Composition of Peel Oil from *Citrus Unshiu*.¹⁾

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α -Pinene, terpinolene, δ -elemene, α -copaene, α - and β -humulene, β -sesquiphellandrene, γ -cadinene, acetate of *p*-menthen-1-ol-9 and acetate of *p*-menthadien-1, 8(10)-ol-9 were newly identified as the volatile constituents of peel oil from *C. Unshiu*. Aroma characteristics of *C. Unshiu* are discussed on the basis of quantitative analysis.

Comparison of aroma patterns of peel oil among various citrus fruits was also carried out with head space analysis.

In the previous paper, four sesquiterpene hydrocarbons, five acetates, five carbonyls and five alcohols were reported as constituents of the aroma of peel oil from *C. Unshiu*. But unknown components remained which seemed to contribute to characteristic aroma of *C. Unshiu*. Therefore, extensive studies on structure determination of the constituents were carried out.

Isolation of various components was done by means of the combination method¹⁾ (Fractional Distillation-Column Chromatography-Gas Chromatography). Isolated pure components

were examined by IR, UV, NMR and MS.

This paper reports 1) the identification of new components since the previous work,¹⁾ 2) determination of peel oil from *C. Unshiu* and 3) comparison of aroma patterns of various citrus fruits.

EXPERIMENTAL

Material. The same peel oil as previously¹⁾ reported was used for identification and quantitative analysis.

Head space analysis was carried out with fresh peel

FIG. 1. Gas Chromatogram of Cold-pressed Peel Oil from *C. Unshiu*.

Column: 23% Ucon 50 HB-270X on Shimalite W 60/80.

Column temperature: 165°C.

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1) Part I. T. Yamanishi, A. Kobayashi, Y. Mikumo, Y. Nakasone, Y. Kita and S. Hattori, *Agr. Biol. Chem.*, **32**, 598 (1968).

from *C. Unshiu*, *C. Limonum*, *C. Junos*, *C. Natsudaidai*, *Fortunella japonica*, *C. amara* and *C. paradisi*.

Apparatus. Gas chromatography to separate the peel oil components was carried out using a Shimadzu Model 1C equipped with a 0.3 mm i. d. $\times$ 225 cm stainless U tube column packed with 23 % Ucon 50 HB 270X on Shimalite W 60/80. For head space analysis, a JEOL Model 810 equipped with a column of 0.02" $\times$ 500' stainless capillary tubing coated with silicon SF-96(50) was used.

Spectrometers for IR, NMR and MS were a Japan Spectroscopic Co. Model IR-S, a JEOL Model 100 J. N. M.-4 H and a Hitachi Ltd. RUM-6 E respectively.

Separation and analysis. Gas chromatogram of whole peel oil is shown in Fig. 1. From the chromatogram we found that low boiling components (region of earlier retention time) amounted to over 90 % by calculating the peak area percentage. Therefore, after removal by a rotary evaporator at 50°C/5 mm of the low boiling fraction, the peel oil was subjected to fractional distillation under reduced pressure, then divided into two fractions; bp 35°C~52°C/3 mm (Fraction 1), and 67°C~79°C/3 mm (Fraction 2). Gas chromatograms of these fractions are shown in Fig. 2.

Since the main substances which contributed to the characteristic aroma of *C. Unshiu* seemed to be contained in Fraction 2; 4.5 g of Fraction 2 was adsorbed on a column of 2.5 cm i. d. $\times$ 30 cm packed with silica-gel (activated at 120°C for 3 hr). Elution

FIG. 2. Gas Chromatograms of the Two Fractions of Cold-pressed Peel Oil from *C. Unshiu*.

was carried out by the same procedure as described previously and the eluate was divided in 51 portions. Each portion was subjected to gas chromatography and individual compounds were collected for identification in capillary tubes cooled with a dry ice-acetone mixture.

