

Isolation and Characterization of Terpenes from *Citrus reticulata* Blanco and Their Comparative Distribution among Other Citrus Species

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By means of gas chromatographic retentions and infrared spectroscopy of isolated fractions, the monoterpenes of tangerine and mandarin oils have been shown to include α -pinene, β -pinene, camphene, myrcene, Δ^3 -carene, α -phellandrene, β -phellandrene, α -terpinene, γ -terpinene, D-limonene, and the aro-

matic hydrocarbon *p*-cymene. Three of these terpenes, Δ^3 -carene, α -phellandrene, and β -phellandrene are reported here for the first time to be constituents of these oils. Qualitative and quantitative comparison is made between the terpene hydrocarbon content of these two oils and other citrus oils.

While there is a large body of information concerning the physicochemical properties of mandarin (*Citrus reticulata* Blanco var. Mandarin) and tangerine (*Citrus reticulata* Blanco var. Tangerine) peel oils, relatively little is known concerning their chemical composition. The hydrocarbon fraction of both Florida and Italian mandarin oils constitutes about 96% of the total oil (Kesterson and Hendrickson, 1953; Kefford, 1959), and the identities of 10 of these terpenes have been reported in the literature (Arnaldo and Cartoni, 1958; Di Giacomo *et al.*, 1963; Kuglar and Kovates, 1963). These are α - and β -pinene, camphene, sabinene, myrcene, γ -phellandrene, D-limonene, γ -terpinene, terpinolene, and the aromatic hydrocarbon *p*-cymene. The compound γ -phellandrene is not mentioned in any of the standard reference works on terpenes; it is therefore concluded that the report of the presence of this compound is in error.

Studies on Florida tangerine oils (Nelson, 1934; Calvarano, 1958; Hunter and Brogden, 1965) indicate nine of the above 10 terpenes to be present, with sabinene being unreported.

Recent research by Attaway *et al.* (1967) revealed that β -ocimene, α -thujene, and sabinene were found in Dancy tangerine peel oils.

This research was directed toward a re-examination of these results and attempts to clarify the true nature of the terpene hydrocarbon fractions of both types of oils.

EXPERIMENTAL

Apparatus. The gas chromatographic equipment used in this study was an Aerograph Model A-90-C equipped with an improved 4-cell katharometer detector, and an F & M Model 609 with a flame ionization detector. All columns were constructed of 1/4-inch stainless steel tubing 10 feet in length unless otherwise noted. For readout, a 1-mv. potentiometric recorder was employed with a chart speed of 20 inches per hour.

Column *A* was 16 feet in length, packed with a mixture of 30% (w./w.) LAC-2-R446 on acid-washed firebrick 100- to 120-mesh. Column temperature was maintained at 120° C. Injector temperature was 170° C.; detector

temperature was 120° C. Helium flow rate was 45 ml. per minute. Sample sizes varied between 7 and 15 μ l.

Column *B* was packed with 20% (w./w.) LAC-2-R446 on acid-washed Sil-O-Cel C-22 40- to 60-mesh. Column temperature was 90° C.; injector temperature was 140° C.; and detector temperature was 90° C. Helium flow rate was 70 ml. per minute. Sample size was 2 μ l.

Column *C* was packed with 10% (w./w.) Ucon 50-HB-2000 on Chromosorb W 42- to 60-mesh. Column temperature was 50° C.; injector temperature was 110° C.; and detector temperature was 130° C. Nitrogen and hydrogen flow rates were each 45 ml. per minute.; and air flow rate was 450 ml. per minute.

Column *D* was packed with 10% (w./w.) SE-30 on Chromosorb W 42- to 60-mesh. Column temperature was 90° C.; injector temperature was 150° C.; and detector temperature was 150° C. Nitrogen and hydrogen flow rates were each 45 ml. per minute. Air flow rate was 380 ml. per minute.

Column *E* was packed with 10% (w./w.) DC-200 silicone oil on Chromosorb W 42- to 60-mesh. Column temperature was 110° C.; injector temperature was 150° C.; and detector temperature was 150° C. Nitrogen and hydrogen flow rates were each 45 ml. per minute. Air flow rate was 380 ml. per minute.

Sample size for columns *C*, *D*, and *E* was 0.01 μ l.

