Chemical and Sensory Evaluation of Trace Compounds in Naturals

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Formerly, say about 25 years ago, little was known about trace constituents present in concentrations below 0.1% (1,000 ppm) in natural isolates, such as essential oils, oleoresins, concretes or absolutes. Today it is possible to characterize compounds in concentrations less than one part per billion (0.001 ppm).

It is general knowledge that rather polar trace constituents, such as acids, phenols, nitrogen compounds and sulphur compounds, can have a characteristic impact on the overall sensory properties of the natural isolate.

Acidic compounds have been found in cassie absolute,[1] costus root, patchouli and olibanum oil.[2] These constituents have been reported to be important for the olfactive properties of the oils in each case.

Substituted phenols occur in almost every essential oil. On one hand these phenols, like thymol and eugenol, can be olfactively characteristic, as in thyme and clove oils. Trace amounts of thymol and eugenol can modify in a positive way the organoleptic quality of citrus oils, such as mandarin, lemon and orange. On the other hand, the lower substituted phenols may have a negative influence on the sensory properties of the oils, as for example in wood oils.

Volatile nitrogen and sulphur compounds are organoleptically important constituents of flavors.[3,4] In the early 1970s, much work was done to determine the sensory properties of these flavor compounds. During the last decade the characterization and determination of the sensory properties of volatile nitrogen and sulphur compounds from natural isolates came more and more into focus. This interest arose from the improvements in the isolation and concentration of volatile trace constituents from natural products, and was initiated by modern chromatographic techniques, such as gas chromatography on high resolution, high precision fused silica capillary columns.

Volatile nitrogen compounds, often called the basic fraction, can be easily isolated from essential oils by an acidic extraction, as recently described by Maurer and Hauser.[5] Almost every essential oil contains some nitrogen compounds as trace constituents. More than 20 years ago[6,7] researchers at Naarden detected a series of olfactively interesting nitrogen compounds in the basic fraction of the following oils: absinthe, angelica, carrot seed, coriander seed, clary sage, celery, galbanum, lavandin, parsley, petitgrain, rosemary, spike lavender and vetiver.

Sulphur compounds occur in almost every natural isolate. Some of these compounds may be formed during isolation; for instance, disulphides from thiols by oxidation during steam distillation. Essential oils contain sulphur compounds in concentrations ranging from less than one part per billion (0.001 ppm) to parts per thousand (1,000 ppm). Often the low concentration of these materials present in the natural isolate (essential oil or extract) does not allow their detection by normal gas chromatographic techniques. Detection is only possible by using the inherent sensitivity and specificity of the flame photometric detector (FPD) for determining of sulphur-containing compounds.[8]

Additionally, determining the sensory properties of the nitrogen and sulphur compounds is complicated because of several factors. First, this determination is a rather subjective task due to intra- and interindividual differences. Second, the qualitative sensory properties of the compounds often are dependent on their concentration. This olfactive dependence on stimulant concentration may be caused by the so-called multiplicity of the compound. Multiplicity of a compound is its ability to trigger different receptor sites at various concentrations. Another aspect of the sensory evaluation of the compounds is that the determination of the odor quality often is influenced by the type of pleasantness or unpleasantness (hedonics). It is known, for instance,[9] that the odors of the alkylpyrazines are generally associated with pleasant roasted food (such as cocoa, coffee, roasted nuts), whereas the odors of alkylpyridines are less pleasant, more amine-like. Moreover, the sensory properties of strong-smelling (intense) trace constituents are dependent on the media in which the properties are determined.

