate. The foliage is becoming more popular and strong scent of leaves is an important characteristic. *E. gunii* proved to be a promising species for cut foliage, with yield of 5.4kg/plant and 7.5 kg/plant in 2nd and 3rd year respectively. *E. globulus* has too large leaves for cut foliage, *E. viminalis* with narrow leaves and broad side shoots does not appeal to florists and *E. rubida*, although very attractive, has a low yield (2.1 kg/plant).

P53

ESSENTIAL OILS OF LEAVES AND FRUITS OF *JUNIPERUS FOETIDISSIMA* Willd.

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Juniperus foetidissima Willd. (Cupressaceae) is known in Turkey as "Kokar Ardıç". Two samples were collected from different localities in Eskişehir-Turkey. Leaves, fruits, seedless fruits, seeds and branches of the two collections were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce oils in 0.32-1.37% yields. The essential oils were analyzed by GC and GC/MS techniques. The main components of these parts were as follow:

Leaves	:	β-thujone (21-22 %), sabinene (11-21 %)
Fruits	:	sabinene (31-35 %), α-pinene (23-39 %)
Seedless fruits	:	sabinene (13-17 %), α-pinene (10-24 %), terpinen-4-ol (5-13 %)
Seeds	:	sabinene (10-20 %), α-pinene (14-17 %)
Branches	:	α-pinene (56-71 %)

P54

THE CHEMICAL COMPOSITION OF THE VOLATILE OILS OF *PINUS BRUTIA, PINUS NIGRA, PINUS SYLVESTRIS* GROWING IN TURKEY

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The genus *Pinus* is represented by five species in Turkey. Among these species only *P.brutia, P.nigra and P.sylvestris* are used to produce timber by the Forestry Department. The needles of these three species were collected periodically from the Antalya, Bursa, Denizli, Içel, Kastamonu, Kütahya, Muğla, Samsun and Sinop provinces.

The composition of volatile oils were analysed by means of GC and GC/MS.

Forty seven constituents were identified in the essential oil of *P.brutia*. The constituents identified in high amounts and their percentages are as follows: α-pinene (10.98-33.45%), β-pinene (8.60-51.97%), Δ3-carene (0.05-11.45%), β-caryophyllene (2.95-10.87%), germacrene-D (2.26-17.63%).

Forty two constituents were identified in the essential oil *of P.nigra*. The constituents identified in high amounts and their percentages are as follows: α-pinene (4.51-49.53%), β-pinene (1.15-43.10%), β-caryophyllene (5.26-21.48%), germacrene-D (1.81-21.38%).

Forty three constituents were identified in the essential oils of *P.sylvestris*. The constituents identified in high amounts and their percentages are as follows: α-pinene (19.44-56.88%), β–pinene (2.87-17.09%), camphene (0.44-16.84%).

This is the first detailed report on the volatile constituents of the needles of *P.brutia, P.nigra and P.sylvestris* growing in Turkey

P55

PRODUCTION OF ROSE ABSOLUTE FROM ROSE CONCRETE AND DETERMINATION OF IT'S PROPERTIES

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Turkey is one of the most important rose oil and rose concrete producer in the world. Especially in the region of Isparta and Burdur, rose-cultivation is densely done and the grown oil-rose flowers (*Rosa damascena* M.) are processed with two different methods (steam distillation and volatile solvent extraction) by domestic and foreign firms.

The products obtained by steam distillation and solvent extraction are named as rose oil and rose concrete, respectively. Neither of the products are consumed domestically, but are exported.

In this study the conditions of absolute production and the yields of rose absolute from rose concretes of four different firms were examined and the physicochemical properties and chemical composition of the product, rose absolute, has been determined by GC and GC/MS.

P56

CALIFORNIA ROSE CONCRETE

Karl Lee
Spagyrica, Partington, Big Sur
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California, a state rich in agricultural diversity, a world-class producer of fine fruits, flowers and produce with a growing international market and appreciation, lacks an agricultural aroma industry of the same world-class quality.

Thirty years ago, California had no varietal wine or grapes available. Innovative thinkers imported and planted the truly great varietals of today's California wine industry. My work suggests an aroma industry is possible in California that is equal in quality to Grasse.

