Quantitative Determination of Volatile Constituents in the Pummelo (*Citrus grandis* Osbeck forma Tosa-buntan)

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The volatile samples of the pummelo (*Citrus grandis* Osbeck forma Tosa-buntan) were obtained by cold-pressed procedure and steam distillation under reduced pressure with subsequent solvent extraction. Extracts were analyzed by glass capillary gas chromatography and mass spectrometric techniques. A total of 28 compounds were identified and quantitated on the basis of two kinds of internal standards. Nootkatone is one of the characteristic components of the pummelo's aroma, in common with the grapefruit. 1,8-Cineole overlapping with limonene on the gas chromatogram was quantitatively determined by mass fragmentography.

The pummelo or shaddock (*Citrus grandis* Osbeck) is commercially grown especially in the Orient, in contrast to the grapefruit, which is recognized as a natural hybrid of the pummelo (Hodgson, 1967) but grown mainly in America. Since the pummelo, in Japanese the buntan or the zabon, was introduced to Japan in the 1770s, it has been selected for commercial use. Now the Tosa-buntan (*Citrus grandis* Osbeck forma Tosa-buntan) grown mainly in southwestern Japan is the most popular variety of the pummelos. It has a pleasant taste with sugar and acid and a refreshing flavor that resembles that of the grapefruit. It is not, however, likely that any study has been carried out on the characteristics of aroma of the pummelo. There is only a report on the horticultural characteristics of Tosa-buntan (Hashimoto, 1976).

Recently the review of Shaw (1979) dealing with the quantitative determination of essential citrus oils suggested that unity of techniques would be important for comparative studies to literature. In order to minimize variations from the native composition, one of the preferable methods for preliminary separation would be a cold-pressed preparation rather than separations including distillation, liquid extraction, etc. Furthermore, the data calculated by weight percent on the basis of an internal standard method would be more accurate. The present study describes quantitative glass capillary gas chromatographic and mass fragmentographic results of Tosa-buntan cold-pressed oil and compares this to those of the steam-distilled oils.

EXPERIMENTAL SECTION

Tosa-buntan was cultivated in the field of Kochi Fruit Tree Experiment Station, Kochi, and harvested in the middle of December. The flavedo was bent to crack the oil glands. Then the crude oil that discharged was centrifuged (4000*g*, 15 min) after being saturated with sodium chloride. The supernatant, dehydrated with anhydrous sodium sulfate, was stored overnight at 5 °C and then filtrated. The yield of cold-pressed oil (CPO) was 1.16% of the flavedo by weight. Oil (SDO) won by steam distillation under 20 mmHg with subsequent solvent extraction was prepared by the procedure reported previously (Kusunose and Sawamura, 1980). This yield was 2.39% of the flavedo by weight.

For quantitative analyses a Shimadzu GC-7A gas chromatograph with FID was used. Separations were performed on a 0.22 mm (i.d.) × 50 m glass capillary column coated with PEG 20M. The column temperature was held at 65 °C for 8 min, raised to 210 °C at 2 °C/min, and finally held at 210 °C for 8 min. The split ratio was 50/1. The flow rate of carrier gas was 1.0 mL/min N_2. The temperature of the injection and detector portion was 230 °C. The peak areas were calculated with a Shimadzu C-RlB integrator, and the results (w/w, %) were recalculated on the basis of detector response factors obtained previously by authentic compounds. Two kinds of internal standard were used: 1-heptanol and methyl myristate.

GC/MS conditions were as follows: apparatus, Shimadzu LKB-9000; column, PEG 20M (0.22 mm (i.d.) × 50 m); carrier gas flow rate, 1.5 mL/min He; temperatures of separator and ion source, 230 and 270 °C, respectively; electron energy, 70 eV for qualitative determination and 20 eV for mass fragmentography.

Each CPO and SDO sample was fractionated into terpenic hydrocarbons and oxygenated substances on silica gel (Wako gel Q-23, Wako Pure Chemical Industries) with *n*-hexane and ethyl acetate as the eluting solvent (Kirchner and Miller, 1952). All eluates were dried over anhydrous sodium sulfate and concentrated.