The identities and sensory properties of a series of volatile acids, phenols, nitrogen compounds and sulphur compounds occurring as minor and trace constitu-

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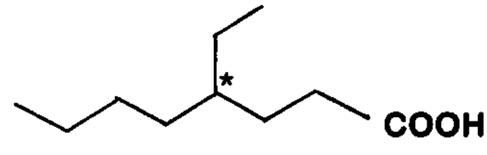
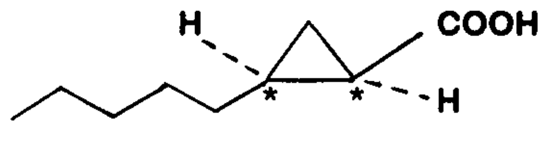
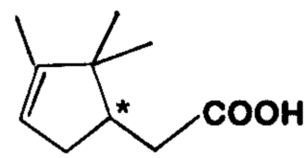


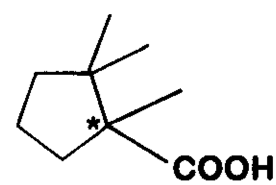
4-ethyloctanoic acid (in costus oil)



2-pentylcyclopropanecarboxylic acid (in patchouli oil)



α-campholytic acid (in olibanum oil)



1,2,2-trimethylcyclopentylcarboxylic acid (in labdanum oil)

Figure 1. Chemical identities of acidic trace constitutents

ents in natural isolates will be discussed in more detail.

Results and Discussion

Acids: Various acidic components were isolated from the essential oils of costus root, patchouli and olibanum.[2] Olfactively, the most important constituent of costus root oil was 4-ethyloctanoic acid (Figure 1). The olfactory properties of this acid proved to be characteristic for the "goaty" odor of the oil. The threshold concentration of 4-ethyloctanoic acid was determined[10] to be 1.8 ppb in water, the lowest value found so far for aliphatic acids. Male subjects tend to differ from female subjects regarding their preference for this acid. Sugiyama et al.[11] have identified 4-ethyloctanoic acid in sebaceous gland secretions from male goats, and observed that this compound exhibited releaser pheromone activity in oestrous female goats. Recently, racemic 4-ethyloctanoic acid was synthesized by Karl et al.[12] and purified via preparative GC. During this operation a strong goaty aroma was perceptible and was persistent for about three weeks. During enantiomer sniffing experiments, 0.6 ng (in air) of the (S)-enantiomer and 1.3 ng of the (R)-enantiomer could be recognized.

Isolating and Analyzing Trace Constituents

Volatile free acids in essential oils were isolated and analyzed according to the basic method described by de Rijke et al.[2] Volatile nitrogen and sulphur compounds in natural isolates were isolated and analyzed according to the methods described by Maurer and Hauser[5] and by Omata,[8] respectively.

GC analyses were carried out using a Carlo Erba MEGA HRGC 5300, equipped with a fused silica column, 50 x 0.32 mm i.d. coated with SE 54 (Hewlett Packard HP- 5; high performance cross-linked 5% phenyl silicone gum phase), film thickness 1.05 micron. Oven temperature programmed, 40-280°C at 4°C/min; injector and FID: 220°C; carrier gas Helium, 120 kPa head pressure. Inlet split 50 ml/min.

GC/MS data were obtained on a Finnigan TSQ70 mass spectrometer directly coupled with a Hewlett Packard 5890 gas chromatograph. Columns used and gas chromatographic conditions were as stated above.

A mixture of the cis and trans isomers of 2-pentylcyclopropanecarboxylic acid was identified in the acidic fraction isolated from patchouli oil. The acid was synthesized and possessed a clear patchouli-like note. A patent was granted to cis-2-pentylcyclopropanecarboxylic acid, and it has been claimed as a fragrance material.[13] Both the cis and trans isomers of this acid consist of (R)- and (S)-enantiomers, which so far have not been isolated.

The most interesting compound olfactively in the acidic fraction isolated from olibanum oil was α-campholytic acid {(2,2,3-trimethylcyclopent-3-en-1-yl)carboxylic acid}. This acid was synthesized and showed a rather strong odor reminiscent of the oil. The acid is also a mixture of two enantiomers.

Phenols: Thymol and eugenol are known organoleptically as character-impact constituents of thyme and clove oils, respectively. Less is known about the sensorial modifying aspects of substituted phenols in citrus oils as published by Kugler et al.[14] and Wilson et al.[15] Minor quantities (<0.1%) of thymol can modify in a positive way the organoleptic qualities of mandarin and tangerine oils, as can similar quantities of eugenol in lemon and orange oils.

