

subjecting known mixtures of aldimine isomers to control conditions. In this manner we established that conjugated aldimines would have been detected if 1% had been present after the original reaction. No evidence was found for further reaction between aldimines or pyrrole products and  $\alpha$ -amino acids.

The branched chain aldimines obtained from furfural and L-leucine or L-valine were remarkably resistant to isomerization and hydrolysis and they were steam distilled practically quantitatively from aqueous mixtures. In contrast to the results with L-leucine and L-valine, alanine produced ethylamine as the only steam distillable product. Consistent with this observation we found that *N*-furfurylideneethylamine (prepared from furfural and ethylamine) underwent rapid hydrolysis and decomposition during steam distillation attempts. The apparent intermediacy of a conjugated aldimine in the furfural-alanine reaction suggests that the protonation step (Figure 3) may be quite sensitive to the steric size and electronegativity of the  $\alpha$  substituent on the amino acid.

**Aroma Properties of Aldimines.** Aldimines possessed strong odors ranging from biting and unpleasant to mild and foodlike. In view of the unique aromas of aldimines derived from L-valine and L-leucine several analogs were synthesized for organoleptic evaluation (Table III). Subtle differences in structure such as the position of the imine double bond had marked effects on aroma, e.g., *N*-furfurylideneisobutylamine produced an unpleasant aroma under conditions where its isomer smelled more chocolate-like.

We concluded that stable aliphatic aldimines may be

playing an important role as isoelectronic analogs of aldehydes in browning reaction flavors. The importance of corresponding cyclic aldimines such as 1-pyrroline (Yoshihawa *et al.*, 1965) and 2-acetyltetrahydropyridines (Buechi and Wuest, 1971) in bread flavor and of trimethyl-3-oxazoline in meat aroma (Chang *et al.*, 1968) has already been well established.

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## Quantitative and Qualitative Analysis of Tangerine Peel Oil

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Seventeen major volatile flavor constituents of cold-pressed tangerine oil were quantitatively and qualitatively analyzed by a gas-liquid chromatograph (glc) with a computing integrator. Quantitative corrections were made for glc detector response factors and high boiling material which would not be eluted from the glc column during the analysis period. The qualitative analy-

sis was extended to include carbonyl constituents, the only group of volatile flavor compounds that had not been systematically investigated in tangerine oil.  $\gamma$ -Elemene is reported as a new citrus component and  $\alpha$ - and  $\beta$ -sinensal and thymol methyl ether are reported as new tangerine oil components.

Since cold-pressed citrus oil makes a major contribution to citrus product flavor (Stanley, 1962), knowledge of oil composition is essential to understanding flavor. Individual constituents and their respective quantities are important to desirable flavor and aroma of citrus oils (Nursten and Williams, 1967).

Earlier studies on citrus oils have reported many qualitative analyses (Nursten and Williams, 1967) but relatively few quantitative analyses (Ziegler, 1971). Our laboratory has identified volatile hydrocarbons and alcohols from tangerine, orange, and grapefruit oils (Hunter and Brogden, 1965; Hunter and Moshonas, 1965, 1966). Similarities in these fractions suggested that the carbonyl fraction has the greatest impact on flavor and aroma characterizing each citrus fruit (Moshonas, 1971). Orange and grapefruit carbonyl fractions have been systematically analyzed

(Moshonas, 1971; Moshonas and Lund, 1969). Some mandarin (tangerine) oil carbonyls have been reported (Di Giacomo and Calvarano, 1970), but this fraction has not been analyzed systematically. The relatively little quantitative analytical information available on citrus oil components usually involves either groups of compounds (Stanley, 1962) or glc area percent for individual components where glc response factors or percent noneluting (high-boiling) material were not determined (Bernhard, 1960; Kugler and Kovats, 1963; Kita *et al.*, 1969; Ziegler, 1971). Stanley, in 1962, determined quantities of a few individual citrus oil components, and Shaw *et al.* in 1971, reported weight percent of the 12 main components in Persian lime oil.

