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Leaf essential oils of three different varieties of Citrus *reticulata* Blanco growing in Egypt

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Because of their economic importance, citrus essential oils are subject to vast investigation. Most of the reported work deals mainly with the study of the fruit oil, including peel and juice aroma. Recently, however, interest has shifted from the volatile oil of the fruit to new sources of volatile oils in the citrus trees. Work has been done on the essential oils extracted from citrus leaves and blossoms.

Kesterson and coworkers and Attaway and coworkers studied the leaf oils of different citrus species by means of gas chromatography.[1,2] Attaway found that leaf oils of *Citrus reticulata* Blanco, varieties "Murcott" and "Dancy" tangerine, were found to consist mainly of hydrocarbons together with linalool as a major oxygenated constituent. Many of the identified constituents commonly occurred in both oils, and certain others were identified in one and not in the other. Of these, thymol was stated to occur in a moderate concentration in the Dancy and terpinen-4-ol in the Murcott. Balbaa and coworkers analyzed the peel and flower oils of *C. reticulata* Blanco (Balady variety) and stated that the oils were composed mainly of terpene hydrocarbons and some oxygenated constituents, of which some alcohols and methyl anthranilate were prominent.[3,4] The same authors mentioned that the steam distilled leaf oils of bitter orange, sweet orange, and lime contain a high concentration of hydrocarbons and are characterized by the presence of methyl anthranilate.[5]

This work was undertaken for the purpose of exploring new sources of citrus oils which may be useful as food flavors and in the fragrance industry. The study deals with the analysis of leaf essential oils of three different varieties of *C. reticulata* Blanco which flourish in Egypt.

Experimental

Materials

The mature leaves of *C. reticulata* Blanco, Balady, Clemantin, and Suntara varieties were obtained from plants grown in the Experimental Farm, Horticultural Department, Faculty of Agriculture, Ein Shams University, Shopra El Khima, Egypt. The leaf samples were authenticated in the same department.

The essential oils were prepared by steam distillation of the fresh leaves collected during the summer of 1977 (June).

Apparatus

Gas chromatograph—Pye Unicam, series 104, model 64, Cambridge, England; gas chromatograph coupled with mass spectrometer—Varian Mat GC/MS system Mat III; infrared spectrophotometer—Spektromom, 2000, MOM Budapest, Hungary.

Methods

Thin layer chromatography (TLC)

Using silica gel GF 254 layers (0.3 mm thick) and hexane-ethyl acetate (90:10) for development (15 to 17 cm), the oils of the three different varieties were analyzed with different reference isolates. Location was done by inspecting the developed plate under UV (366 mμ), spraying with vanillin reagent, and keeping it in a hot air oven at 100°C for 5 minutes. To locate anthranilate esters, the plate was sprayed first with Dragendorff's reagent before location with the vanillin reagent. The esters acquire an intense orange color.

Gas liquid chromatography (GLC) and gas chromatography coupled with mass spectrometry (GC/MS)

The entire oil of each variety under investigation was analyzed by GLC and GC/MS under the following conditions:

Column: GLC—1.5 m x 0.4 mm coiled glass columns packed with 5% XE 60 on Chromosorb W 80/100 mes; GC/MS—3 m x 3 mm coiled inox column packed with 5% Carbowax 20 M on varaport 80/100 mesh.

Carrier gas: GLC—nitrogen flow rate, 55 ml/min; GC/MS—helium flow rate, 15 ml/min.

Temperature of column: GLC—70°C kept isothermal for 5 min and then increased by 12°C/min till 200°C; GC/MS—from 60° to 200°C with 4°C/min increase.

Injector port temperature: 240°C; detector oven temperature: 265°C in both cases.

Attenuation: GLC—20 x 10^{2}; GC/MS—

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variable according to the peak area of each compound.

Sample size (10% v/v solution of each sample in methanol): GLC—0.2 μl; GC/MS—5 μl.

Chart speed: 1 cm/min in both techniques.

In the case of the coupled technique, approximately one third of the columns effluent was diverted to the mass spectrometer by a special device, while the remaining effluent was analyzed with a flame ionization detector.

Infrared spectroscopy (IR)

A thin film of each oil under investigation was made between two sodium chloride discs to obtain the respective IR spectrum.