In his famous book *The Scent of Orchids*, Kaiser[16] devoted a paragraph to flower scents with a "spicy-floral" image and mentioned the phenolic compounds: p-cresol, vinyl guaiacol, chavicol, eugenol, isoeugenol and vanillin. All these compounds show a positive effect on the overall olfactive properties of certain flowers. On the other hand,

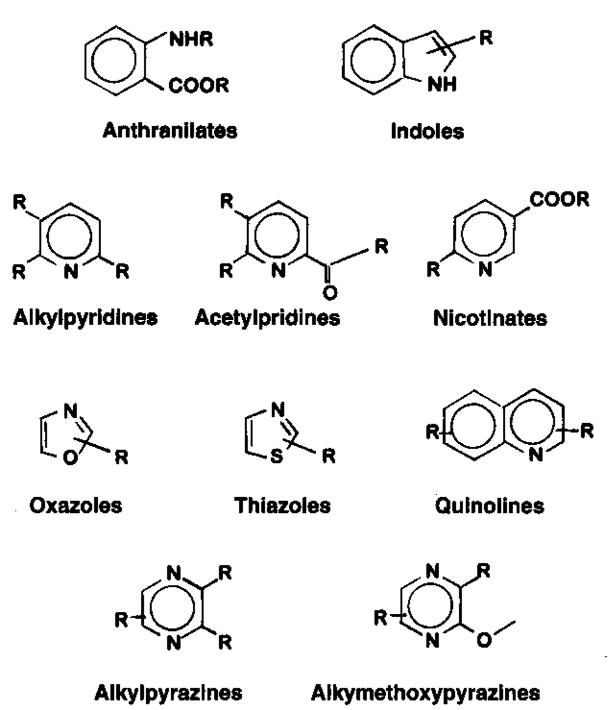


Figure 2. Structures of identified trace nitrogen compounds

lower substituted phenols (such as creosote) may be formed from plant cell material by overheating during isolation of the natural products. These phenols can have a negative effect on the sensory properties of the end product, as for instance in cedarwood, rosemary and spike lavender oil.

Nitrogen and sulphur compounds: Volatile nitrogen and sulphur trace constituents were identified in or isolated from 60 essential oils. The extracts were sensory evaluated by a group of five perfumers and flavorists. The most interesting extracts olfactively and organoleptically were selected for further investigation. The identities of the nitrogen compounds are alkyl-substituted pyridines and pyrazines, 2-alkyl-3-methoxypyrazines, 2-acetylpyridine, 2-alkylthiazoles and substituted quinolines, methyl anthranilate and indole (Figure 2 and Table I).

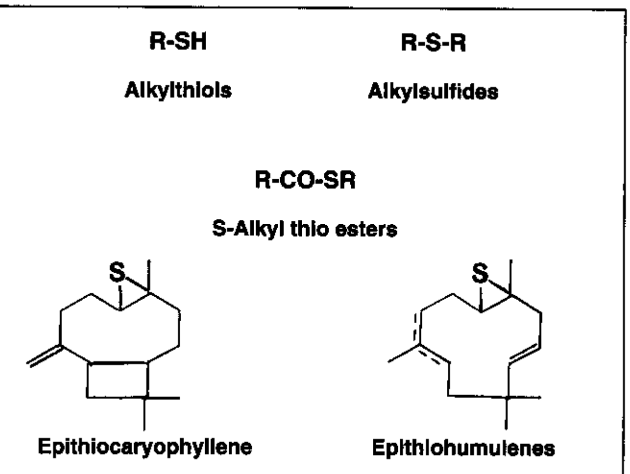


Figure 3. Identified trace sulphur compounds

The identities of the most frequently occurring sulphur compounds are alkylthiols, dimethyl mono- and disulphides, dipropyl sulphide, dialkyl thio esters, epithiocaryophyllene and epithiohumulene (Figure 3 and Table II).