RESULTS AND DISCUSSION

Physical values of CPO and SDO, respectively, were determined as follows: refractive index at 20 °C, 1.4768, 1.4737; specific gravity at 25 °C, 0.8469, 0.8382; specific rotation at 20 °C, + 92.69°, +89.94°. Since each value of CPO was slightly higher than those of SDO, CPO may include a small amount of higher boiling point or nonvolatile materials.

The gas chromatogram of CPO is shown in Figure 1, in which about 60 peaks were detected. After the fractionation of CPO, 25 peaks were detected from the fraction of hydrocarbons and more than 100 peaks from the fraction of oxygenated substances. Similar results were obtained in SDO. For quantitative determination a single component was generally used as an internal standard. However, the use of two internal standards well separated from each other on a gas chromatogram would be more desirable, since the elution time of all oil components from start to end takes as long as 80 min. In Figure 1 the area of 1-heptanol is within peaks 1–19, and that of methyl myristate appears after peak 20. A total of 28 components were identified by relative retention times as well as GC/MS, as listed in Table I.

The predominant component in CPO was limonene. γ-Terpinene and myrcene were major components, followed by α- and β-pinene. These hydrocarbons amounted to about 98%. Among minor components β-cubebene was isolated in the Tosa-buntan oil, although cubebenes are rare components in citrus oils. Among minor oxygenated components decanal, linalool, nootkatone, and citronellal were comparatively predominant.

Comparison of the major components in CPO with those in SDO did not show great differences. These two types

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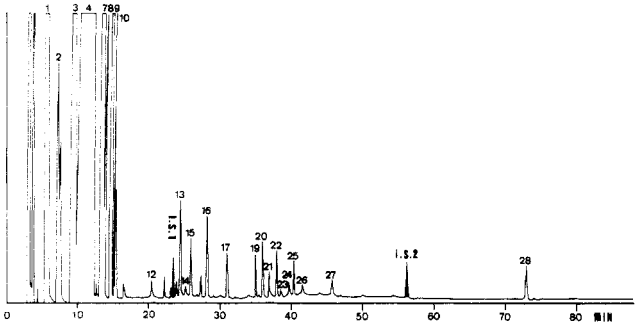

Figure 1. Gas chromatogram of cold-pressed Tosa-buntan oil. The peak numbers correspond to the numbers in Table I. I.S. 1 and I.S. 2 represent *n*-heptanol and methyl myristate, respectively.

Table I. Quantitative Analysis (Weight Percent) of Tosa-Buntan Oil

peak no.	component	cold-pressed	distilled
1	α-pinene	1.22	1.47
2	β-pinene	0.91	1.24
3	myrcene	3.53	4.44
4	limonene	86.19	85.30
5	1,8-cineole	0.0035	0.0035
6	ocimene	tr[a]	tr
7	γ-terpinene	5.78	5.96
8	*p*-cymene	0.03	0.04
9	terpinolene	0.10	0.13
10	2-carene	0.18	0.19
11	isooctyl acetate	tr	tr
12	nonanal	0.05	0.06
13	citronellal	0.18	0.17
14	*n*-octyl acetate	0.08	0.07
15	decanal	0.29	0.32
16	linalool	0.24	0.29
17	terpinen-4-ol	0.04	0.04
18	β-caryophyllene	0.01	0.03
19	β-terpineol	0.05	0.02
20	α-terpineol	0.05	0.04
21	β-cubebene	0.04	0.03
22	geranial	0.08	0.02
23	carvone[b]	tr	tr
24	Δ-cadinene[b]	0.03	0.02
25	neryl acetate	0.03	0.02
26	perillaldehyde	tr	tr
27	carveol	0.04	0.04
28	nootkatone	0.21	0.03

[a] Less than 0.01%. [b] Tentative identification.

of oils were equivalent in limonene, but α-pinene, β-pinene, and myrcene appeared at higher concentrations in SDO than in CPO. It was more characteristic that nootkatone was about one-seventh less in SDO as compared with CPO. In the present experiment CPO had the strongly characteristic aroma of Tosa-buntan, while SDO had a weaker aroma. The stronger aroma was formed in the residue after steam distillation. A 3-h distillation seems to be insufficient to obtain the characteristic component of Tosa-buntan. The result suggests, however, that the characteristic components of Tosa-buntan aroma would be substances with higher boiling points. Hence, we suppose that one of the characteristic components contributing its aroma could be nootkatone, which is a major flavor contribution component of grapefruit (MacLeod and Buigues, 1964; Nursten, 1975). Tosa-buntan is recognized as a close relative to the grapefruit, so nootkatone may be a common constituent in these species.

