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THE ORIGIN OF CITRUS FLAVOR COMPONENTS—II.

IDENTIFICATION OF VOLATILE COMPONENTS FROM CITRUS BLOSSOMS*

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Abstract—Oils were extracted from the petals, stamens, and pistils of blossoms from two citrus species and from the petals and pistils of blossoms from a third by steam distillation. Analyses were performed using the techniques of gas–liquid chromatography, thin-layer chromatography, i.r. spectroscopy, and mass spectrometry. Thirty-eight compounds were isolated and identified.

INTRODUCTION

THE present series of papers is devoted to a study of volatile oils extracted from various tissues of the citrus tree, with the eventual objective of determining the mechanisms by which these compounds are formed. The first paper[1] described the analysis of oils extracted from the mature leaves of eleven citrus varieties. This paper describes the composition of oils extracted from the petals, stamens, and pistils of grapefruit and tangerine blossoms, and the petals and pistils of orange blossoms. Comparisons of the chemical compositions of the various parts are made. The chemical makeup of the pistil oils is of particular interest because the fruit is formed from this part of the blossom.

RESULTS

Comprehensive analyses of the petal oils from all three species namely; *Citrus sinensis*, *C. reticulata*, and *C. paradisi*, were carried out with a minimum of difficulty since relatively large quantities of these oils (up to 700 μl) were obtained in the petal extractions. One or more of the techniques of mass spectrometry, thin-layer chromatography, and i.r. spectroscopy were used in combination with gas–liquid chromatography to identify the specific compounds separated. Unfortunately, however, stamens from all three varieties and pistils from orange flowers yielded only minute amounts of oil, insufficient for isolating compounds for identification. In the case of orange stamen oil there was not enough for a satisfactory chromatogram, so no information on this material is presented.

Thirty-eight compounds have been positively identified in one or more of the oils studied. There were twenty-one additional peaks to which no identification has been assigned. The identified compounds are shown in Tables 1 and 2 divided on the basis of hydrocarbons and non-hydrocarbons. The number in the first column of the tables refers to the number of the peak as it appears on one or more of the gas chromatograms shown in Figs. 1–3. This facilitates correlating the chromatograms with data in the tables. Numbers along the

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[1] J. A. ATTAWAY, A. P. PIERINGER and L. J. BARABAS, *Phytochem.* 5, 141 (1966).

abscissas of the chromatograms indicate elapsed time in minutes for each chromatographic run.

Of the compounds isolated and identified, phenylacetaldehyde was found to be most reminiscent of the odor of orange blossoms.

DISCUSSION

Although (+)-limonene normally accounts for 90 per cent or more of the total makeup of citrus peel oils, a recent study of citrus leaf oils[1] showed that limonene is a comparatively minor component of the leaves. The major terpene hydrocarbon peak from the leaf oils was

TABLE 1. RELATIVE CONCENTRATIONS OF HYDROCARBONS FROM CITRUS FLOWER PARTS

Number	Compound	Orange		Grapefruit			Tangerine		
		Petal	Pistil*	Petal	Pistil	Stamen*	Petal	Pistil	Stamen*
1	α-Pinene	VS	W-M	M	W-M	--	S	M-S	VS
2	Unknown	VW	VW	VW	VW	–	VW	VW	VW
3	β-Pinene	W	W	W	W	–	S	M-S	VS
4	Sabinene	VS	VS	VS	VS	W	W	W	W
5	Myrcene	VS	VS	VS	VS	VW	VS	S	VS
6	Car-3-ene	M-S	W	VW	VW	--	VW	–	W-M
7	Unknown	VW	–	–	–	-	-	–	–
8	α-Terpinene	VS	M	VW*	VW*	--	W	W	W-M
9	(+)-Limonene	VS	VS	VS	VS	W-M	VS	VS	VS
10	β-Phellandrene	M	W-M	M	S	–	W-M	M	S
11	β-Ocimene	VS	VS	VS	VS	W	VS	VS	VS
12	γ-Terpinene	M-S	W	M-S	M	–	M	W-M	W
13	p-Cymene	W	–	W	–	–	W	W-M	–
14	Terpinolene	VS	M	W	VW*	–	M	M-S	VS
15A	Unknown	W	W-M	W	--	-	–	–	W
16	p-Isopropenyltoluene	-	–	–		–	M-S	W	W
16A	Unknown, mol. wt. = 134	–	–	–	--	-	W	W	W-M
25	β-Caryophyllene	W-M	VS	VW*	W-M	W	VW*	VW*	M
31	Valencene	VW*	M-S	VW*	–	M	--	VW*	W-M

* Indicates petal constituents identified by GLC only.