From the literature[3,4] and from my own investigations, one can observe that white flower oils (e.g. jasmine, neroli and hyacinth) tend to contain more nitrogen compounds and red flower oils (e.g. rose) tend to contain more sulphur compounds. In general nitrogen compounds are found in oils from the plant families Umbelliferae (Apiaceae), Labiatae (Lamiaceae) and Oleaceae; whereas sulphur compounds are found in the plant families Liliaceae (Allium species), Compositae (Asteraceae) and Rosaceae. In some Compositae species (e.g. absinthe and chamomile) trace nitrogen compounds as well as sulphur compounds have been found.

Thomas and Bassols[17] found that cold-pressed Florida orange (Valencia cultivar) oil contained 16 substituted pyridines, the main one of which is 3-hexylpyridine at approximately 20 ppb. Alkyl-substituted pyridines were also detected in Brazilian orange (Pera cultivar) oil. The flavor threshold concentration of 3-hexylpyridine in water was found to be 0.28 ppb. Flavorists described 3-hexylpyridine as having a fatty, citrus orange note.

Table I. Volatile nitrogen compounds in essential oils

Oil	Odor descriptions of basic fraction	Identified nitrogen compounds (number of compounds)
absinthe	strong green note, somewhat pea-like	quinoline alkylmethoxypyrazines (4)
angelica seed	intensive green, pyrazine-like	alkylmethoxypyrazines (4)
carrot seed	green, pyridine-like	alkylpyridines (6) alkylpyrazines (10) quinoline
coriander seed	intensive green, pea-like, more pyrazine than pyridine connotation	alkylpyrazines (13) alkylmethoxypyrazine 2-acetylpyridine 2-isobutylthiazole
clary sage	strongly green, tobacco-like	alkylpyridines (2) quinolines (2) alkylmethoxypyrazines (4)
celery seed	green bean, vegetable-note, rye bread connotation	2-acetylpyridines (2) alkylpyrazines (5)
galbanum	intensive green, green bell pepper, galbanum-like	alkylmethoxypyrazines (11) tetramethylpyrazine
lavandin	intensive note, fresh, green	alkylpyridines (5) alkylpyrazines (6) quinoline
parsley seed	characteristic rye bread note	alkylpyridines (3) alkylpyrazines (9) alkylmethoxypyrazines (4)
petitgrain	strongly green, leafy	alkylpyridines (6) 2-acetylpyridine alkylpyrazines (2) alkylmethoxypyrazines (3)
rosemary	interesting, musty leaf-like	alkylpyridines (2) 2-acetylpyridine alkylmethoxypyrazine dimethylpyrazine
spike lavender	intensive green note	alkylpyridines (13) acetylpyridines (3) alkylpyrazines (7) quinoline
vetiver	intensive vetiver-like, pyrazine note	alkylpyrazines (11)

Table II. Volatile sulphur compounds in essential oils

Oil	Number of compounds in oil	Concentration (ppm)	4,5-Epithiocaryophyllene & epithiohumulene (ppm)
rose	20	600	500
absinthe	10	300	100
pepper	6	200	50
patchouli	5	100	50
helichrysum	5	50	30
cassia	4	40	15
clove	3	10	5

Ishihara et al.[18] recently found 38 nitrogen-containing compounds, including 11 new pyridine derivatives, in spearmint and peppermint oil. A major component was 2-acetyl-4-isopropenyl-pyridine, which possesses a powerful grassy-sweet and minty odor.

Surburg et al.[19] investigated the isolation of bioactive volatile compounds from plants.

Recently Maurer[20] (for the late A.F. Thomas) wrote an interesting article on alkaloids, bases and essential oils. He mentioned the identities and organoleptic qualities of a series of alkaloids, such as substituted pyridines in tobacco flavor, juniper oil, sweet orange peel oil and jonquil absolute. One may speculate whether the substituted pyridines and pyrazines are already present in the living plants or if they are formed after cutting and/or during isolation of the volatile compounds. It is commonly known that alkylpyrazines are formed in food flavors (e.g. cocoa, coffee, nuts) during processing of the food.