The present study reports both qualitative and quantitative analysis of tangerine oil. Qualitative composition of the important flavor fraction, the carbonyls, was determined. Quantitative glc analysis of the 17 major tangerine oil components afforded their weight percentages after glc response factors and percent of high-boiling components

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Table I. Quantitative Analytical Data for Tangerine Oil

Compound	Synthetic mixture			Tangerine oil		Means of identification
	Tangerine oil, glc area % <sup>c,d</sup>	Known wt %	Area % <sup>c,d</sup>	Calcd wt %	Corrected wt %	
$\alpha$ -Pinene	1.254	1.017	0.690	0.850	0.814	ir, ms, rt
Myrcene	2.757	2.126	2.487	2.356	2.257	ir, ms, rt
Limonene	90.840	92.490	91.142	92.212	88.339	ir, ms, rt
$\gamma$ -Terpinene	2.859	2.612	3.204	2.330	2.232	ir, ms, rt
Linalool	0.996	1.146	1.259	0.906	0.868	ir, ms, rt
Citronellal	0.090	0.090	0.060	0.135	0.129	ir, ms, rt
Decanal	0.222	0.260	0.334	0.172	0.165	ir, ms, rt
$\alpha$ -Terpineol	0.111	0.100	0.166	0.067	0.064	ir, ms, rt
Thymol methyl ether <sup>a</sup>	0.130	0.086	0.113	0.099	0.095	ir, ms, rt
Geranial	0.059	0.070	0.055	0.075	0.072	ir, ms, rt
Perillaldehyde	0.088	0.098	0.082	0.105	0.101	ir, ms, rt
Thymol	0.035	0.044	0.036	0.043	0.041	ir, ms, rt
$\gamma$ -Elemene <sup>a</sup>	0.103			0.161 <sup>b</sup>	0.154	ir, rt
$\beta$ -Elemene	0.048			0.075 <sup>b</sup>	0.072	ir, rt
$\Delta$ -Elemene	0.068	0.050	0.032	0.106	0.101	ir
Elemol	Trace	Trace	Trace	Trace	Trace	ir, rt
$\alpha$ -Sinensal <sup>a</sup>	0.101	0.260	0.139	0.190	0.182	ir, rt

<sup>a</sup> Reported for the first time in tangerine peel oil. <sup>b</sup> Based on detector response factor obtained for its isomer,  $\gamma$ -elemene. <sup>c</sup> Mean of two determinations. <sup>d</sup> Assumes all of injection sample is glc volatile.

were determined. Three compounds new to tangerine, thymol methyl ether and  $\alpha$ - and  $\beta$ -sinensal, and one compound new to citrus,  $\gamma$ -elemene, were identified.

#### EXPERIMENTAL SECTION

**Samples.** Tangerine cold-pressed oil was obtained from a Florida plant and stored at 40°F. Authentic samples of individual compounds were either obtained from commercial sources or isolated previously from other citrus essential oils at this Laboratory (Moshonas, 1971; Moshonas and Lund, 1969), with thymol methyl ether being the only exception.

Thymol methyl ether was prepared by treating 150 mg of thymol in 1 ml of tetrahydrofuran with diazomethane in ether prepared from 2.14 g of Diazald (deBoer and Backer, 1954). After 36 hr at room temperature, the reaction mixture was concentrated and the residue separated by glc on an F&M Model 500 instrument equipped with a thermal conductivity detector and a 0.20 in. i.d.  $\times$  18 ft 20% Carbowax 20M on 60–80 mesh Gas Chrom P column held isothermally at 200° for 16 min, then raised to 225° at 30°/min. Helium flow was 100 ml/min and injection port and detector were at 260°. Thymol methyl ether eluting in 12 min and thymol in 46 min were present in a 1:5 ratio, as determined by hand integration (height  $\times$  width at half height) of the glc curve.

**Spectroscopic Measurements.** Mass spectra (ms) were obtained with a Bendix Model 3012 (TOF) mass spectrometer at 70 eV and infrared spectra (ir) were obtained on a Perkin-Elmer Infracord Model 137-A as oil films.

**Quantitative Analysis.** Weight percent of nonvolatiles was determined by distilling 430 g of tangerine oil in a rotary evaporator at 44° and 0.9 mm until most of the terpene hydrocarbons (99% limonene-*d*) were removed. The residue, 60 g, was distilled further in a 2-in. molecular still at 150° and 0.4 mm, yielding 18 g of residue that represented 4.2% of the starting cold-pressed oil.