– = not present in identifiable quantities. VW = Less than 5 per cent recorder scale deflection, W = 5–20, W-M = 20–40, M = 40–60, M-S = 60–80, S = 80–100 and VS = over 100 per cent (peak off scale).

a mixture of sabinene and β-pinene. In most cases this mixture was largely sabinene although two of the eleven citrus varieties showed a somewhat greater amount of β-pinene. Ocimene also gave a large peak. In citrus blossom oils (+)-limonene was one of several major terpene hydrocarbon peaks, but did not approach the preponderant concentration found in the peel. In "Hamlin" orange petal oil (Fig. 1) the combination sabinene-β-pinene peak (peaks 3–4), mostly sabinene according to thin-layer chromatographic analysis, was the major one followed in order of concentration by (+)-limonene (9), ocimene (11), and myrcene (5). This was also shown to be true in "Hamlin" pistil oil (Fig. 1), "Marsh" grapefruit petal oil (Fig. 2), and "Marsh" grapefruit pistil oil (Fig. 2).

Orange petal and pistil oils were found to be almost identical qualitatively, and were very similar quantitatively particularly with respect to the terpene hydrocarbons. Tables 1 and 2

and Fig. 1. The major differences were found in the compounds with retention times higher than linalool (21). The petal oil contained significantly greater quantities of nerolidol (36), an unknown (38B), and farnesol (39), while the pistil oil was much richer in the sesquiterpene

TABLE 2. RELATIVE CONCENTRATIONS OF NON-HYDROCARBONS FROM CITRUS FLOWER PARTS

Number	Compound	Orange		Grapefruit			Tangerine		
		Petal	Pistil*	Petal	Pistil	Stamen*	Petal	Pistil	Stamen*
14A	Unknown, mol. wt.=150	–	–	W	–	–	VW	W	–
15	Methyl heptenone	–	–	W	W	–	–	–	–
17	*t*-Linalool oxide	–	–	M	–	–	–	–	–
17A	Unknown, mol. wt.=154	W-M	W-M	W	W	–	–	–	–
18	*c*-Linalool oxide	–	–	W-M	–	–	–	–	–
19	Citronellal	W	VW	W	W	–	–	–	–
20	Benzaldehyde	W	VW	W	–	–	W	–	–
21	Linalool	VS	VS	VS	VS	VS	VS	VS	W
21A	Octanol	M*	W	W-M*	–	–	–	–	M
21B	Unknown	W-M	W	W	W	–	–	W-M	–
21C	Unknown	–	–	–	–	–	W-M	W-M	–
22	Thymyl methyl ether	–	–	–	–	–	M-S	W-M	VS
23	Terpinen-4-ol	VS	VS	VS	W	–	W-M	W-M	W
24	Phenylacetaldehyde	M	W-M	M-S	W	VW	M-S	–	W-M
26	Neral	M	W-M	M	–	–	–	VW	–
27	α-Terpineol	M	W-M	M	W*	–	W-M	W-M	–
27A	Unknown	W	M	–	–	–	–	–	–
28	Geranial	W	W-M	M	–	W-M	–	–	W
29	Citronellol	W*	W	W-M	VW*	W	–	VW*	VW
29A	Unknown	–	VW	–	–	–	–	–	M
30	Nerol	W	W	W-M	VW*	–	VW*	VW*	M
30A	Unknown	–	VW	–	–	–	–	–	VS
33	Geraniol	VW*	VW	W-M	–	–	W*	VW*	VW
34	Benzyl alcohol	M-S	VS	–	VW*	–	M	M	VS
34A	Unknown	–	W	–	–	M	–	–	VW
35	Phenylacetonitrile	W*	M-S	W-M	–	VW	W	W	VW
35A	Unknown	W-M	W	VW	–	–	W-M	VW	VW
36	*cis*-Nerolidol	VS	VS	VS	S	VS	M	W*	S
36A	Unknown	–	W-M	–	–	–	–	–	–
36B	Unknown, mol. wt.=164	–	W-M	–	–	–	W	VW	W-M
36C	Unknown	VW	W	–	–	VS	–	–	–
37	Thymol	–	–	–	–	–	VS	VS	W
38	Methyl anthranilate	W-M	W	W	–	W	–	–	–
38A	Unknown	M	VS	W	VW	W	–	–	W
38B	Unknown	M	–	–	–	S	–	–	–
39	Farnesol	S	W-M	W	–	VS	VW	VW*	VS
40	Indole	VW	W-M	VW	–	W	W	VW	VS
41	Unknown	M	M	VW	W	W-M	W	VW	S
41A	Unknown	–	–	W	–	–	–	–	–
42	Unknown	W	W	–	VW	W	W	–	W-M

