

Constituents of Yuzu (*Citrus junos*) Oil

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Eight monoterpenic hydrocarbons, 15 sesquiterpenic hydrocarbons, 6 aliphatic aldehydes, 1 aromatic aldehyde, 3 terpenic aldehydes, 1 terpenic ketone, 1 aliphatic ketone, 1 phenol, 1 phenol ether, 8 terpenic esters, 3 aliphatic alcohols, 9 monoterpenic alcohols, 9 sesquiterpenic alcohols, 1 aromatic alcohol, 5 fatty acids and 2 coumarin compounds were identified in cold-pressed peel oil of Yuzu (*Citrus junos*) based on gas chromatography, infrared spectrometry, nuclear magnetic resonance spectrometry and capillary GLC connected with fast-scan mass spectrometry. Structures of the sesquiterpenic hydrocarbons, γ -elemene and bicycloelemene are discussed.

Yuzu is a type of sour orange which originated in central China and has been propagated in our country since the 10th century, or earlier. The fruits, have a pleasant aroma, are used as the raw material for vinegar and seasonings.

Many investigations have been done on constituents of citrus oils of Western origin, but little is known about the composition of domestic citrus oils. Ohta and Hirose¹⁾ studied the constituents of the peel oil of "Natsukan" (*Citrus natsudaikai*) and identified twenty six compounds. Maekawa and his co-workers²⁾ reported analytical results on the peel oils obtained from "Kabusu" (*C. aurantium* Linn. from *Kabusu*), "Sudachi" (*C. sudachi*), "Daidai" (*C. daidai*) and "Iyokan" (*C. iyo*). Kadota and Nakamura³⁾ investigated the components of "Hyuga-natsu" (*C. tamurana*). More recently, Yamanishi and her co-workers⁴⁾ made quan-

titative analyses of the peel oleoresin and cold-pressed peel oil from "Unshu mikan" (*C. unshu*).

This paper presents full analytical results of the cold-pressed peel oil of Yuzu orange.

EXPERIMENTAL

Apparatus.

Gas Chromatography (GLC). Preparative separations were performed using a Varian Model 90-P fitted with a 20 ft \times 1/4 inch O.D. aluminium column packed with 10% Carbowax 20 M on celite material. Fractions eluted from the gas chromatograph were collected in glass tubes of 1 mm I.D. and 30 cm long. A Hitachi gas chromatograph K-53, with flame ionization detector fitted with a 45 m \times 0.25 mm I.D. stainless steel capillary column coated with polypropylene glycol-2000, was used for the typical gas chromatogram of each fraction and for the final analyses of the components. In the analysis of fatty acids a column, 3 mm I.D. by 4 m stainless steel tube packed with a mixture of 7% polyethylene glycol adipate and 1% phosphoric acid on celite, was employed.

Mass spectrometry. Mass spectrometry was carried out with a Hitachi Mass spectrometer RMU-6 type under the following conditions; ionization voltage, 80 eV, ion source temperature 250°C, ion accelerative voltage 2 kV and inlet vapor temperature 150°C.

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

2) K. Maekawa, M. Kodama, M. Kushii and M. Mitamura, *ibid.*, **31**, 373 (1967).

3) T. Kadota and T. Nakamura, *J. Food Sci. and Tech.*, **14**, 137 (1967).

4) T. Yamanishi, A. Kobayashi, Y. Mikuma, Y. Nakasone, M. Kita and S. Hattori, *Agr. Biol. Chem.*, **32**, 593 (1968).

Capillary GLC and fast-scan mass spectrometry method (GC-MS). Analysis via direct connection of the capillary GLC with fast-scan mass spectrometer was done using the mass spectrometer mentioned above and the same gas chromatograph containing a 0.5 mm \times 45 m capillary column coated with polypropylene glycol-2000. Helium was adopted as the carrier gas. The effluent from the column was introduced into a ionization chamber after passing through a carrier gas separator designed by Bieman. The mass spectrum was scanned at 20 to 200 mass units in 3 seconds. Part of the total ion current was multiplied and recorded as a monitor of the gas chromatography.

Liquid chromatography. Mallinckrodt's silicic acid was used as the adsorbent, and a hexane solution of ether (3%, 5%, 10% and 30%) and methanol was used as the elute solvent.

Infrared spectrometry. Hitachi's EPI-G2 type infrared spectrometer with a beam condenser using a NaCl cell was used.