Mookherjee et al.[21] studied the main constituents, including nitrogen compounds, emitted by living and picked flowers. They showed that there were definite differences in the chemical composition of the living and picked flowers. For instance, indole, which occurred in relatively high concentration in the headspaces of living flowers, decreased significantly in the headspaces of dead flowers, and alkylpyrazines, which occurred in the headspaces of dead flowers, were not detected in the headspaces of living flowers. It may be possible that alkylpyrazines are formed after picking of the plant material. Alkylmethoxypyrazines are probably present in living plants.

Sonnenberg et al.[22] investigated the formation of alkyl-substituted pyridines by model reactions of reactive natural unsaturated carbonyl compounds with ammonia. They mentioned that, because essential oils or plant extracts contain both the carbonyl compounds and the corresponding pyridines, it may be possible that these pyridines are formed as a result of the isolation process (Figure 4).

Various reactions may account for the formation of volatile sulphur compounds in natural isolates. The precursors of volatile sulphur compounds are sulphur-containing amino acids, such as cysteine and methionine. It seems that epithio-derivatives, such as from caryophyllene and humulene, are directly formed from the sesquiterpene hydrocarbons and sulphur. Some sensorially interesting sulphur compounds are the addition products of hydrogen sulphide to unsaturated compounds, such as isoprenyl de-