Wilson and Shaw (1980) determined quantitatively 32 components in the cold-pressed grapefruit oil. There were great differences in γ-terpinene between Tosa-buntan and grapefruit: 5.78% and 0.12%, respectively. They also found a low level (0.02%) of nootkatone in a small sample of oil obtained in November. It was, however, discussed that the nootkatone content would increase up to 0.75–0.81% during the processing season (May–June). The level of nootkatone in Tosa-buntan harvested at the best season was about 3 times that in the grapefruit. Acetate esters are supposed to be important to the aroma of grapefruit oils (Moshonas, 1971). The grapefruit oil contained four acetate esters (Wilson and Shaw, 1980), 0.17% in all, while the buntan CPO contained 0.11% of three acetate esters.

Table II. Percentage[a] Intensities of Fragment Ions of Limonene and 1,8-Cineole

m/z	limonene	1,8-cineole	*m/z*	limonene	1,8-cineole
132	0.0	NF[b]	136	2.3 (M^+)	1.0
133	0.0	NF	137	0.3	0.1
134	0.0	0.5	154		5.0 (M^+)
135	0.0	0.0			

[a] Peak intensity percentage of total intensity. [b] NF = not found.

Recently 1-*p*-menthene-8-thiol was found to be an extremely powerful characteristic component of grapefruit (Demole et al., 1982). Assuming that the characteristic aroma of the buntan is similar to that of the grapefruit and the botanical relation is close between the two varieties, 1-*p*-menthene-8-thiol may occur in the Tosa-buntan oil as well. There are several citrus fruits closely related to Tosa-buntan, and their aroma is similar to that of Tosa-buntan. We are now further investigating the aroma from aspect of chemotaxonomy.

While 1,8-cineole is abundant in eucalyptus oils, small amounts also occur in citrus oils. Usually gas chromatography of citrus oils reveals the greatest peak to be limonene, which often hinders the very small peak of 1,8-cineole because of close retention times. Although 1,8-cineole can be qualitatively isolated from a fraction where hydrocarbns were eliminated by silica gel column chromatography, it is almost impossible to quantitate. That is one of the reasons why 1,8-cineole is not reported in recent papers dealing with the quantitative analysis of citrus oils. Since 1,8-cineole content was formed to be approximately $^1/_{25000}$th of that of limonene in the Tosa-buntan oil, it was impossible to separate and quantitate 1,8-cineole peak under the present gas chromatographic conditions. Therefore, we applied mass fragmentography.

Proper fragment ions were determined as given in Table II. As for 1,8-cineole, the parent ion (M^+ 154) was selected as a single ion monitoring peak, because its intensity was rather strong in addition to the rigidity of identity. As for limonene the *m/z* 133 fragment peak was selected, because the intensity was the slightest and 1,8-cineole had no fragment peak of *m/z* 133. As limonene is present in the oil sample at high concentration, the total intensity of collected ions became enormous and resulted in overlapping the capacity of the computer (Shimadzu Chromatopack 90), even if the fragment peak of limonene was not apparently so high on the mass spectrum.

The mass fragmentogram of Tosa-buntan oil is shown in Figure 2. The difference in retention time between limonene and 1,8-cineole was only about 0.02 min, but the two-channel (*m/z* 133 and 154) mass fragmentography gave perfect separation of them. 1,8-Cineole content was calculated from the ratio of the ion intensities of *m/z* 154 to 133. The precision of reproducibility was 4.5%, and this figure was close to 4.2% regarding the mass fragmentographic analysis of cholestane (Middleditch and Desiderio, 1973). The calibration curve gave good linearity through the origin. Consequently 1,8-cineole contents were determined in CPO and SDO to be 0.0035%.