Nuclear magnetic resonance spectrometry. JEOL's JNM-60 Spectrometer with tetramethylsilane as an internal standard was used. Samples were measured in carbon tetrachloride solutions.

Materials. Yuzu oil was obtained by compressing the peels of unripe fruits collected in Tokushima Pref. at the beginning of October, 1966 using a rolling

press. Physical constants for the oil are as follows; d^{20}_4 0.8515, n^{20}_D 1.4752, $[\alpha]^{20}_D$ +73.4, aldehyde contents as decyl aldehyde 0.03%.

Procedure. One kg of oil was extracted three times with 5% NaOH solution (total 300 ml) to remove acidic components and was fractionally distilled by means of Podbielniack's 25 mm Heli-Grid column under reduced pressure until most of the monoterpene hydrocarbons were taken off. The residue was transferred to an ordinary distillation flask and distillation was continued under 1 mm Hg pressure to remove the non-volatile fraction from the higher boiling fraction. This higher boiling fraction was subjected to column chromatography on silica-gel to afford a hydrocarbon fraction and a fraction of oxygenated compounds. The procedures described above are summarized in Fig. 1.

Each fraction was subjected to further fractionation by means of column chromatography. Compounds contained in every sub-fraction were isolated in a pure state using preparative GLC and were identified by comparing their IR and MS spectra and their GLC retention times with authentic samples.

To analyze the sesquiterpenoid alcohols, which were minor components of the oxygenated compounds of Yuzu oil, another lot of original oil was supplied. Two kg of oil was distilled under reduced pressure (1 mm Hg) to remove the lower boiling components. Throughout distillation, the bath temperature was no

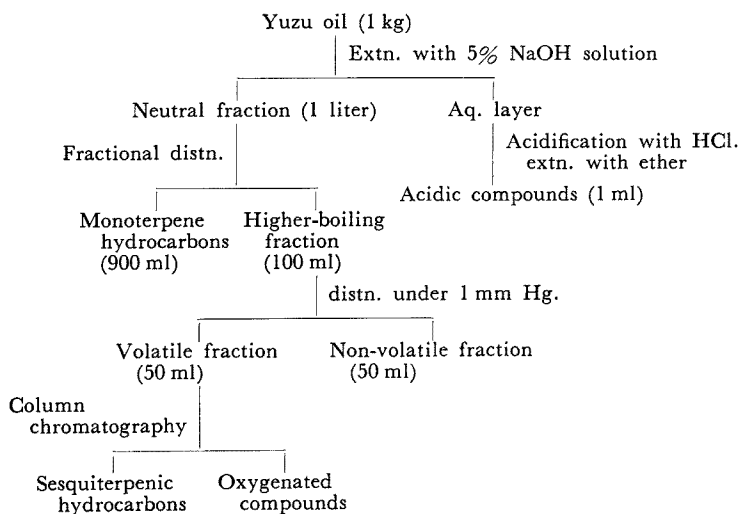


FIG. 1. Process for the Treatment of Yuzu Oil.

allowed to rise above 60°C in order to prevent isomerization and/or decomposition. The distillation residue was kept in a refrigerator over night, after which crystals of coumarin compounds were filtered out. The filtrate (total 116 g) was subjected to column chromatography on silica-gel to obtain the alcoholic fraction. For the final isolation of the compounds, repeated separation by preparative GLC was used.

RESULTS AND DISCUSSION

Monoterpene hydrocarbons

Like other citrus oils, limonene is the major component in Yuzu oil, with a content of nearly 80%. α -Pinene, β -pinene, myrcene, γ -terpinene, terpinolene and *p*-cymene were also isolated along with limonene. *p*-Isopropenyl toluene was identified by comparing its IR and MS spectra and the RTs of GLC with those of an authentic sample synthesized from *p*-methylacetophenone. Traces of low boiling oxygenated compounds, *i.e.* *n*-hexyl aldehyde, *n*-amyl alcohol and *n*-hexyl alcohol, were found in this fraction.

Low-boiling oxygenated compounds

The gas chromatogram shown in Fig. 2 is

an analysis of the oxygenated compounds fraction, which is the most important fraction in the characteristic flavor of Yuzu oil.