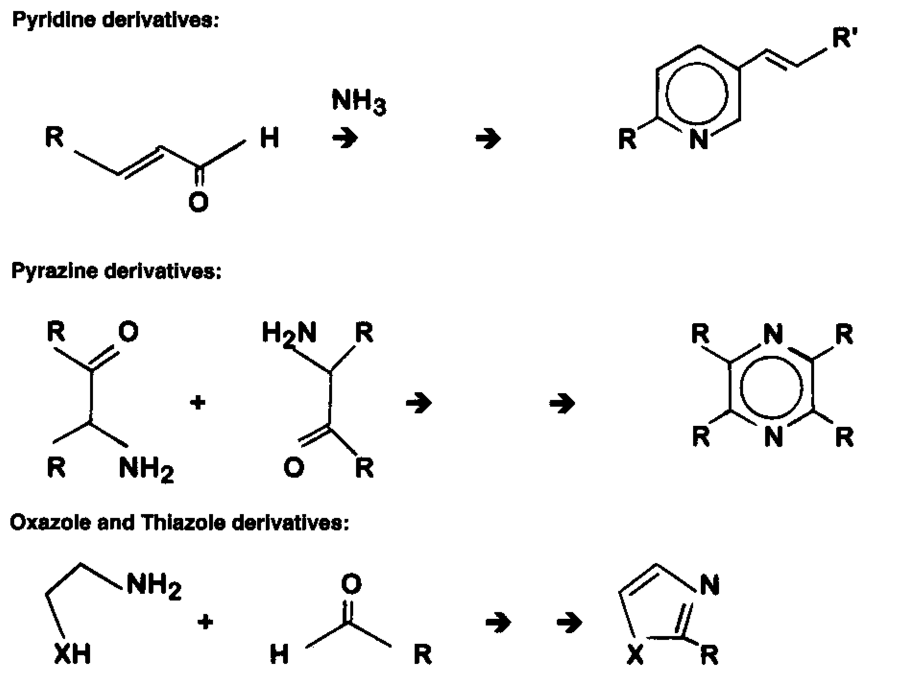


Figure 4. Possible formation of nitrogen compounds

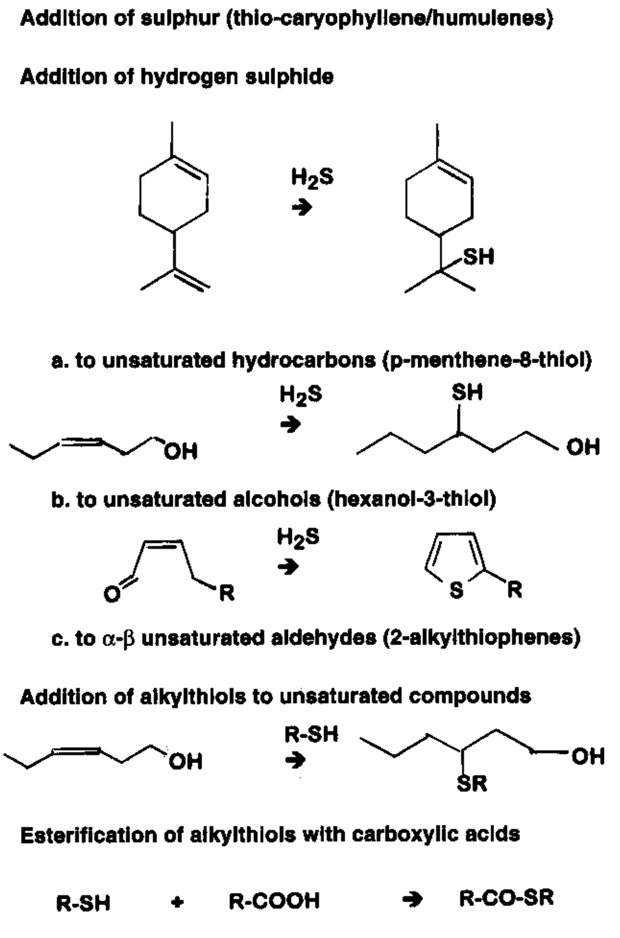


Figure 5. Formation of some volatile sulphur compounds

rivatives (ether, ester), (Z)-3-hexenol, limonene and isopulegone. These compounds have been found in black currant buds, passion fruit, grapefruit and buchu oil. Many olfac-tively interesting sulphur com-pounds can be derived from the addition of hydrogen sulphide or methylmercaptan to reactive unsaturated compounds, such as ionones and damascenones (Figure 5).

Threshold values: The threshold values of substituted pyrazines in water can vary from 100 ppm for methylpyrazine, to 0.1 ppm for 2-ethyl-5-methylpyrazine, to 0.0004 ppm for 2-ethyl-3,6-dimethylpyrazine. The values for the alkylmethoxypyrazines are significantly lower, ranging, for example, from 0.004 ppm for 2-methoxy-3-methylpyrazine down to 0.000002 ppm for 2-isobutyl-3-methoxypyrazine.

For alkylpyridines the threshold values in water range from 0.02 ppm for 5-ethyl-2-methylpyridine and 2-acetylpyridine to 0.0006 ppm for 2-pentylpyridine and 0.0003 ppm for 2-hexylpyridine. For 2-isobutylthiazole a threshold value in water of 0.0035 ppm was found.

Threshold values of volatile sulphur compounds can be extremely low. A threshold value of 30-60 ng/L was found for 1-methoxy-3-methyl-3-butanethiol, the character-impact compound of black currant buds, in sunflower oil. 3-Methyl-2-buten-1-thiol possessed a threshold value in water of 0.2 ng/L and the grapefruit compounds (+)-(R)-1-p-menthene-8-thiol and (–)-(S)-1-p-menthene-8-thiol had threshold values in water of 0.02 ng/L and 0.08 ng/L, respectively.

The isolated volatile acids, phenols, and sulphur and nitrogen compounds demonstrate a relatively high polarity. In other words, these compounds can easily release or attract protons and therefore may be reactive at the polar end of olfactory receptor sites.

Summary

Trace constituents present in essential oils, concretes and absolutes at concentrations of less than 0.1% (1,000 ppm) include acids (in costus, patchouli and olibanum oil), phenols (in mandarin and cedarwood oil), nitrogen compounds (in lavandin and petitgrain oil) and sulphur compounds (in buchu, galbanum, black currant bud and rose oil). These constituents can possess high odor intensities and low threshold values. These compounds reveal characteristic olfactive and organoleptic properties, or are able to modify the overall sensory properties of the end product in a significant way.