See Table 1 for interpretation of the symbols.

β-caryophyllene. The pistil oil also had more of the unknowns 15A, 27A, 36A, 36B, and 38A, as well as citronellol (29), valencene (31), benzyl alcohol (34), phenylacetonitrile (35), and indole (40). The presence of large quantities of the sesquiterpenes, β-caryophyllene and valencene, in the pistil oil was interesting.

Grapefruit petal and pistil oils were also similar qualitatively although slightly more different quantitatively than the corresponding orange blossom oils (Fig. 2). In a comparison of grapefruit petal and pistil oils some differences were evident in the middle region of the chromatogram just before and after linalool (21). The petal oil had significant quantities of linalool oxides (17 and 18), benzaldehyde (20), terpinen-4-ol (23), geranial (28), citronellol (29), geraniol (33), and phenylacetonitrile (35) which were either missing or greatly reduced in the pistil oil. However, the pistil oil had a greater quantity of β-caryophyllene as was the case in the pistil oil from the orange blossoms. In the high retention time region of the chromatogram, after nerolidol (36), the petal oil had significantly more methyl anthranilate (38) and farnesol (39) which were missing in the pistil oil. These petal constituents were found to be important contributors to the aroma.

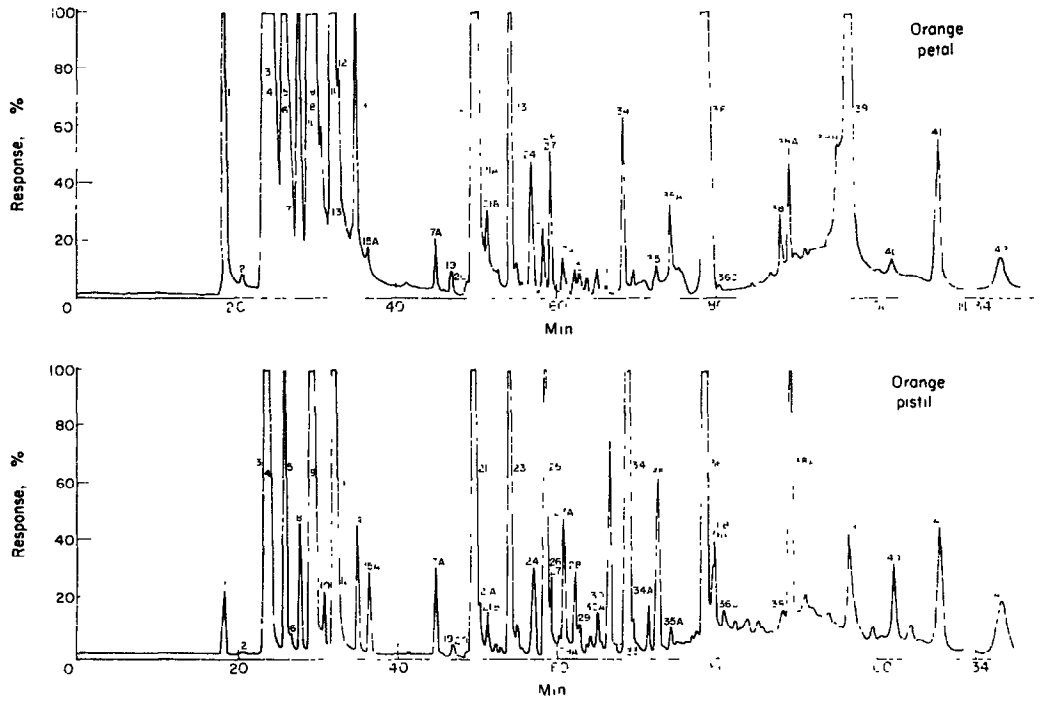

FIG. 1. GAS CHROMATOGRAMS OF ORANGE PETAL AND PISTIL OILS.