Infrared spectra were determined using a Beckman IR-5 spectrophotometer.

Samples. Nine samples were employed in this study. A sample of pure spirits of gum turpentine was included for comparison as this material contains many of the terpene hydrocarbons present in citrus oils. Sample 1. Cold-pressed tangerine oil (Florida Citrus Canners Cooperative, Lake Wales, Fla.). Sample 2. Cold-pressed tangerine oil, Dancy variety (Sunkist Growers, Inc., Ontario, Calif.). Sample 3. Cold-pressed tangerine oil, blended varieties (Sunkist Growers, Inc., Ontario, Calif.). Sample 4. Oil mandarin Sanderson's (Dodge and Olcott, Inc., New York, N.Y.). Sample 5. Pure spirits of gum turpentine (Turpentine and Rosin Factors of Texas, Inc., El Paso, Tex.). Sample 6. Temple orange oil (Florida Citrus Canners Cooperative, Lake Wales, Fla.). Sample 7. Cold-pressed Sicilian lemon oil (Sunkist Growers, Inc., Ontario, Calif.). Sample 8. Cold-pressed lime oil (Florida Citrus Canners Cooperative, Lake Wales,

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Fla.). Sample 9. Cold-pressed grapefruit oil (Florida Citrus Canners Cooperative, Lake Wales, Fla.). The physical properties of the oils were determined and are presented in Table I.

Procedures. All samples were deterpenated by a modified Kirchner procedure which has been described in detail (Clark and Bernhard, 1960; Kirchner and Miller, 1952).

Determination of the evaporation residue was made as described by Guenther (1949).

Fractions eluted from the gas chromatograph were collected in glass tubes 0.1-mm. I.D. and 30 cm. long bent in a double U shape to inhibit aerosol formation and facilitate condensation. During the collections, the lower two thirds of the tube was immersed in a mixture of dry ice-ethanol contained in a Dewar flask (-75° to -77° C.). The collected fractions were dissolved in Spectrograde carbon tetrachloride and introduced into sodium chloride cavity cells of 0.1-mm. path length for determination of infrared spectra.

RESULTS AND DISCUSSION

Prior to the investigations described herein, each of the samples was examined using a gas chromatograph equipped with a hydrogen flame ionization detector. Five different stationary liquid phases were employed in this preliminary study (columns *A* through *E*) to allow the best estimate to be made of the nature and identity of the compounds present in these various oils. Tentative assignment of the various peak identities was made by determination of the corrected retention volumes (Ambrose *et al.*, 1958) of known compounds and comparison with those for the unknown peaks. In this manner, a tentative identification of a large number of the components present was achieved. Confirmation of these results was further enhanced by cross-comparison of retention volumes with those obtained using other stationary liquid phases. In addition, an enrichment procedure in which known compounds were added, one at a time, to fresh portions of the terpene hydrocarbon fractions was employed. These enriched fractions then were subjected to re-examination by gas chromatography. While these procedures presented an excellent over-all view of the compositional patterns of the samples, they did not provide a rigorous identification of each of the components. As a considerable body of such information already exists in the literature (Clark and Bernhard, 1960; Bernhard, 1960, 1961; Hunter and

Brogden, 1965), these data were not included in this publication.

A chromatographic column of moderate length and relatively high efficiency was constructed which would permit collection of eluted fractions and subjection of these fractions to further physical analyses. For this reason, an instrument equipped with a katharometer detector was used for the investigation reported herein.

Examination of Table I reveals that the physical properties of the three tangerine oils examined were markedly similar in most respects, with the exception that sample 1, cold-pressed Florida tangerine oil, had a slightly higher evaporation residue. In contrast, the cold-pressed mandarin oil examined was of a lighter color, had a greater specific gravity, and lower optical rotation and evaporation residue. The tangerine oils were more closely akin to the sample of Temple orange oil in regard to their physical properties. The lime and grapefruit oils included in this comparison showed a high evaporation residue which undoubtedly was due, at least in part, to their high content of coumarin compounds (Stanley and Vannier, 1957).