References

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1. E Demole et al, Sur les constituants odorants de l'essence absolue de Cassie, *Helv Chim Acta* **52** 24-32 (1969)
2. D de Rijke et al, Acidic components in essential oils of costus root, Patchouli and Olibanum. *Phytochemistry* **17** 1661-1666 (1978)
3. MH Boelens and LJ van Gemert, Volatile character-impact sulphur compounds and their sensory properties, *Perf & Flav* **18** 29-39 (1993)
4. MH Boelens and LJ van Gemert, Characterization and sensory properties of volatile nitrogen compounds from natural isolates, *Perf & Flav* **19** 51-65 (1994)
5. B Maurer and A Hauser, New pyridine derivatives from essential oils, *Chimia* **46** 93-95 (1992)
6. HJ Takken et al, Olfactive properties of a number of polysubstituted pyrazines, *J Agric Food Chem* **23** 638-642 (1975)
7. R Timmer, Research Department Naarden International, private communication (1973)
8. A Omata et al, New sulphur components of Bulgarian rose oil, in *Flavors and Fragrances: A World Perspective*, BM Lawrence, BD Mookherjee and BJ Willis, eds, Amsterdam: Elsevier Sci Publ BV (1988) pp 707-714
9. RG Buttery et al, Roasted lamb fat: Basic volatile components, *J Agric Food Chem* **6** 1227-1229 (1977)
10. H Boelens et al, Threshold values of and human preferences for 4-ethyloctanoic and 3-methyl butanoic acid, *Perf & Flav* **8** 71-74 (1983)
11. T Sugiyama et al, Unusual fatty acids with specific odor from mature male goat, *Agric Biol Chem* **45** 2655-2658 (1981)
12. V Karl et al, Stereoisomeric flavour compounds LXVIII, 2-, 3-, and 4-Alkyl-branched acids, Part 2: Chirospecific analysis and sensory evaluation, *Chirality* **6** 427-434 (1994)
13. US Pat 3,926,860, RL Chappell (1975)
14. E Kugler and E Kovats, Zur Kenntnis des Mandarineschalen-Oels, *Helv Chim Acta* **46**1480-1499 (1963)
15. CW Wilson et al, Importance of thymol, methyl N-methylanthranilate, and monoterpene hydrocarbons to the aroma and flavor of mandarin cold-pressed oils, *J Agric Food Chem* **29** 494-496 (1981)
16. R Kaiser, *The Scent of Orchids*, Amsterdam: Elsevier Sci Publ BV (1993) p 44
17. AF Thomas and F Bassols, Occurrence of pyridines and other bases in orange oil, *J Agric Food Chem* **40** 2236-2243 (1992)
18. M Ishihara et al, Pyridine derivatives and basic components in spearmint oil (*Mentha gentilis* f. *cardiaca*) and peppermint oil (*Mentha piperita*), *J Agric Food Chem* **40** 1647-1655 (1992)
19. H Surburg et al, *Bioactive Volatile Compounds from Plants*, ACS Symposium series 525, Washington DC: American Chemical Society (1993) pp 168-186
20. B Maurer and AF Thomas, Alkaloids, bases and essential oils, *Perf & Flav* **19** 19-27 (1994)
21. BD Mookherjee et al, Fruits and flowers: Live vs dead - Which do we want?, in *Flavors and Fragrances: A World Perspective, Proceedings of the 10th Int Congr of Essent Oils*, BM Lawrence, BD Mookherjee and BJ Willis, eds, Amsterdam: Elsevier Sci Publ BV (1988) pp 415-424
22. S Sonnenberg et al, Pyridines as constituents of essential oils and plant extracts. Possible pathways by model reactions of reactive carbonyl compounds with ammonia. Poster no. 52 from the 24th Int Symp on Essential Oils, Berlin (1993)