The chromatogram revealed the presence of more than sixty compounds. Predominant compounds in the oxygenated fraction are linalool, α -terpineol, terpinen-4-ol, and thymol. Minor components are *n*-octyl aldehyde, *n*-decyl aldehyde, *n*-dodecyl aldehyde, perillaldehyde, citral, carvone, methyl heptenone, citronellal, perillyl acetate, citronellyl formate, citronellyl acetate, *n*-nonyl alcohol, citronellol, geraniol, nerol, *p*-mentha-1,8-diene-4-ol, *cis*-carveol, *trans*-carveol, cuminaldehyde, cuminalcohol and thymol methyl ether.

As the composition of some of the column chromatographic fractions were rather complicated, analyses using combined capillary GLC and fast-scan mass spectrometry were adopted. By this method, nonyl aldehyde, undecyl aldehyde, terpinyl acetate, geranyl formate, geranyl acetate and *p*-menthadien-1, 8(10)-ol-9 acetate were also confirmed.

Sesquiterpenic hydrocarbons

The gas chromatogram of the sesquiterpenic hydrocarbons of Yuzu oil is shown

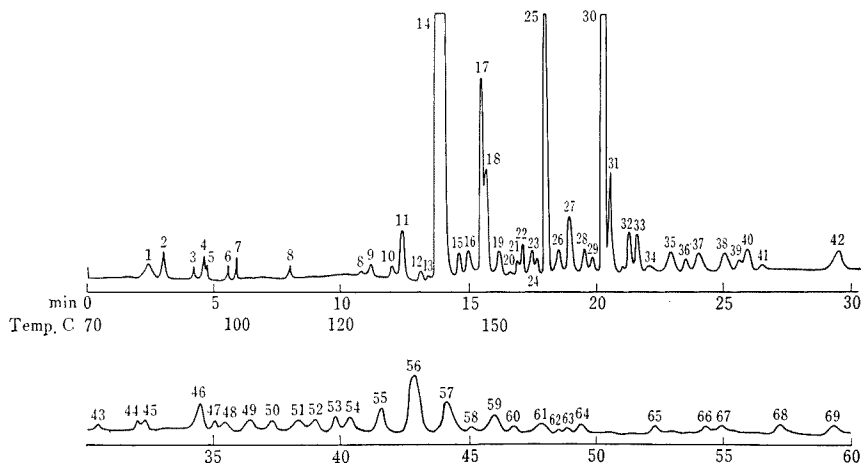


FIG. 2. Gas Chromatogram of Oxygenated Compounds.

Column temp., 70~150°C, programming rate 5°C/min.

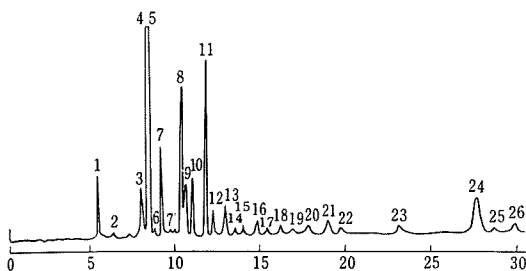


FIG. 3. Gas Chromatogram of Sesquiterpenic Hydrocarbons.

Column temp., 150°C.

in Fig. 3. δ -Elemene, α -ylangene, β -ylangene, caryophyllene, α -copaene, β -bisabolene, β -elemene, humulene, β -farnesene, α -muurolene, γ -cadinene, α -curcumene and calamenene were identified, respectively, by comparing their IR and MS spectra with those of authentic samples.

Peak 11 was a mixture of humulene and a hydrocarbon whose IR spectrum was very similar to that of β -elemene, exhibiting absorption at 3080, 1635, 1010 and 890 cm^{-1} ($-\text{CH}=\text{CH}_2$ and $-\text{C}=\text{CH}_2$). On hydrogenation with platinum oxide in acetic acid it absorbed three moles of hydrogen affording a saturated hydrocarbon whose IR and MS spectra and Rt of GLC corresponded to those of elemene, which was derived from β -elemene by catalytic hydrogenation. The NMR spectrum of the compound showed signals at τ 8.35 and τ 8.92 due to three methyl groups attached to double bonds and at τ 8.94 (3H, s) due to a tertiary methyl group. A multiplet centered at τ 5.2(4H) is due to four protons of a pair of end methylene groups. A quartet centered at τ 4.25(1H, $J=10.5$ and 18 Hz) is attributed to a single β -vinyl proton.