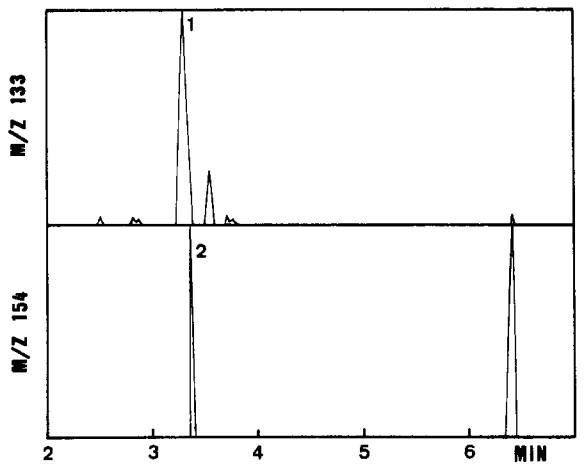

Figure 2. Mass fragmentogram of Tosa-buntan oil. Magnetic mass was m/z 131.0 from PFK. Peak 1 was limonene (R_t = 3.33 min), and peak 2 was 1,8-cineole (R_t = 3.35 min).

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Registry No. α-Pinene, 80-56-8; β-pinene, 127-91-3; myrcene, 123-35-3; limonene, 138-86-3; 1,8-cineole, 470-82-6; ocimene, 29714-87-2; γ-terpinene, 99-85-4; *p*-cymene, 99-87-6; terpinolene, 586-62-9; 2-carene, 554-61-0; isooctyl acetate, 31565-19-2; nonanal, 124-19-6; citronellal, 106-23-0; *n*-octyl acetate, 112-14-1; decanal, 112-31-2; linalool, 78-70-6; terpinen-4-ol, 562-74-3; β-caryophyllene, 87-44-5; β-terpineol, 138-87-4; α-terpineol, 98-55-5; β-cubebene, 13744-15-5; geranial, 141-27-5; carvone, 99-49-0; Δ-cadinene, 483-76-1; neryl acetate, 141-12-8; perillaldehyde, 2111-75-3; carveol, 99-48-9; nootkatone, 4674-50-4.

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Novel Monoterpene Diols and Diol Glycosides in *Vitis vinifera* Grapes

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The β-D-glucopyranoside and the 6-*O*-α-arabinofuranosyl-β-D-glucopyranoside of (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol were isolated from *Vitis vinifera* cv. Riesling grape juice and their structures determined by spectral methods. Additionally, the diastereoisomeric 3,7-dimethylocta-1,6-diene-3,5-diols and (*E*,*E*)-2,6-dimethylocta-2,6-diene-1,8-diol were identified for the first time in grapes, and these three diols together with (*E*)- and (*Z*)-2,6-dimethylocta-2,7-diene-1,6-diol were quantified both as free monoterpenes and as glycosides in the juice of eight grape varieties. Model experiments were carried out on (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol and the 3,7-dimethylocta-1,6-diene-3,5-diols to assess their roles as potential precursors of flavorants in juice and wine. The first compound, although largely resistant to acid hydrolysis, yielded (*E*,*E*)-2,6-dimethylocta-2,6-diene-1,8-diol as the major product and *p*-menth-1-en-9-al and 3,9-epoxy-*p*-menth-1-ene as trace products. The mixture of 3,7-dimethylocta-1,6-diene-3,5-diols decomposed to give (*E*)-2,6-dimethylocta-3,7-diene-2,6-diol, hotrienol, and nerol oxide, all of which are known wine components.

The significance of volatile monoterpenes to the flavor and varietal character of some cultivars of *Vitis vinifera* grapes is well documented (Strauss et al., 1986). In addition to those monoterpenes occurring free in the fruit, further volatiles are formed in juices by acid hydrolysis of free monoterpene polyols and glycosides that are also present. Enzymic studies have indicated that the monoterpene polyols can themselves be glycosylated in the fruit (Wilson et al., 1984), although the nature of these glycosides and the particular oxygens of the polyols that are glycosylated have, until lately, been uncertain. These points were recently clarified with the report, from this laboratory, of the occurrence in grape juice of two glycosides **1d** and **1e** (Figure 1) of (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol (**1a**) (Strauss et al., 1987).

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