Grapefruit stamen oil, however, showed an entirely different picture (Fig. 2). Whereas the petal and pistil oils contained several terpene hydrocarbons in major concentration, there were almost no peaks in this region of the chromatograms from the stamen oil. The grapefruit stamen oil actually had only three identifiable major peaks, all of which corresponded to terpene alcohols. These were linalool (21), nerolidol (36), and farnesol (39).

The oils from "Dancy" tangerine petals, pistils, and stamens, Fig. 3, showed more similarities one to another than petal, pistil, and stamen oils from other varieties. The stamen oil possessed large quantities of the terpenes α-pinene (1), β-pinene (3), myrcene (5), (+)-limonene (9), and β-ocimene (11) which were also major components of the oils from petals and pistils. The compounds *p*-isopropenyltoluene (16), thymyl methyl ether (22), and thymol (37) were found in all three tangerine blossom oil parts but not in orange or grapefruit

blossoms. This was also true of leaf oils[1] where these three compounds were found only in the mandarin varieties. Most of the *p*-isopropenyltoluene was in the tangerine petals with little being found in the pistils and stamens. β-Pinene was present in larger concentration than sabinene which was the reverse of the findings for orange and grapefruit. Large quan-

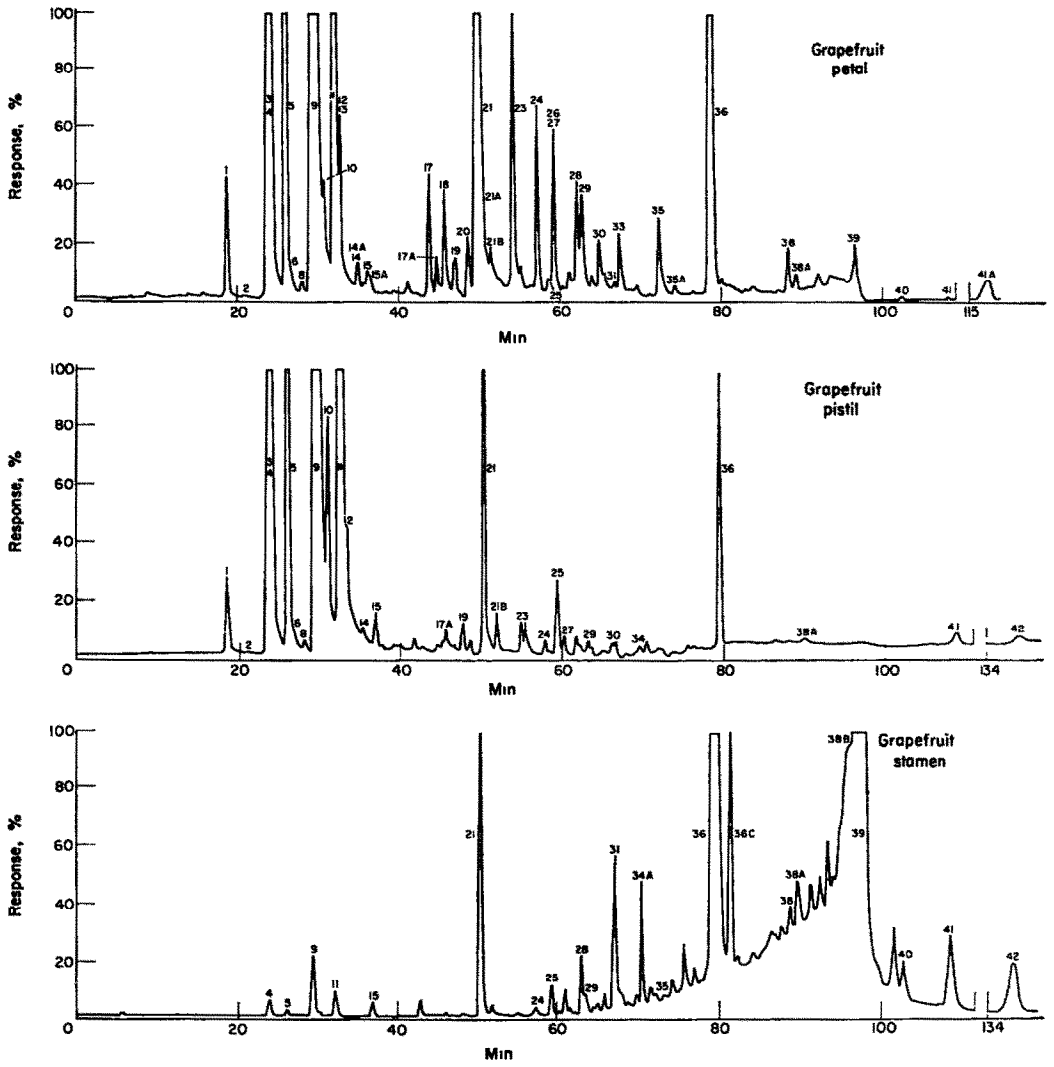

FIG. 2. GAS CHROMATOGRAMS OF GRAPEFRUIT PETAL, PISTIL AND STAMEN OILS.

tities of nerolidol and farnesol were found in the tangerine stamen, but the linalool content was unusually small.

Both tangerine and grapefruit stamen oil contained a larger number of unidentified compounds than did tangerine and grapefruit petal and pistil oils. Several of the unidentified peaks were major ones and could be identified if a sufficient quantity of the oil could be obtained.

Several aromatic compounds which had not previously been reported in either leaf or peel oils were detected in the blossom oils. Among these were benzaldehyde, phenylacetaldehyde, benzyl alcohol, phenylacetonitrile, and methyl anthranilate.