Spectral and chemical data suggest that the compound corresponds to the structure of I or I'. The τ value 8.94 for the angular methyl group is nearly that of β -elemene, indicating that both angular methyl groups are in the same situation, even though the value is rather high in comparison to the

ordinary value for the methyl group on the carbon atom adjacent to a double bond. Formula I was proposed by Southerland⁵⁾ for the compound which appeared on the gas chromatogram of the dehydration product of elemol phenylurethane, by heating at 200°C, and was named γ -elemene without isolating it in the pure state. Brown and his co-workers⁶⁾ reported its preparation from germacrene by Cope rearrangement. No literature on its isolation from a natural source has appeared, although a compound identical with our hydrocarbon in its IR and MS spectra was isolated from the volatile oil of *Mosla punctulata* Nakai by Ohta and Hirose⁷⁾ and from the oil of *Kadsura japonica* by Morikawa and Hirose.⁷⁾

Upon ozonolysis of the compound isolated from *Mosla punctulata* followed by esterification with diazomethane, Ohta obtained a diketester of molecular weight 212. This result is well explained by formula I, as shown in Fig. 4. The compound represented by formula I' would give α -diketocarboxylic acid which would decarboxylate easily to the diketone with a molecular weight of 154.

The IR spectrum of the compound represented by peak 5 was nearly the same as that of bicycloelemene which was isolated from mint oil of Bulgarian origin. It was assigned to II for its structure by Vlahov and Ognyanov⁸⁾ because it disclosed two distinct methylene groups $-\text{C}=\text{CH}_2$ and $-\text{CH}=\text{CH}_2$, at 3095, 1638, 895 and 910 cm^{-1} . A cyclopropane ring was posited from its NMR spectrum in which a broad multiplet at τ 9.19 to 9.50(2H), attributed to the methine protons on cyclopropane ring, appeared. On hydrogenation

5) M. D. Southerland, *Aust. J. Chem.*, **17**, 1270 (1964).

6) E. D. Brown, M. D. Solomon, J. K. Sutherland and A. Torre, *Chem. Comm.*, **1967**, 111.

7) Unpublished data.

8) R. Vlahov and I. Ognyanov, *Riechstoff u. Aromen*, **17** [8], 315, 316, 318 (1967). cf. V. Herout, R. Vlahov and I. Ognyanov, *Coll. Czech. Chem. Comm.*, **32**, 808 (1967).

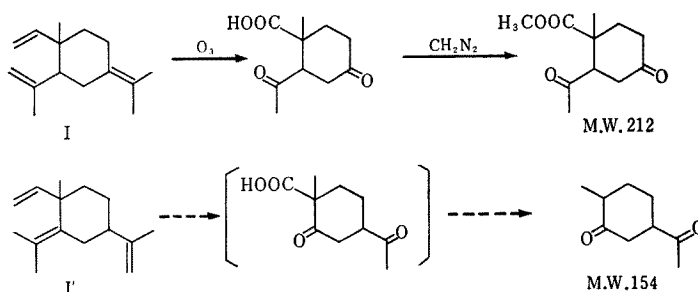
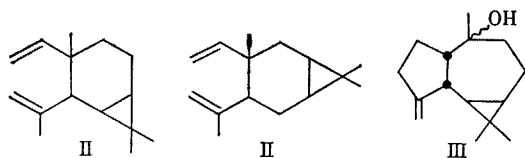


FIG. 4.

with platinum oxide in acetic acid it first gave a tetrahydro-compound, which absorbed one more mole of hydrogen to afford a hexahydro-compound on further hydrogenation. The hexahydro-compound was proven identical with elemene prepared from β -elemene by means of IR and MS spectra and Rt of GLC. Signals in the NMR spectrum at τ 8.98(6H) and 9.04(3H) were attributed to three tertiary methyl groups. One of them, τ 8.98 was assigned to the angular methyl group in the same situation as that of β -elemene and γ -elemene, which showed signals at an unusually higher field. In the region τ 6.60 to 6.97(4H) signals due to terminal methylene protons and terminal protons on the vinyl double bond were shown. A quartet at τ 4.30(1H, $J=10.2$ Hz and 18 Hz) was attributed to a β -proton of vinyl double bond. These facts suggest that the compound is bicycloelemene (II), or has the formula II'. The former is more plausible from the biological point of view.