FIG. 3. Gas Chromatograms of Monoterpene Hydrocarbons, Sesquiterpene Hydrocarbons and Esters from *C. Unshiu*.

Column: 23 % Ucon 50 HB-270X on Shimalite W 60/80.

Column temperature: (1) 145°C; (2), (3), (4) 165°C.

- (1) Monoterpene hydrocarbons.
- (2) Sesquiterpene hydrocarbons (The second portion of effluents from the silica-gel column).
- (3) Sesquiterpene hydrocarbons (The fifth portion of effluents from the silica-gel column).
- (4) Esters.

RESULTS

Monoterpene hydrocarbons

Five monoterpene hydrocarbons were found in Fraction 1. Completely pure compounds were obtained by gas chromatography at 145°C (Fig. 3). Peak 1 was proved to be α -pinene by comparison of its IR and gas chromatographic data with those of the authentic sample. Peak 5 was confirmed as terpinolene by comparison of IR, MS and NMR spectra. The remaining three peaks were identified as myrcene (peak 2), *d*-limonene (peak 3) and *p*-cymene (peak 4) as previously reported.

Sesquiterpene hydrocarbons

Eight sesquiterpene hydrocarbons were identified. In Fig. 3, peak 9 was proved to be δ -elemene by comparison of its IR (Fig. 4) with that reported by J. A. Wenniger *et al.*²⁾ and by analysis of its NMR. Peak 11 was identified as α -copaene based on the coinci-

dence of its IR and NMR with those described by J. A. Wenniger *et al.*²⁾ and by S. Dev *et al.*³⁾ Peak 26 was proved to be a mixture of

FIG. 4. Infrared Spectra of Peak 9 and Peak 32.

(1) Peak 9 (δ -Elemene), (2) Peak 32 (β -Sesquiphellandrene).

FIG. 5. Infrared Spectra of Peak 33 and Peak 36.

- (3) Peak 33 (Acetate of *p*-menthen-1-ol-9).
 —Peak 33, ----Synthetic compound.
 (4) Peak 36 (Acetate of *p*-menthadien-1,8-(10)-ol-9).
 —Peak 36, ----Synthetic compound.

2) J. A. Wenniger, R. L. Yates and M. Dolinsky, *J. A. O. A. C.*, **50**, 1331 (1967).

3) V. H. Kapadia, B. A. Nagasampagi, V. G. Naik and S. Dev, *Tetrahedron*, **21**, 607 (1965).

α - and β -humulene by comparison of its IR and MS with those described by S. Dev⁴⁾ and by G. L. K. Hunter *et al.*⁵⁾ Peak 32, one of the major sesquiterpene hydrocarbons in the peel oil, was identical (IR (Fig. 4) and UV ($\lambda_{\max}$ 232 m μ)) with β -sesquiphellandrene as reported by D. W. Connell *et al.*⁶⁾ In addition, its MS showed M⁺ ion $m/e=204$ and its NMR showed a secondary methyl (τ 9.11 doublet, $J=6$ cps). β -Sesquiphellandrene has never before been reported as a component of citrus fruits. Peak 35 was identical (IR) with γ -cadinene which was reported by J. A. Wenniger *et al.*²⁾ Peak 19 and 20 were identified as β -elemene and caryophyllene, respectively, by comparison of their IR, MS and gas chromatographic data with those of authentic samples.

Oxygenated compound

The compound represented as peak 33 (Fig. 3) was identified as acetate of *p*-menthen-1-ol-9 by comparison of its IR and gas chromatographic data with those of the synthetic product (Fig. 5). No one has ever found this acetate as natural product before, although *p*-menthen-1-ol-9 has been found in Valencia orange peel oil by G. L. K. Hunter *et al.*⁷⁾ The preparation of this acetate was by hydroboration of *d*-limonene, followed by acetylation.* Peak 36 was identified as acetate of *p*-menthadien-1, 8(10)-ol-9 by comparing of its IR (Fig. 5), MS, NMR and gas chromatographic data with those of the authentic compound ($[\alpha]_D+58.5^\circ$) which was synthesized, in one step from 4 R-*d*-limonene, using SeO₂-Ac₂O reagent.** The same compound, which

TABLE I. IDENTIFICATION AND QUANTITATIVE VALUE OF THE CONSTITUENTS OF COLD-PRESSED PEEL OIL FROM *C. Unshiu*.