The environment for my study is the Central Coast of California above the fog, lined with well-drained hills of sandy-clay soil high in limestone. The exposure is severe with east, south and west sun--a natural site for a rose study.

Three rose varietals of importance were selected following a twenty-year acclimation evaluation. It is not surprising that after twenty years of neglect and harsh environment, the survivors are: *Rosa Damascena Trigintipetala*, the standard of *Rose Otto; Rosa Gallica Officinalis*, the rose of medicine; and, a moss rose so oily it coats one's fingers with sticky resin.

Evaluation of these rosa chemotypes will hopefully stimulate a more extensive study of these roses with detailed component analysis and comparison with the **Bulgarian, French and Moroccan concretes.**

Renewable botanical resources are the wave of the future.

P57

THE ESSENTIAL OILS OF *DICTAMNUS ALBUS* L. FROM THREE DIFFERENT REGIONS OF TURKEY

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A single species of the genus *Dictamnus* (Rutaceae) is recorded in the flora of Turkey. *D. albus* L. grows in the north and west Anatolia (1). This species is locally known as "akgiritotu" and "gazelotu" and is used in folk medicine as antipyretic, nervetonic and digestive.

Aerial parts of *D. albus* collected from Samsun, Bursa and Safranbolu provinces of Turkey were subjected to hydrodistillation for 3h using a Clevenger-type apparatus. The essential oils so obtained were analysed by GC and GC/MS techniques.

Oil from the Samsun material contained dictagymnine, feniculine, methyl chavicol and anethole as major constituents (2), while β-pinene, limonene, cis-β-ocimene, γ-terpinene were main compounds in the Bursa and Safranbolu materials.

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P58

INVESTIGATIONS ON THE EXTRACTION OF ESSENTIAL OIL FROM DIFFERENT HERBAL DRUGS DURING AQUEOUS EXTRACTION

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Reliable indications and recommendations for an appropriate dosage should be the basis of a rational phytotherapy. For that reason especially traditional formulations of crude drugs, like aqueous extracts, have to be examined critically. According to a market research for the year 1994, concerning the western part of the Federal Republic of Germany, about one 1.2 billion DM have been turned over for tea and related products in retail trade (1). About 300 million DM of this amount were spent for „non black tea".

After extensive investigations about storage stability of herbal drugs, it was very interesting for us to know, if the crude drugs' content of essential oil is important for the quality of the aqueous extract prepared out of it. Only little is known about the extraction yield of the lipophilic essential oil components into aqueous extracts.

Different methods in view of a quantitative isolation of the essential oil compounds of aqueous extractions were tested for an easy and rapid GLC analysis. A new microdistillation technique (Capillary Distiller 5325, Fa. Eppendorf) was shown to produce good results.

Sage leaves (Salviae folium, DAB 1996) and Peppermint leaves (Menthae pip. folium, DAB 1996) are commonly used for the preparation of aqueous extracts for oral application. The German Pharmacopoeia explicitly demands a quality of sage, which is rich in thujon. As a consequence of its well known toxicity, the grade of aqueous extraction for this substance is of considerable importance.

Depending on the mode of extraction, the particle size and the quality of the herbal drug, ranges of extraction from 15 % to nearly 50 % of the essential oil can be found in the aqueous extracts. Remarkable differences between the total essential oil and the lipophilic components of the aqueous extract were found (see also 2,3). In contrast to other components of the essential oil of Salviae folium, thujon can be found in higher proportions.

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We are grateful for financial support by PhytoLab GmbH & Co.KG, Vestenbergsreuth and BfArM, Berlin

P59

GC-ANALYSIS OF NEROLI OIL ON CHIRAL COLUMNS

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In recent years a large number of racemic adulterations of different essential oils has been detected by GC-analysis on cyclodextrin phases (Ref. 1/2). If this method is to be used in the European Pharmacopoeia, general applicability is necessary. A two-step temperature gradient must enable the separation of all important components by a single one-dimensional GC-run. It must be possible to separate these components on commercially available columns and to check them by easily accessible substances as standards.