This was further proven by the distinct similarity of the mass spectral patterns between γ -elemene and this compound (Figs. 5 and 6). The characteristic fragments at m/e

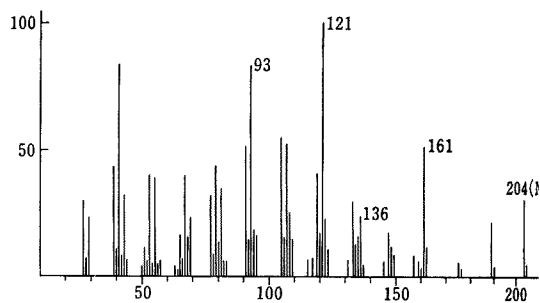
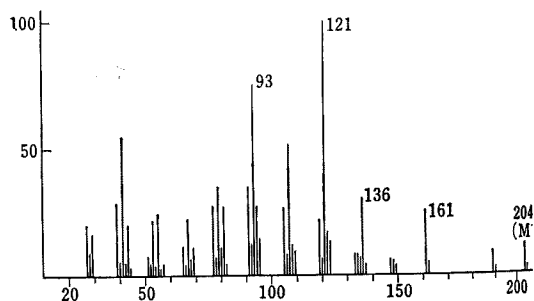
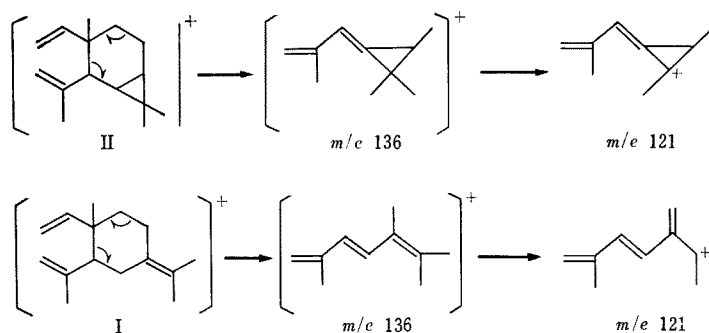
FIG. 5. Mass Spectrum of γ -Elemene.

FIG. 6. Mass Spectrum of Bicycloelemene.

121 and at 136 are shown in Fig. 7.

Thomas⁹⁾ reported the isolation of a similar hydrocarbon from the essential oil of "Hinoki" leaves (*Chamaecyparis obtusa* Sieb. et Zucc. f. *formosana*). He is of the opinion that its constitution also corresponds to formula II. The IR spectrum of bicycloelemene obtained

9) A. F. Thomas, *Reichstoffe u. Aromen*, 16, 42 (1966).

FIG. 7. Fragmentation of Bicycloelemene and γ -Elemene.

from Yuzu oil was slightly different from Thomas's, although the mass spectra had a marked similarity. It is reasonable to conclude that both substances may have analogical constitutions, however, they differ in their steric arrangement.

Sesquiterpenic alcohols

Figure 8 is a gas chromatogram of the sesquiterpenic alcohol fraction of Yuzu oil. Narolidol (peak C) was identified by comparing its IR spectrum with that of an authentic sample.¹⁰ The IR spectrum of the compound represented by peak E was identical with that of elemol.¹⁰ The NMR spectrum showed signals at τ 9.05(3H, s, $\text{CH}_3\text{-C-}$),

τ 8.86(6H, s, $(\text{CH}_3)_2\text{-C-OH}$), τ 8.32(3H, s, $\text{CH}_3\text{-C=C}$), τ 5.04~5.44(4H, m, $\text{CH}_2\text{-C-}$), and τ 4.23(1H, q, H-C=C-) supporting this structure. The crystalline compound of mp 75°C (M.W. 222) represented by peak G showed the same IR spectrum with that of globulol.¹⁰ The NMR spectrum exhibited the signals of two methine protons on a cyclopropane ring at τ 9.2~9.6, and no signal below τ 7.0.

Thymol (peak F) was isolated from this fraction too. The compound from peak H (M.W. 220) was confirmed to be spathulenol by means of its IR spectrum.¹¹ The NMR spectrum disclosed signals of two methine protons on a cyclopropane ring at τ 9.1~9.6 and of two protons of terminal methylene at τ 5.36(s). On hydrogenation with platinum oxide in acetic acid it absorbed one mole of hydrogen yielding a saturated alcohol with a molecular weight of 222.