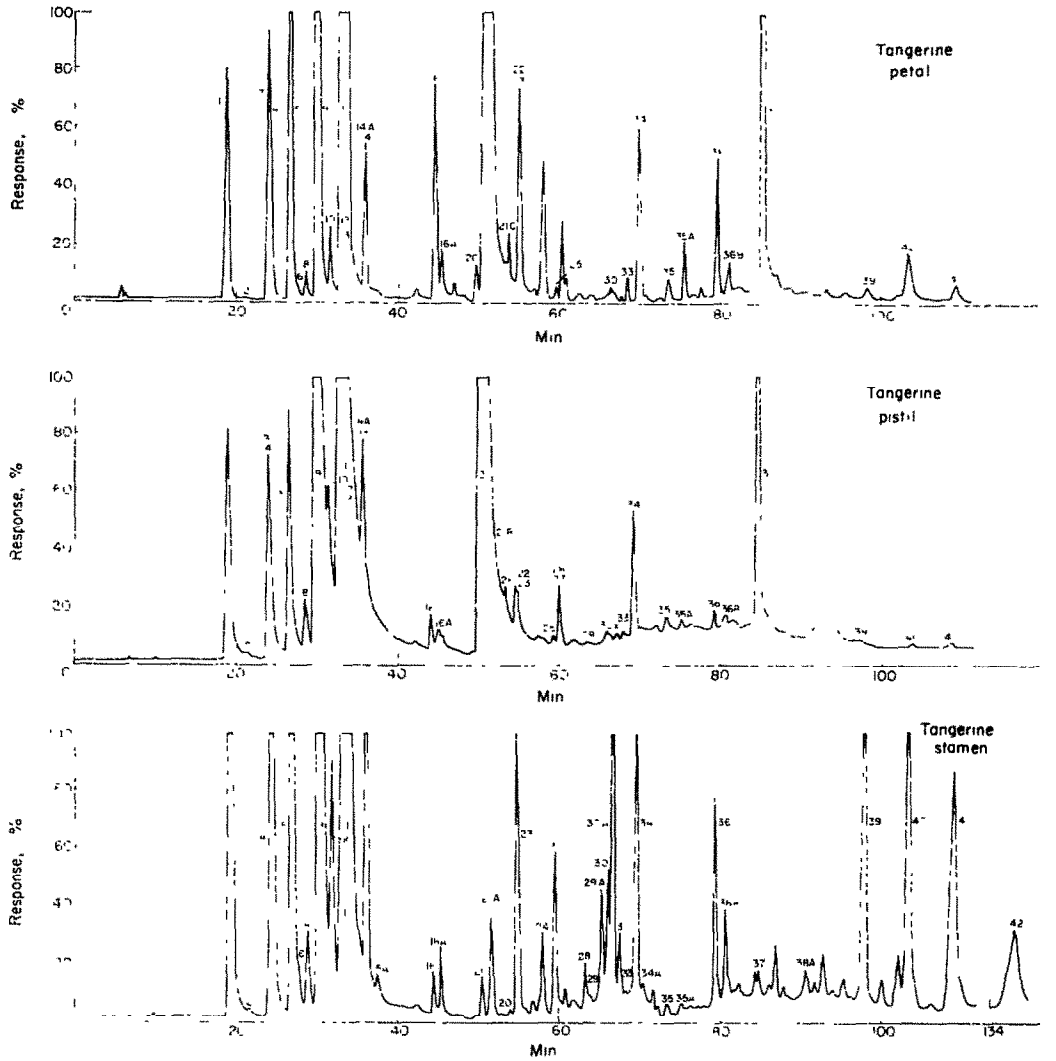

FIG. 3. GAS CHROMATOGRAMS OF TANGERINE PETAL, PISTIL AND STAMEN OILS.

EXPERIMENTAL

Preparation of Samples

Complete flowers (1500–1800 g) were collected from the cultivars "Hamlin" orange (*C. sinensis*), "Dancy" tangerine (*C. reticulata*), and "Marsh" grapefruit (*C. paradisi*). Grapefruit blossoms were approximately twice the weight of tangerine blossoms, while orange blossoms were of intermediate weight. Blossoms were harvested directly from the tree, and carefully divided into petals, stamens, and pistils on the same day they were picked.

Harvest dates were: "Hamlin" orange, 25 March 1965; "Dancy" tangerine, 31 March 1965; and "Marsh" grapefruit, 5 April 1965.

Each portion was kept in the refrigerator until the next day and then steam distilled.

Flower parts were placed in round-bottom flasks (500 ml for stamens and pistils, 4 l. for petals), deionized water added and heated to boiling. The distillate was collected in a modified Clevenger trap for the removal of the volatile oil. Grapefruit petals yielded 700 μl of oil, which was the largest amount obtained from any of the oils studied. Oil samples were stored, under nitrogen, in a freezer.

Gas–Liquid Chromatography