The results obtained from examination of the terpene fraction of cold-pressed Florida tangerine oil were in agreement with those previously described in the literature (Table II), except for the presence of terpinolene which could not be detected. It was experimentally impossible to distinguish between β -pinene, sabinene, and myrcene using the chromatographic conditions described for column *A*. To separate these three terpenes, column *B* was employed and the fractions were collected from this column for subsequent identification. Column *B* did not yield suitable resolution of the remaining terpenes, and thus was not employed in subsequent surveys. The presence of sabinene, β -ocimene, or α -thujene was not detected in any of the tangerine or mandarin oil samples examined. In addition to the compounds previously described in the literature, the present studies revealed the presence of three hitherto unreported terpenes, Δ^3 -carene and α - and β -phellandrenes.

Using column *A*, examination of the other tangerine oils revealed that they were qualitatively similar to the Florida tangerine oil with the exceptions that the blended oil contained no detectable amounts of Δ^3 -carene and the unknown peak 26.

Mandarin oil was also qualitatively similar to the tangerine oils, except that the unknown peak 26 was absent.

Table I. Physical Properties of Samples

Sample	Color	Specific Gravity, 25°/25°	n_D^{20} ^c	$[\alpha]_D^{25}$ ^d	Evaporation Residue, %
C. P. ^a Florida tangerine oil	Deep orange	0.8474	1.4731	+92.4°	4.140
C. P. Dancy tangerine oil	Deep orange	0.8488	1.4720	+92.1°	3.618
C. P. tangerine oil (blended varieties)	Deep orange	0.8492	1.4725	+91.8°	3.931
C. P. mandarin oil	Light yellow	0.8556	1.4737	+71.5°	2.555
Pure spirits of gum turpentine	Colorless	0.8678	1.4694
C. P. Temple orange oil	Reddish orange	0.8440	1.4733	+97.5°	3.35
C. P. Sicilian lemon oil	Light yellow	0.8510	1.4750	+62.1°	...
C. P. lime oil	Greenish yellow	0.8860	1.4855	+41.3°	13.00
C. P. grapefruit oil	Yellow	0.8520	1.4752	+92.1°	7.02

^a Cold-pressed.

Table II. Retention Data for Terpenes

Compound	Peak	Known	C. P. Florida Tangerine Oil	C. P. Dancy Tangerine Oil	C. P. Tangerine Oil (Blended Varieties)	C. P. Mandarin Oil	Turpentine	C. P. Temple Orange Oil	C. P. Sicilian Lemon Oil	C. P. Lime Oil	C. P. Grapefruit Oil
Solvent	1-16
α -Pinene	17	0.488	0.488	0.487	0.487	0.488	0.481	0.490	0.490	0.491	0.489
Camphene	18	0.636	0.637	0.637	0.638	0.638	0.621	0.632	0.640	0.639	0.641
β -Pinene	19	0.731	0.747	0.747	0.746	0.746	0.739	0.739	0.744	0.744	...
Myrcene	19	0.745	0.747	0.747	0.746	0.746	0.739	0.739	0.744	0.744	0.752
Δ^3 -Carene	20	0.811	0.811	0.812	...	0.811	0.811	0.824
α -Phellandrene	21	0.881	0.879	0.879	0.880	0.882	0.880	0.874	0.882	0.881	0.886
α -Terpinene	22	0.916	0.919	0.918	0.918	0.912	0.921	0.923	0.921	0.922	0.923
D-Limonene	23	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
β -Phellandrene	24	1.097	1.101	1.099	1.099	1.097	1.096	1.103	1.106	1.104	1.105
γ -Terpinene	25	1.222	1.218	1.220	1.220	1.218	...	1.197	1.222	1.223	1.227
Unknown	26	...	1.305	1.305	1.313	1.312
<i>p</i> -Cymene	27	1.434	1.437	1.432	1.435	1.433	...	1.442	1.428	1.423	1.423

Data presented as relative corrected retention volumes. Retentions relative to D-limonene. For conditions of operation, see text, column A.