Peak H contained another sesquiterpenic alcohol of molecular weight 220. Its NMR spectrum exhibited signals at τ 8.98(6H, s, $(\text{CH}_3)_2\text{-C-}$), τ 8.79(3H, s, $\text{CH}_3\text{-C-OH}$), τ 9.2~9.6(2H, m, cyclopropane ring protons) and at τ 5.36(2H, s, $\text{CH}_2\text{-C-}$). On catalytic hydrogenation with platinum oxide in acetic acid, dehydration and hydrogenation took place

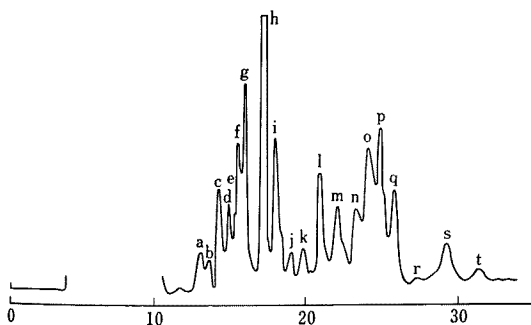


FIG. 8. Gas Chromatogram of the Sesquiterpenic Alcohol Fraction.

Column temp., 150°C.

10) O. Motle, M. Horák, J. Pliva and F. Sörm, *Terpenspektren Teil II.* (1963).

11) The IR spectrum of spathulenol, obtained from *Eucalyptus spathulata*, was kindly donated by Dr. R.C. Bowyer of the Royal Perth Hospital, Western Australia.

together and producing the saturated hydrocarbon, allo-aromadendrane. From these results we have tentatively assigned the formula III for this alcohol.

IR spectra of compounds represented by peak L and peak M corresponded to those of T-cadinol and T-muurolol, respectively. Both compounds were first isolated from *Taiwania cryptomeides* Hayata by Lin.¹²⁾

The IR spectrum of the compound from peak O coincided with that of β -eudesmol.¹³⁾ The NMR spectrum exhibited signals at τ 9.24 (3H, s, $\text{CH}_3\text{-C-}$), τ 8.83 (6H, s, $(\text{CH}_3)_2\text{-C-OH}$), τ 5.32 and 5.56 (2H, $\text{CH}_2=\text{C-}$) confirming the structure. α -Cadinol (peak P) and juniper camphor (peak S) were identified by their IR spectra in comparison with those of authentic samples.¹⁰⁾

The identification of the compounds represented by the remaining peaks in Fig. 8 was impossible because of their limited amounts.

Fatty acids and non-volatile compounds

The acidic fraction of Yuzu oil was analyzed using gas chromatography. Capric acid, lauric acid, myristic acid, palmitic acid, and stearic acid were identified by their retention times.

On chromatographic separation of the non-volatile fraction of Yuzu oil two crystalline

compounds, mp 67°C and mp 98°C were isolated. The former compound, $\text{C}_{19}\text{H}_{22}\text{O}_3$, was deduced to be aurapten (7-geranoxycoumarin) from its melting point and from the following spectroscopic properties. IR; $\nu_{\text{max}}^{\text{liq}}$ cm^{-1} : 1720 (conj. lactone), 1610 (conj. C=C) 1580 and 1500 (aromatic). NMR; τ : 8.36 and 8.32 (3H each, s, $(\text{CH}_3)_2\text{C=C}$), 8.21 (3H, s, $\text{CH}_3\text{-C=C-}$), 7.80~7.90 (4H, d, $-\text{CH}_2\text{-CH}_2\text{-}$), 5.40 (2H, d, $J=6.6$ Hz, $\text{C=CH-CH}_2\text{-O}$), 4.95 (1H, m, $-\text{C=CH-}$), 4.50 (1H, m, $-\text{C=CH}$), 3.88 (1H, d, $J=9$ Hz, $-\text{O-CO-CH=C-}$, α -ethylenic proton of carbonyl group on coumarin ring) and 2.49 (1H, d, $J=9$ Hz, $-\text{O-CO-CH=CH-}$, β -ethylenic proton of carbonyl group on coumarin ring).