The development of an appropriate a chiral GC-method is difficult, due to the overlapping peaks of different ingredients in these oils. The elution sequence of the single components with cyclodextrin phases shows a strong variability depending on temperature (Ref. 3). A number of cyclodextrin phases have been examined, and the results with Neroli oil are reported. Different amounts of the enantiomeres of the main component linalool are detected in Neroli oils of different commercial origin. Similar results are found in so-called "artificial" Neroli oils. Three different adulterations must be taken into account:

- addition of racemic linalool,
- addition of (-)-linalool from sources rich in (-)-linalool (e.g. the essential oil from the wood of Bursera delpechiana),
- the artificial "composition" of Neroli oil.

A chiral GC-analysis represents a convenient method for a Neroli oil monograph.

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Acknowledgement: This investigation was supported by a grant from the Bundesinstitut für Arzneimittel und Medizinprodukte, Berlin.

P60

ESSENTIAL OIL ANALYSIS OF PLANTS FROM CAMEROUN BY GC/FID AND GC/MS

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The essential Oils of various plants from Cameroun were analyzed by GC/FID and GC/MS to identify the volatiles responsible for applications in traditional folk medicine of Cameroun as well as their interesting odour impressions for perfumery.

Using different GC/MS systems (GC-QP5000, GC-MSD, GCQ) the essential oils of *Callistemon rigidus* (Myrtaceae, leaves and seeds), *Dorstenia psilurus* (Moraceae, roots), *Hyptis suaveolens* (Lamiaceae, leaves and flowers), *Irvingia gabonensis* (Irvingaceae, fruits) and *Laggera pterodonta* (Asteraceae, leaves) were investigated. Especially mono- and sesquiterpenes are dominating among the nearly 200 different constituents of these oils.

After olfactoric evaluation (by professional perfumers) and quantification (by GC/FID measurements) each compound was correlated to a possible odour impression as well as to a reported medical application of the corresponding oil. Also the composition of the investigated essential oils is presented and discussed for a possible use of them in fine perfumery applications.

P61

A SHORT SYNTHESIS OF NEW KETONE WITH AMBER-LIKE ODOR FROM LONGIFOLENE

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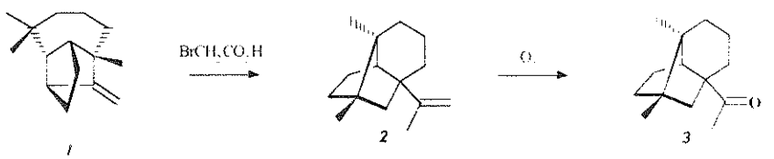
Using a longifolene-rich fraction isolated by distillation for the residue of Polish turpentine oil, the three-step synthesis of ketone **3** from longifolene **1** via acid-catalysed isomerisation, followed by ozonolysis of alloisolongifolene **2** is presented.



The title ketone **3** possess very interesting olfactory activitity by showing an amber-like odor, which belongs to the most appreciated scents in perfumery. Synthesis, structure and spectral details are presented.

SYNTHESIS AND ODOR-STRUCTURE-RELATIONSHIP OF SEVERAL SATURATED AND UNSATURATED KETONES

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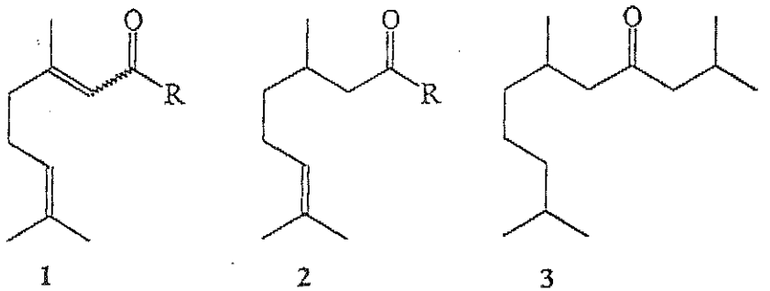
The mixture of sesquiterpene ketones identified from solitary bees[1] was found to be pleasantly smelling. For the investigation of odor-structure-relationship these compounds of type 1-3 were synthesised and olfactorily evaluated.