Constituents were separated for identification by injecting 100- $\mu$ l samples of tangerine oil onto an F&M Model 810 gas chromatograph equipped with a glass-lined injection port, a thermal conductivity detector, and a 0.20 in. i.d.  $\times$  20 ft column packed with 20% UCW-98 on 60–80 mesh Gas Chrom P. The oven temperature was programmed from 90 to 140° at 1°/min, then to 225° at 4°/min with a helium flow of 75 ml/min. When separation of individual compounds was not complete, the mixture was rechromatographed on a 0.20 in. i.d.  $\times$  20 ft column packed with 20% Carbowax 20M on 60–80 mesh Gas

Chrom P. For all runs, injection temperature was 250° and detector temperature was 290°.

Quantitative glc analyses were carried out on a Hewlett-Packard Model 7620A gas chromatograph connected to a computing integrator. Samples of cold-pressed tangerine oil (100  $\mu$ l each) were injected directly onto a 0.20 in. i.d.  $\times$  20 ft column packed with 20% UCW-98 on 60–80 mesh Gas Chrom P and run under the similar glc conditions as above, with on-column sample injection being the only difference. The integrator parameters were: integration delay, 1700 sec; peak width, 20; slope sensitivity, 40; minimum area, 50; and running time, 7000 sec. The printout from the integrator gave retention time and area percent for each peak.

The column was regenerated between runs by injecting 8  $\mu$ l of the silanizing agent Silyl 8 at 225° and maintaining that temperature for 10 min. This treatment restored resolution and baseline stability by removing the nonvolatiles from the column (Shaw *et al.*, 1971).

A synthetic mixture of purified constituents ranging from 4.4 mg to 9.249 g and weighed to 0.001 mg was prepared and run under the same glc conditions as the oil in order to calculate glc response factors. The compounds were obtained from commercial sources or from cold-pressed citrus oils and purified by column and gas chromatography to at least 99% purity.

**Carbonyl Analysis.** Tangerine oil (1680 g) was distilled in a rotary evaporator at 44° and 0.9 mm to remove most (1468 g) of the terpene hydrocarbons. The 212 g of residue was distilled by a molecular still at 105° and 0.4 mm. A 63-g fraction of distillate was collected for further analysis. The short exposure time of the oil to the 105° temperature (7 to 10 sec) minimized the possibility of decomposition which could occur at higher temperature or long exposure to heat. Molecular still distillate (10 g) was separated into three fractions on a 1 in.  $\times$  17 in. 9° water-jacketed column containing 60–100 mesh Florisil deactivated by addition of 6% water. The fractions were eluted successively with 300 ml of hexane to remove the hydrocarbons (8.5 g), 400 ml of a 50–50 mixture of hexane and ethyl ether to remove the nonalcoholic oxygen-containing compounds (1.0 g), and 300 ml of ethanol to remove the alcohols (0.5 g). Although most of the carbonyl-containing compounds were in the hexane-ether fraction, all three fractions were analyzed.

Analysis of fractions was carried out on an F&M Model 810 gas chromatograph using the 20% Carbowax 20M column under the conditions discussed above with the fol-

**Table II. Carbonyl Compounds Identified in Tangerine Peel Oil**

Octanal	Perillaldehyde
Decanal	$\alpha$ -Sinensal <sup>a</sup>
Undecanal	$\beta$ -Sinensal <sup>a</sup>
Dodecanal	Carvone
Neral	Geranyl acetate
Geranial	Neryl acetate
Citronellal	Thymol methyl ether <sup>a,b</sup>

<sup>a</sup> Isolated and identified as a component of tangerine peel oil for the first time. <sup>b</sup> Isolated in the carbonyl fraction.

lowing exceptions. The oven temperature was programmed from 100 to 225° at 1°/min and the helium flow was 60 ml/min. When separation of individual compounds was not complete, the mixed components were rechromatographed on the 20% UCW-98 column.