The latter compound, mp 98°C, $\text{C}_{17}\text{H}_{16}\text{O}_5$, showed absorptions in its IR spectrum at 1730 (conj. lactone), 1600 (conj. C=C), 1580 and 1470 cm^{-1} (aromatic) and NMR signals at τ 8.27 (6H, s, $(\text{CH}_3)_2\text{C=C-}$), τ 5.87 (3H, s, $\text{CH}_3\text{-O-}$), τ 3.76 (1H, d, $J=9.3$ Hz), τ 3.05 (1H, d, $J=2.4$ Hz), τ 2.43 (1H, d, $J=2.4$ Hz) and τ 1.96 (1H, d, $J=9.3$ Hz), indicating the presence of a methoxyl group and a $\gamma\gamma'$ -dimethyl allyl ether group on a furocoumarin skeleton. The UV spectrum of this compound showed absorptions at 271 $\text{m}\mu$ ($\log \epsilon=4.8$) and 314 $\text{m}\mu$ ($\log \epsilon=4.5$) corresponding to the reported value for phellopterin, which was isolated from

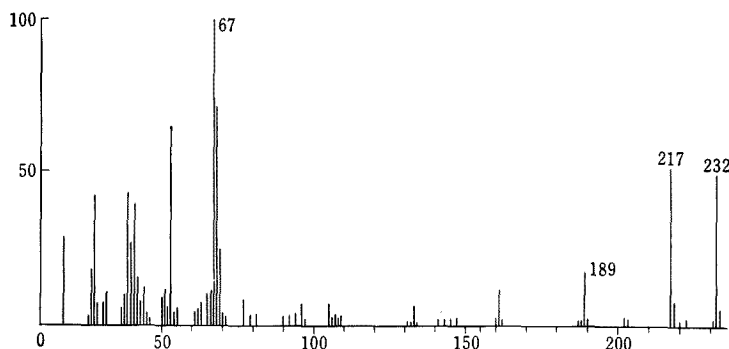


FIG. 9. Mass Spectrum of Phellopterin.

12) Y. T. Lin, *Chem. Comm.*, **1967**, 565.

13) F. J. McQuillin, *J. Chem. Soc.*, **1956**, 2973.

TABLE I. COMPOUNDS IDENTIFIED IN YUZU OIL

Compounds	% of volatile components	Peak No. in GLC.	Methods of identification	Compounds	% of volatile components	Peak No. in GLC.	Methods of identification
α -Pinene	1.4		IR, GLC	Linallyl acetate	+	2~12	GLC, MS
β -Pinene	0.5		IR, GLC	Citronellyl formate	+	2~22	GLC, MS
Myrcene	2.2		IR, GLC	Terpinyl acetate	+	2~19	GLC, MS
Limonene	79.4		IR, GLC	Geranyl formate	+	2~28	GLC, MS
γ -Terpinene	9.5		IR, GLC	Geranyl acetate	+	2~32	GLC, MS
Terpinolene	+		IR, GLC	Perillyl acetate	+		GLC, MS
p -Cymene	0.2		IR, GLC	p -Menthadien-1, 8(10)-ol-9 acetate	+		IR, MS
p -Isopropenyl toluene	0.02	{2~10 3~2	IR, GLC, MS	<i>n</i> -Nonyl alcohol	+		GLC, MS
β -Elemene	0.40	3~8	IR, GLC, MS	<i>n</i> -Amyl alcohol	+		GLC
γ -Elemene	0.51	3~11	IR, NMR, MS	<i>n</i> -Hexyl alcohol	+		GLC
δ -Elemene	0.30	3~4	IR, NMR, MS	Cumin alcohol	+	2~46	GLC, MS
α -Ylangene	+	3~6	IR, GLC	Citronellol	+	2~28	IR, GLC
β -Ylangene	+	3~7'	IR, GLC	Linalool	0.87	2~14	IR, GLC
α -Copaene	0.30	3~7	IR, GLC	Geraniol	+	2~37	IR, GLC
β -Farnesene	0.86	{3~12 2~30	IR, MS	Nerol	+	2~36	GLC
β -Bisabolene	+	3~13	IR	p -Menth-1, 8-diene-4-ol	+	2~21	IR
α -Muurolene	+	3~15	IR, MS	α -Terpineol	0.12	2~25	IR, GLC
γ -Cadinene	+	3~16	IR, MS	Terpinene-4-ol	0.02	2~17	IR, GLC
α -Curcumene	+	3~17	IR, MS	<i>trans</i> -Carveol	+	2~33	IR
Calamenene	+	3~21	IR, MS	<i>cis</i> -Carveol	+	2~35	IR
Caryophyllene	0.30	3~10	IR, GLC, MS	Thymol	0.15	{2~56 8-f	IR, MS
Humulene	+	3~11	IR, GLC, MS	Nerolidol	+	8-c	IR
Bicycloelemene	0.71	3~5	IR, MS, NMR	Elemol	+	8-e	IR, NMR
Thymol methyl ether	0.02	2~31	GLC, MS	Globulol	+	8-g	IR, NMR
<i>n</i> -Hexyl aldehyde	+		GLC	Spathulenol	+	8-h	IR, NMR
<i>n</i> -Octyl aldehyde	0.02	2~8	GLC, MS	T-Cadinol	+	8-l	IR
<i>n</i> -Nonyl aldehyde	+	2~9	GLC, MS	T-Muurolol	+	8-m	IR
<i>n</i> -Decyl aldehyde	0.04	2~15	GLC, MS	β -Eudesmol	+	8-o	IR, NMR
<i>n</i> -Undecyl aldehyde	+	2~23	GLC, MS	α -Cadinol	+	8-p	IR
<i>n</i> -Dodecyl aldehyde	+	2~32	GLC, MS	Juniper camphor	+	8-s	IR
Cumin aldehyde	+	2~32	GLC, MS	Capric acid	+		GLC
Perillaldehyde	0.03	2~33	GLC, MS	Lauric acid	+		GLC
Citral	0.01	2~27	IR, GLC	Myristic acid	+		GLC
Carvone	0.02	2~27	IR, GLC	Palmitic acid	+		GLC
Methyl heptenone	+	2~10	GLC	Stearic acid	+		GLC
Citronellal	+	2~11	GLC	Aurapten			IR, NMR, MS
Citronellyl acetate	+	2~23	GLC, MS	Phellopterin			IR, NMR, MS