[1] W.Francke, *Pure & Appl. Chem.*, 61, 539 (1989)

SYNTHESES AND OLFACTORY PROPERTIES OF ALCOHOLS AND KETONES WITH DECALIN SKELETON

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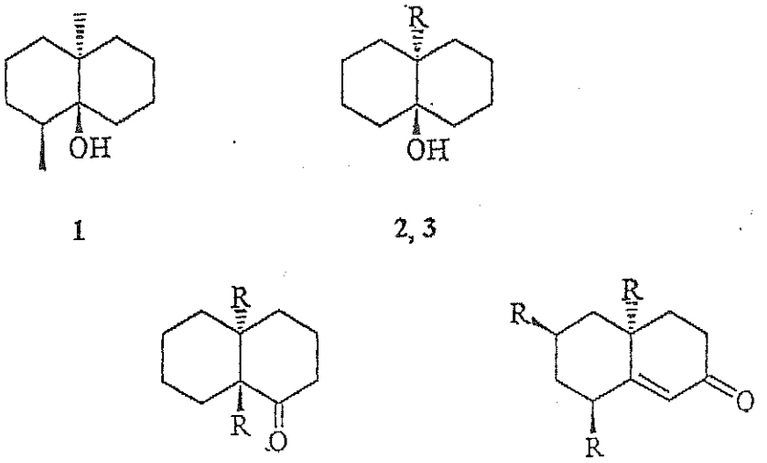
Geosmin (1), a compound with earthy odor, is one of the strongest fragrance chemicals which is known today (threshold 0.1 ppb). It is formed by various soil microorganisms of the Streptomyces family and Myxobacteria.
For better knowledge of the relationship of structure and earthy odor, the syntheses and olfactory properties of the two *nor*-geosmins 2 and 3 and several derivatives with decalin skeleton and different functional groups such as the saturated 4 and 5 and the α,β-unsaturated ketones 6-9 are presented.



R = H or Me

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ANTIHEPATOXIC EFFECT OF LIPOSOMIZED PREPARATION CONTAINING VOLATILE OIL OF *CURCUMA XANTHORRHIZA* AND CURCUMINOIDS OF *CURCUMA DOMESTICA* ON LIVER DAMAGE INDUCED BY CCL_4

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Curcuma xanthorrhiza Roxb and *Curcuma domestica* Val. have been used long in traditional medicine. The rhizomes are used traditionally for tonic, stomache, rheumatism, and liver disorder. The main components are volatile oil and curcuminoids.

The effect of antihepatotoxic effect of Curcuma Complex and Curcuma Complex plus on mice liver damage induced by CCl_4 has been carried out. Curcuma Complex (CC) consisted of Volatile oil (isolated from *Curcuma xanthorrhiza*) and curcuminoids (isolated from *Curcuma domestica*). Curcuma Complex Plus (CCP) is liposomized preparation of CC. Antihepatotoxic effect were examined histopathologically

Ninety mice were randomly divided into 6 groups. Group I was natural control group; group II received CCl_4 only; group III received CCl_4 and CC of dosis 3; Group IV received CCl_4 and CCP of doses 1 and group V received CCl4 and CCP of dosis 2 and group VI received CCl_4 and CCP of dosis 3.

The results showed that CCP of doses 2 (liposomized CC preparation of 0,14 mg volatile oil/kg b.w and 0,572 mg curcuminoid/kg b.w) had the best antihepatotoxic effect compared with CC (0,28 mg volatile oil/kg b.w and Curcuminoid 1,144 mg/kg b.w) and CCP of dosis 1 (0,07 mg vol.oil/kg b.w and Curcuminoid 0,286 mg/kg b.w) and CCP of dosis 3 (0,28 mg vol.oil/kg b.w and Curcuminoid 1,144 mg/kg b.w).

However the antihepatoxic effect of CCP can not stop the process of liver fibrosis caused by CCl_4 toxicity twice a week for three weeks.

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GLOBAL VISION LEADERSHIP TRAINING

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This paper is a survey of the efforts and progress made in the USA since 1988 to develop university level training in the growing and processing of aromatic and medicinal plants for use in fragrance, flavor and pharmaceutical applications, and how TBAM might be instrumental in helping the Americas obtain the kind of education in aromatic extraction technology and basic training in perfumery that is needed everywhere on this continent.