The analytical gas chromatograms were prepared using instrumentation and conditions designed to produce better sensitivity and resolution than that shown on the chromatograms in the previous paper[1]. Packed columns of 4% Carbowax 20 M on 60–80 mesh Gas-Chrom Z, 50 ft × $\frac{1}{8}$ in. were used on an F & M Model 810 dual column gas chromatograph equipped with dual flame ionization detectors. Nitrogen, at a flow rate of 17 ml/min and inlet pressure of 100 lb/in^2, was the carrier gas. The column temperature was held isothermal at 70° for 10 min after sample injection, and then programmed to 210° at a rate of 2°/min, and then held at 210° for a period of 70 min. The normal sample size was 0·35–0·40 μl.

Semi-preparative gas chromatography for collection of cuts to be used for thin-layer, mass spectrometry, and i.r. analyses were conducted using the F & M Model 720 instrument with a thermal conductivity detector according to the procedure described previously.[1]

Thin-layer Chromatography

Thin-layer analyses were carried out on Silica Gel G chromatoplates using the vanillin–H_2SO_4, $KMnO_4$–H_2SO_4, and dinitrophenylhydrazine spray reagents as reported earlier.[1]

Infrared Spectroscopy

Infrared spectra were determined using a Perkin–Elmer Infracord recording infrared spectrophotometer employing the capillary film technique.

Mass Spectrometry

Mass spectral analyses were run using the Bendix Time of Flight Mass Spectrometer as reported earlier[1].

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