## RESULTS AND DISCUSSION

The major volatile flavor constituents in tangerine oil were quantitatively determined by glc analysis, which included corrections for glc response factors and percent of high-boiling substances which did not appear on the glc curve. A synthetic tangerine oil mixture based on these data had a citrus-like aroma but lacked a true tangerine aroma. This observation indicates the importance to flavor of minor constituents in these complex oils. Qualitative analysis of volatile carbonyl constituents completes our systematic analysis of tangerine oil. Comparing the carbonyl constituents of tangerine with those of other citrus oils helps to understand the compounds responsible for the characteristic flavor of each citrus.

Quantitative analytical results for cold-pressed tangerine oil are listed in Table I. Column 1 lists each of the 17 constituents isolated and identified, and column 7 lists the means of identification when compared with authentic samples. The sesquiterpene hydrocarbon  $\gamma$ -elemene, which is a major constituent reported for the first time as a citrus component, was identified by matching the ir spectrum with the high-resolution ir spectrum published by Wenninger *et al.* in 1967. The fine detail and matchup of every absorption band in the spectra assured positive identification.

Column 2, Table I, lists area percent of each compound as computed by the integrator from the glc curve and is considered an approximate weight percent of each compound. From these approximate weight percent values, a synthetic mixture with known weight percent of each component was prepared (column 3) and run under identical glc conditions to afford area percent values (column 4) used to determine the response factor for each compound. These response factors were then used to calculate weight percent of each tangerine oil constituent eluted from the glc (column 5). Thus, weight percent in the synthetic mixture (column 3) divided by area percent of that compound in the synthetic mixture (column 4) times area percent in tangerine oil (column 2) affords weight percent (column 5) (Keulemans, 1959). The glc response factor of  $\gamma$ -element was used to calculate weight percent of its two isomers,  $\beta$ - and  $\Delta$ -elemene, because of their similarity and the difficulty of obtaining these sesquiterpene hydrocarbons.

Distillation results show that only 95.8% of the tangerine oil injected into the glc could be expected to elute from the column. By calculating 95.8% of each value in column 5 in Table I, corrected weight percent of each oil constituent was determined and is listed in column 6. Since the level of peel oil in tangerine juice is approximately 0.030% (Scott and Veldhuis, 1966), the percentage of each of these 17 compounds in juice can be calculated readily.

No meaningful flavor evaluation of citrus oils can be made without accurate quantitative data. This report does not provide the complete answer to tangerine oil flavor but does provide the identity and an accurate determination of the quantity of each major volatile constituent. It also points out two major constituents unique to tangerine, thymol methyl ether and  $\gamma$ -elemene, which might be used to determine tangerine oil purity. Surprisingly, one major constituent,  $\alpha$ -sinensal, with an odor threshold in parts per billion (Dougherty, 1971) had not been previously found as a tangerine flavor component. The 14 identified compounds from the systematic qualitative analysis of the carbonyl constituents of tangerine oil are listed in Table II. Particular care was taken to prevent decomposition and/or rearrangement of compounds by using procedures such as vacuum and molecular distillation and water-cooled column for liquid chromatography to keep heat contact to a minimum. Compounds identified include ten aldehydes, two esters, one ketone, and one ether (thymol methyl ether) which was listed because it is the first time this compound has been isolated from tangerine. The carbonyls are particularly important in flavor because each has a potent and distinctive odor. Even the minor ones can influence the full natural flavor and aroma of tangerine oil. The quantitative portion of this study has shown  $\alpha$ -sinensal, geranial, citronellal, decanal, and perillaldehyde to be the major carbonyls, thereby showing the remaining carbonyls listed in Table II to be minor but important flavor constituents. The thymol methyl ether also was found in large quantity. Important differences are apparent when these major tangerine carbonyls are compared with the carbonyls estimated to be in major quantity in orange oil (octanal, nonanal, decanal, citronellal, neral, geranial, carvone,  $\alpha$ - and  $\beta$ -sinensal, and nootkatone) and grapefruit oil (nootkatone, geranyl acetate, neryl acetate, octyl acetate, 1,8-*p*-menthadien-2-yl acetate, citronellyl acetate, 1,8-*p*-menthadien-9-yl acetate, decanal, and perillaldehyde), indicating that the carbonyls may contribute the largest impact on the characteristic flavor of each fruit (Moshonas, 1971; Moshonas and Lund, 1969). Thus, the major tangerine oil carbonyls contain fewer aldehydes than orange and fewer esters than grapefruit oil.

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