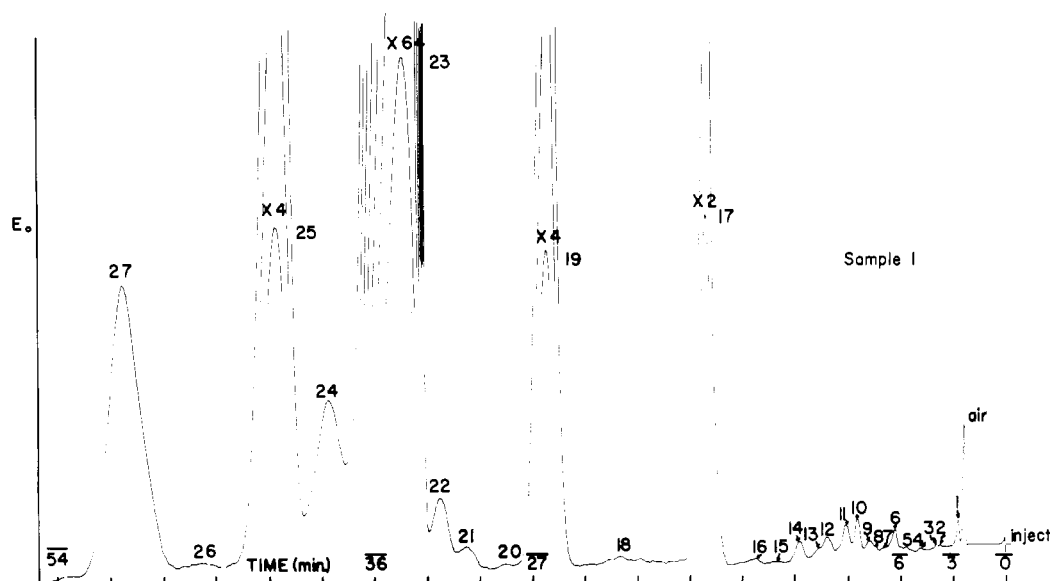


Figure 1. Gas chromatogram of terpene hydrocarbon fraction of cold-pressed Florida tangerine oil using column A (see text)

Peak identities presented in Table II

Table III. Average Per Cent Composition of Samples Used in This Study

Terpene	C. P. Florida Tangerine Oil	C. P. Dancy Tangerine Oil	C. P. Tangerine Oil (Blended Varieties)	C. P. Mandarin Oil	Turpentine	C. P. Temple Orange Oil	C. P. Sicilian Lemon Oil	C. P. Lime Oil	C. P. Grapefruit Oil
α -Pinene	0.62	0.99	0.76	1.53	86.0	12.9	1.30	1.19	1.61
Camphene	0.014	0.016	0.020	0.14	1.13	1.20	0.493	0.49	0.170
β -Pinene,									
Myrcene	1.72	2.06	1.69	3.01	3.92	15.9	8.02	8.34	1.41
Δ^3 -Carene	0.004	0.004	...	0.056	4.92	0.141
α -Phellandrene	0.026	0.054	0.063	0.59	0.106	0.188	0.221	0.220	0.843
α -Terpinene	0.089	0.080	0.094	0.35	0.114	0.201	0.672	0.761	0.145
D-Limonene	93.6	92.7	92.8	78.5	1.56	67.0	75.6	64.4	95.0
β -Phellandrene	0.386	0.392	0.433	0.512	1.18	0.94	0.776	0.899	0.662
γ -Terpinene	2.72	3.44	3.75	13.4	0.077	0.143	11.8	21.7	0.494
<i>p</i> -Cymene	0.823	0.350	0.432	1.91	0.708	0.382	1.08	1.93	0.375

Based on 3 replicate determinations. Values derived from integration of areas under appropriate peaks.

These results were also in agreement with the literature except for the presence of terpinolene, which was not detectable. Once again, the three terpenes Δ^3 -carene, and α - and β -phellandrene were found, and are reported here for the first time as constituents of mandarin oil.

The presence of all of the terpenes listed in Table II was confirmed by comparison of the spectra of known terpenes with those of the trapped fractions eluted from the gas chromatograph with but one exception. Sufficient amounts of peak 18 (camphene) could not be obtained from the tangerine or mandarin oil to obtain unequivocal spectral comparisons.

Figure 1 indicates a typical chromatogram of the terpene hydrocarbon fraction obtained from Florida tangerine oil using column A. Chromatograms of the other tangerine oils and the mandarin oil were qualitatively similar in nature.