Both Aromatherapy and Perfumery are a combination of Art and Science. Therefore the Aroma Trades need staff with broad cultural background and inter-disciplinary perspective. In France today there are two schools of perfumery, Roure-Givaudan in Grasse, and at Versailles, a government and fragrance industry sponsored school. Both accept French speaking students with broad liberal arts backgrounds from all over the world. However, despite great need, there is no university associated training in perfumery and aromatics available to the general public in the USA.

Following World War II, the global fragrance industry became overloaded with chemists and technicians, trained in scientific discipline, but lacking in history, philosophy and the arts. By the mid sixties the industry realized that chemistry alone was not adequate foundation for perfumery. Therefore, Stephan Arctander, a visionary leader, began recruiting aspiring perfumers with liberal arts backgrounds for the two year course on perfumery & essential oils he taught at Rutgers Univerity, College of Pharmacy in Newark, New Jersey, USA.

The course was taught until 1969. No high level training in perfumery has been available in the USA since then. Consequently, there is now a backlog of pent-up demand for training in perfumery in North America, as well as in all the countries of the Middle East, Asia, Africa, S.E. Asia and the Far East where English is a *lingua franca*. As France is the only country where there is a school of perfumery open to the public, they are inundated with requests for some kind of instruction in the English language. It is time the English speaking world took action to meet this demand.

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ESSENTIAL OIL INDUSTRY IN IRAN

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The world production of the essential oils is estimated to be 45000 tons a year. The developing countries have an active role in this field. We are going to discuss crucial problems in Iran and suggest suitable methods in this regard. The main point are as follows.

1- Information

In the Essence industry the dispersion of information seems more evident. Each plant needs an information pyramid containing different species, their geographical condition, manner of cultivation, processing and harvesting and also their application, suitable technology and their quantity and effective substances and so on...

1- Industrial Structure

The main points to be covered thoroughly are as follows:

a) The existence of internal and foreign markets.

b) The rationalization of production lines and quality control.

c) Requirements for the acauisition of tools and equipments and final products in rural production units and cultivation, industrial complexes.

3- Training

The manner of promoting the culture of application of technology and information through an efficient system. Special organizations are engaged in this section. Some general methods are suggested to be applicable from rural up to the university level.

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THE USES OF ESSENTIAL OILS IN FOOD INDUSTRY

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Essential oils are the volatile oils obtained from plants or from parts thereof by (1) water distillation (2) steam distillation (3) enzymatic action followed by steam distillation, or (4) water and steam distillation.

In Food Industry, essential oils are widely used as a herb and spice extract instead of direct usage of plants. The main purpose of this is to render food products more attarcive to human taste. Essential oils are widely used in food production due to their antimicrobial, antioxidative, coloring and flavoring properties.

Some examples of spices that contain important essential oils used in Food Industry are cloves, turmeric, cumin, caraway, cardamom, coriander, cinnamon, fennel, fenugreek, nutmeg and mace.

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ESSENTIAL OILS AND PLANT SUBSTANCES - AN ALTERNATIVE OF SYNTHETIC GROWTH ENHANCERS IN ANIMAL FEEDING

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The administration of synthetic growth enhancers came under criticism because of the suspected development of vancomycin-resistant enterococci. In some EU countries, the administration was prohibited (Avoparcin Germany, 1997). As with growth enhancers the daily growth rate of the animals can be increased by 5-9% the farmers demand secure and economic alternatives. Traditional compounds of active substances (Bio-tonic, Livol) as well as new preparations with essential oils (CRINA, Micro-plus) are commercially available. Tests in Brandenburg have shown that it is possible to store freshly harvested grain (18% moisture) with an addition of 0.2% *Origanum*, *Caraway* and *Clove* oil, resp. In fodder acceptance tests grain which was stabilized by essential oils was added. Forty days pig fattening tests (38 kg-72 kg) have demonstrated the following fodder utilization.

	Control	*Origanum* oil 0.1%	*Cinnam.* oil 0.1%
Daily weight increase, g	882	882	892
Feed per animal and day, kg	2.50	2.36	2.42
Feed per kg weight increase, kg	2.83	2.69	2.71

Alternative preparations of high efficiency and acceptance will be developed by the combined use of essential oils and prebiotic inulin.

IGV is interested in integrating the work into European research projects and is searching for partners within the 4th framework programme of EU.

AUTHOR INDEX