Identification of constituents

Tentative identification of the oil constituents was made by comparing the mass spectra with published data and confirming by TLC, retention times, and enrichment with authentics. The IR spectrum of each oil points to the chemical groups the main constituents.

Results and discussion

The leaves of the three investigated varieties were found comparatively rich in essential oil content, reaching up to 1.2% in the fresh samples. The oil of the Balady variety solidified in the consenser during its distillation, owing to the presence of a very high percentage of dimethyl anthranilate.

TLC, GLC, and GC/MS of the oil samples gave good information about their constitution. On inspecting the developed chromatograms under UV at 366 nm, the Balady oil (B.oil) showed a prominent violet spot corresponding to dimethyl anthranilate which gave an intense orange color with Dragendorff's reagent. This compound was detected in minor concentration in Clemantin oil (C.oil) and was lacking in Suntara oil (S.oil). The GLC analysis using two different liquid phases, the polar Carbowax 20 M and the non-polar XE 60, showed that the different samples were resolved into many peaks (up to about 25 in number), many of which were analyzed by mass spectrometry.

Using the different techniques for identification, the following constituents could be tentatively identified in the essential oils of the three different varieties of *C. reticulata* Blanco (see Table I).

Table I. Identified constituents, main fragments of their spectra, and their relative percentage in the oils of the three investigated varieties of Citrus reticulata Blanco

No.	M^+	Mass spectra m/e	Compound	Relative % B.	C.	S.	Ref.
1.	136	93, 121, 79, 80, 68	alpha-pinene	3	3	5	7
2.	136	93, 41, 69, 121, 136	beta-pinene	2	traces		7
3.	136	93, 77, 79, 80, 69, 121	sabinene	---	10	20	7
4.	136	93, 79, 80, 121, 105	delta-3-carene	---	5	10	7
5.	136	41, 69, 93, 105, 121	myrcene	1	2	---	7
6.	136	68, 93, 107, 121, 79	limonene	6	3	1	7
7.	136	93, 80, 121, 105	beta-ocimene	---	5	---	7
8.	136	93, 77, 121, 80	alpha-phellandrene	12	---	---	7
9.	134	119, 134, 91	p-cymene	4	2	---	7
10.	136	93, 79, 107, 121, 136	alpha-fenchene	---	1	---	7
11.	154	93, 71, 41, 55, 80	linalool	---	30	35	8
12.	196	93, 71, 41, 55, 196	linalyl acetate	---	traces		---
13.	154	71, 93, 111, 43, 86	terpinen-4-ol	---	20	25	8
14.	154	59, 93, 81, 121, 68	alpha-terpineol	---	3	---	8
15.	154	69, 55, 81, 69, 81	isopullegol	---	2	---	8
16.	165	133, 134, 105, 106, 77, 78	dimethyl anthranilate	60	5	---	---
17.	151	119, 91, 120, 92	methyl anthranilate	3	---	---	---
18.	204	69, 93, 133, 161	caryophyllene	---	traces		9
19.	204	69, 93, 120, 133, 55, 81, 161, 189	beta-farnesene	traces		---	9
20.	204	93, 80, 121, 67, 136, 55	humulene	---	+	---	9

m/e are arranged according to their intensities in the respective spectrum; B. = Balady oil; C. = Clemantin oil; and S. = Suntara oil.

Terpene hydrocarbons constitute up to 28%, 31%, and 35% of the total makeup of the B., C., and S.oils respectively. The members which could be identified are α- and β-pinenes, sabinene, Δ3-carene, myrcene, limonene, β-ocimene, α-phellandrene, p.cymene, and α-fenchene. α-Pinene and limonene are the only hydrocarbons which occurred in the three in-

vestigated oils. β-Pinene and α-phellandrene were detected only in B.oil, β-ocimene and α-fenchene in C.oil, while S.oil lacked these constituents. Sabinene and Δ3-carene were located in C. and S.oils, myrcene and p.cymene in B. and C.oils only. α-Phellandrene constituted the most dominant hydrocarbon of B.oil, while sabinene and Δ3-carene represent the major part of the hydrocarbons of the C. and S.oils. In the S.oil, the concentrations of these constituents are double those in the C.oil. Also, the concentrations of limonene and p.cymene in the B.oil are double those in the C.oil. Myrcene, α-fenchene, and limonene were detected as minor constituents (1% each) in the B., C., and S.oils respectively.