A)			
Peak No.	Constituent	Peak area (%)	
1	α -Pinene	0.77	
2	Myrcene	1.95	
3	<i>d</i> -Limonene	93.47	
4	<i>p</i> -Cymene	1.62	
5	Terpinolene	1.11	
	Others	1.08	
B)			Weight % in peel oil*
6	Linalool		3.0
7	Octyl acetate		1.4
8	Decanal		4.0
9	β -Elemene		5.3
10	Isopulegol		0.7
11	α -Copaene		5.7
12	Decyl acetate		0.3
13	an Alcohol		0.1
14	α -Terpineol		3.0
15	an Aldehyde		2.4
16	an Alcohol		trace
17	Citronellyl acetate		4.1
18	Undecanal		trace
19	β -Elemene		27.0
20	Caryophyllene		3.6
21	Citronellol		0.8
22	Terpinyl acetate		0.2
23	<i>d</i> -Carbone		0.5
24	Neryl acetate		5.6
25	an Alcohol		0.6
26	$\alpha\beta$ -, Humulene		4.7
27	Perillaldehyde		0.3
28	<i>trans</i> -Carveol		0.9
29	Dodecanal		trace
30	Geranyl acetate		3.8
31	<i>cis</i> -Carveol		1.0
32	β -Sesquiphellandrene		16.9
33	Acetate of <i>p</i> -menthen-1-ol-9		0.1
34	an Sesquiterpene hydrocarbon		0.3
35	γ -Cadinene		2.2
36	Acetate of <i>p</i> -menthadien-1, 8(10)-ol-9		0.3
37	Perillyl acetate		0.7

* excluding monoterpene hydrocarbons

4) S. Dev, *Tetrahedron*, **9**, 1 (1960).

5) G. L. K. Hunter and W. B. Brogden, *J. Anal. Chem.*, **36**, 1122 (1964).

6) D. W. Connell and M. D. Sutherland, *Aust. J. Chem.*, **19**, 283 (1966).

7) G. L. K. Hunter and M. G. Moshonas, *Anal. Chem.*, **37**, 378 (1965).

* Separation of two diastereomers is now under investigation.

** Extensive studies on this reagent are now in progress.

FIG. 6. Gas Chromatographic Comparison of Aroma Patterns among Five Varieties of Citrus Fruits (Head Space Analysis).

Column: $0.02'' \times 500'$, SF 96(50)

Column temperature: $50^{\circ}\text{C}(10 \text{ min}) \xrightarrow{2^{\circ}\text{C}/\text{min}} 120^{\circ}\text{C}(30 \text{ min}) \xrightarrow{2^{\circ}\text{C}/\text{min}} 200^{\circ}\text{C}$.

(1) *C. Aurantium* L. var. *Unshiu* Makino (Unshiu-mikan).

(2) *C. medica* L. subsp. *Limonum* Hook f. (Lemon).

(3) *C. Aurantium* L. subsp. *Junos* Makino (Yuzu).

(4) *C. Aurantium* L. subsp. *Maritima* (Orange).

(5) *C. Aurantium* L. subsp. *Sinensis* (Tangerine).

FIG. 7. Gas Chromatographic Comparison of Aroma Patterns among Five Varieties of Citrus Fruits (Head Space).

Column: 0.02"/500' SF 96(50).

Column temperature: $50^{\circ}\text{C}(10\text{ min}) \xrightarrow{2^{\circ}\text{C}/\text{min}} 120^{\circ}\text{C}(30\text{ min}) \longrightarrow 200^{\circ}\text{C}$.