There were some striking quantitative differences between the three tangerine oils and the mandarin oil. α -Pinene concentrations varied considerably among the four oils (Table III), being large in mandarin oil as compared with the tangerine oils. The camphene concentration was similar in the three tangerine oils but seven to 10 times as great in the mandarin oil. Further examination of Table III reveals additional differences. Two striking differences between tangerine and mandarin oil are shown by the relative concentrations of D-limonene and γ -terpinene. D-Limonene concentration is very much lower in mandarin oil than in tangerine oil, and γ -terpinene concentration is about four times greater.

In pointing out the quantitative differences between the oils, one must note that Attaway (1967) found considerable variation in the content of the individual terpenes with maturity. The oils used in the present study were blended oils which presumably would exhibit a mean value for these components (being composed of a number of individual oils of different stages of maturity). Only a small sampling of oils are presented in this study; thus these comparisons may not be entirely valid for all cases. The differences in relative terpene concentration are further reflected in the specific optical rotations of the four oils (Table I). Undoubtedly, the lower optical rotation of mandarin oil is due to the quantitative differences in both the terpene hydrocarbon content and the terpenoid content of the oil.

The relative distribution of terpene hydrocarbons among other common citrus oils (orange, lemon, lime, and

grapefruit) and turpentine is also presented in Table III. The present results suggest that all of the samples examined contained virtually the same terpenes, except that Δ^3 -carene was not found in the lime, lemon, and grapefruit oils. The sabinene or terpinolene content of the latter five oils was not followed in Table III, since no evidence for their presence in tangerine and mandarin oils was found. The samples differ markedly in their relative concentrations of these materials. Table III indicates that the terpene hydrocarbon D-limonene is the major constituent (> 60%) of the terpene hydrocarbon fraction of the citrus oils examined, while, in contrast, α -pinene is the major component of the noncitrus oil examined. Although present in the smallest amounts in citrus oils (<0.2%), Δ^3 -carene is the second largest constituent of the turpentine used in this study. Other such comparisons can also be made readily.

Ikeda *et al.* (1961) have demonstrated, in the case of lemon oils, that *p*-cymene arises from oxidation of γ -terpinene. As each of the citrus oils examined shows an appreciable concentration of both of these compounds, it seems reasonable to assume that oxidation has occurred and that this oxidative mechanism probably is common to all citrus oils. Turpentine shows no γ -terpinene content and no *p*-cymene.

LITERATURE

- Ambrose, D., Keulemans, A. I. M., Purnell, L. H., *Anal. Chem.* **30**, 1582 (1958).
Arnaldo, L., Cartoni, G. P., *Ricerca Sci.* **28**, 1192 (1958).
Attaway, J. A., Pieringer, A. P., Barabas, L. J., *Phytochemistry* **6**, 25 (1967).
Bernhard, R. A., *J. Chromatog.* **3**, 471 (1960).
Bernhard, R. A., *J. Food Sci.* **26**, 401 (1961).
Calvarano, M., *Essenze Deriv. Agrumari* **28**, 107 (1958).
Clark, J. R., Bernhard, R. A., *Food Res.* **25**, 389 (1960).
Di Giacomo, A., Rispoli, G., Tracuzzi, M. L., *Riv. Ital. Essenze-Profumi Piante Offic.-Aromi-Sapono-Cosmet.* **45**, 269 (1963).
Guenther, E., "The Essential Oils," Vol. I, pp. 256-61, Van Nostrand, New York, 1949.
Hunter, G. L. K., Brogden, W. B., *J. Food Sci.* **30**, 383 (1965).
Ikeda, R. M., Stanley, W. L., Vannier, S. H., Rolle, L. A., *Food Technol.* **15**, 379 (1961).
Kefford, J. F., *Advan. Food Res.* **9**, 334 (1959).
Kesterson, J. W., Hendrickson, R., *Univ. of Florida Agr. Expt. Sta. (Gainesville) Bull.* **521** (1953).
Kirchner, J. G., Miller, J. M., *Ind. Eng. Chem.* **44**, 318 (1952).
Kuglar, E., Kovates, E., *Helv. Chim. Acta* **46**, 1480 (1963).
Nelson, E. K., *Am. Perfumes Essent. Oil Rev.* **29**, 347 (1934).
Stanley, W. L., Vannier, S. H., *J. Assoc. Offic. Agr. Chemists* **40**, 582 (1957).

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