Phellopterus littoralis by Noguchi *et al.*¹⁴⁾
Structural formula IV for this compound was

further confirmed using its mass spectral pattern (Fig. 9), the characteristic fragments of which are represented in Fig. 10.

Compounds found in Yuzu oil in this study are listed in Table I. Percentages were

14) K. Noguchi and M. Anan, *Yakugaku Zasshi*, **60**, 57 (1940).

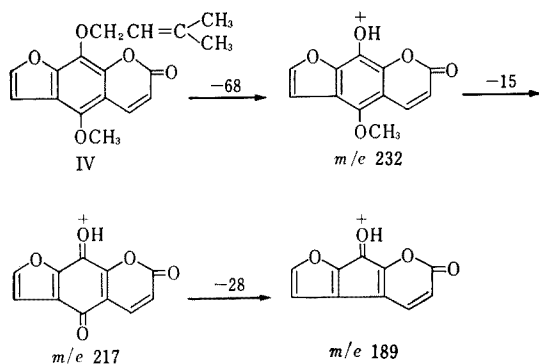


FIG. 10. Fragmentation of Phellopterin.

estimated via gas chromatographic analysis of the original oil and analysis of the various chromatographic fractions.

The characteristic odor of Yuzu fruits seems to be distributed in both lower and higher boiling oxygenated fractions. Among the many compounds present in lower-boiling oxygenated fraction, thymol and perillaldehyde characterize the flavor of Yuzu.

Although the fraction obtained by gas chromatographic separation of oxygenated sesquiterpenoids contained a compound whose odor was reminiscent of Yuzu, its content

was too small for further treatments, *e.g.* separation and identification.

The gas chromatogram of the hydrocarbon fraction, which was obtained by the repeated extraction of oxygenated compounds with a mixture of ethanol and water, was quite simple in its sesquiterpenic regions as compared to those in Fig. 3. This strongly suggests that some unstable precursor of the sesquiterpenic hydrocarbons in Yuzu oil was present in the original oil and was isomerized into more complex mixtures by distillation and/or chromatographic separation. A report on this subject will be published separately.

Stevens and his co-workers¹⁵⁾ isolated two sesquiterpenoid aldehydes, α - and β -sinensal from cold-pressed orange oil. MacLeod *et al.*¹⁶⁾ found nootkatone, an important sesquiterpene ketone, in grapefruit. It is interesting to note that in contrast to citrus oils, the oxygenated sesquiterpene fraction of Yuzu oil consisted mainly of various alcohols and a few carbonyl compounds.

15) K. L. Stevens, R. E. Lundin and R. Teranishi, *J. Org. Chem.*, **30**, 1690 (1965).

16) W. D. MacLeod and N. M. Buigues, *J. Food Sci.*, **29**, 565 (1964).