(1), (2) and (3) are the same as in Fig. 6.

(4) *C. Aurantium* L. subsp. *amara* Forst. (Seidai)

was recently found in *C. Natsudaïdai* by Y. Hirose *et al.*,⁸⁾ has $[\alpha]_D^{25} + 88.7^\circ$. Therefore, the configuration at C-4 of this *d*-form is determined as R by this synthesis. These two acetates have very strong sweet aromas which seemed to contribute to the characteristic flavor of *C. Unshiu*. All identified compounds and their quantities are listed in Table I.

Head space analysis

The analysis was carried out by the injection of 7.5 ml of vapor above the crushed peel of various citrus fruits. The gas chromatogram is shown in Fig. 6. Peak assignment was carried out with component references, which were identified in the peel oil, as shown in Table II. Three fruits (*C. Unshiu*, *C. Junos*, and *C. Natsudaïdai*) showed similar gas chromatographic patterns. From calculation of the peak area, these fruits contained *d*-limonene at about 85 %, while *C. Limonum* contained it at about 54 %. On the other hand, α -pinene and myrcene were present in larger amounts in *C. Limonum* (1.4 % and 2.4 %, respectively)

than in the other fruits. *F. japonica* showed a very simple pattern compared with other fruits.

In order to find more detailed differences between fruits, 60 ml of head space vapor was injected. As shown in Fig. 7, only small differences in the region of the monoterpene hydrocarbons (tR; 55~80 min) were recognized, while large variations were observed in the region of very volatile components (tR; up to 50 min from the beginning). Significant differences were found in the region of high boiling components (tR; later 80 min), which seem to be the main difference of character between these fruits. *C. Junos* and *C. Limonum* also showed very similar patterns in the high boiling region.

DISCUSSION

Peel oil from *C. Unshiu* contains about 98 % monoterpene hydrocarbons which have general citrus like aromas. In comparing peel oil from *C. Unshiu* with that of *C. Mandarin** as reported by Kugler *et al.*,⁹⁾ *C. Unshiu* contained many more esters and sesquiterpene hydrocarbons than did *C. Mandarin** (esters; 36 times, sesquiterpene hydrocarbons; 10 times). Therefore, the characteristic aroma of *C. Unshiu* seems to be due to its larger contents of sesquiterpenes and esters as well as to the complexity of its various components.

Head space analysis seems a possible technique for quality examination of citrus products.

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9) E. Kugler und E. sz. Koväts, *Helv. Chim. Acta*, **46**, 1480 (1963).

* *Citrus reticulata* Blanco, var. "Mandarine", syn. *Citrus nobilis* var. *dericosa* Swingle "Mandarine"

TABLE II. PEAK ASSIGNMENTS FROM GAS CHROMATOGRAMS, FIG. 6 AND FIG. 7

Peak No.	Substance	Peak No.	Substance
1	Acetaldehyde	27	Myrcene
2	Methanol	28	<i>n</i> -Octanal
3	Ethanol	30	β -Carene
4	Isopropanol	31	<i>p</i> -Cymene
5	<i>n</i> -Propanol	32	<i>d</i> -Limonene
8	Isobutanol	33	<i>n</i> -Octanol
10	Isopentanal		Terpinolene
11	<i>n</i> -Butanol	34	<i>n</i> -Nonanol
12	<i>n</i> -Pentanal	35	Linalool
14	Isoamylalcohol	37	<i>n</i> -Decanal
19	<i>n</i> -Hexanol		Octyl acetate
23	α -Pinene	48	δ -Elemene
24	β -Pinene	49	β -Elemene
25	<i>n</i> -Heptanal	52	Caryophyllene

8) Y. Ohta and Y. Hirose, *Agr. Biol. Chem.*, **30**, 1196 (1966).