Sesquiterpene hydrocarbons were located in the oils of the three varieties in trace amounts. Caryophyllene was identified in C. and S.oils, farnesene in B. and S.oils, and humulene in C.oil only. Identification of these sesquiterpenes was based on mass spectral analysis and comparison with published data.

Oxygenated constituents constitute from 60% to 65% of the investigated oils. B.oil was found very rich in dimethyl anthranilate (up to 60%). These findings agree with Guenther on mandarin leaf oil[6] and differ from Attaway and coworkers' findings on Dancy and Murcott tangerine varieties.[2] The presence of this compound as a major constituent lets the oil solidify in the condenser during its distillation and fluoresce very strongly even in daylight. Reviewing the current literature, no explanation of the fragmentation pattern of this compound was found. The parent peak occurred at m/e 165 ($M^{\cdot}$), the base peak at m/e 133 and 134 (M-CH_3OH and M-OCH_3), and characteristic peaks at m/e 105, 106, 77, and 78. The proposed pattern is shown in figure 1.

Methyl anthranilate, which was located in the peel and flower oils of mandarin, was identified as a minor constituent in the leaf oil of the Balady variety. Its mass spectrum showed the parent peak at m/e 151 (M^+), the base peak at m/e 119 (M-32) due to the loss of CH_3OH, and characteristic peaks at m/e 120 (M-OCH_3) and m/e 91 (M-CH_3OH-CO).

Linalool and terpinen-4-ol, on the other hand, represent the highest makeup of the C. and S.oils. α-Terpineol, linalyl acetate, and isopullegol were identified in C.oil only.

The presence of linalool and terpinen-4-ol as major constituents in S.oil is in accordance with Attaway and coworkers' findings in the Murcott leaf oil.[2] The investigated oils, on the other hand, lacked thymol and thymyl methyl ether, two prominent constituents in the Dancy leaf oil mentioned by the same authors.

The IR spectra of the three samples showed that the B.oil is of aromatic nature and composed mainly of esters as shown by the prominent absorption bands at 1520, 1560, and 1580 cm^{-1} and

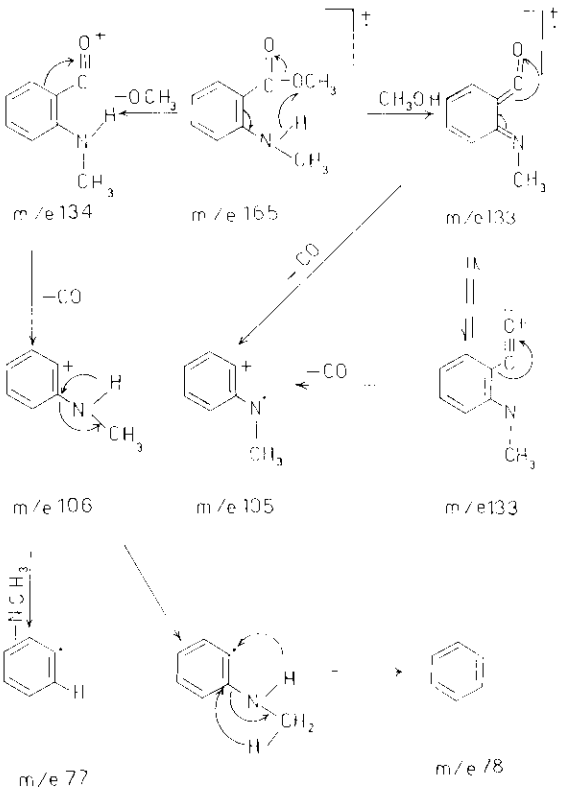

Figure 1. Proposed fragmentation pattern of dimethyl-anthranilate.

the very strong band of carbonyls at 1680 cm^{-1}. Those of C. and S. varieties showed a strong absorption band at 3450-3500 cm^{-1} which is due to the prominent occurrence of hydroxylic compounds.

In conclusion, the occurrence of N. methyl anthranilic acid methyl ester (dimenthyl anthranilate) in the Balady oil on mandarin in a high concentration imparts a delicate and fine fragrant aroma to the oil which makes it most suitable as a flavoring agent. The Clemantin and Suntara mandarin oils, containing high concentrations of linalool and terpinen-4-ol, could be of value in the fragrance and cosmetic industries.

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