BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 484—487 (1970)

A Comparative Study of the Constituents of Volatile Oils of *Zanthoxylum*

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(Received July 18, 1969)

The constituents of the volatile oils of three *Zanthoxylum* species were studied on the basis of the preceding exhaustive analysis of fruit oil from the Japanese pepper. It seems that the fragrance quality among the genera *Zanthoxylum* depends on the content of citronellal in the essential oil. That is, the unripe fruit oil of Japanese pepper with the strongest characteristic aroma contained the largest amount of citronellal. Ripe fruit oil of *Z. simulans* containing a trace amount of citronellal had a poor flavor.

According to Engler,[1] the genus *Zanthoxylum* (15 species) is distributed in the temperate regions of Asia and North America, and the genus *Fagara* (200 species), in the tropics. However, there has been much confusion in the nomenclature because of meager morphological distinction between these genera. A number of *Faraga* species are alternatively named *Zanthoxylum*, and *vice versa*. Since the differences of distribution and chemical constituents have been recognized, these two genera may be differentiated from the chemotaxonomic point of view. In our country, there are two endemic species, *i.e.*, *Z. piperitum* (Linn.) DC and *Z. planispinum* Sieb. et Zucc., and one horticultural species, *i.e.*, *Z. piperitum f. inerme* (Makino) Makino. Generally, the essential oils of *Zanthoxylum* constitute a complex mixture of terpenic compounds and are superior in fragrance. On the other hand, the essential oils of *Fagara* consist of aliphatic ketones and phenolic ethers and are inferior in aroma.

In previous communications, we reported on the compositions of volatile oils obtained from the fruits[2] and the young leaves[3] of *Zanthoxylum piperitum f. inerme*. This paper will present the results of our comparative study of the constituents of volatile oils from the ripe fruits of *Z. planispinium* (Fuyu-sanshoo in Japanese) and from the ripe fruits of *Z. simulans* (Hana-sanshoo, of Red Chinese origin), on the basis of the preceding exhaustive analysis of *Z. piperitum f. inerme* (Asakura-sanshoo).[2]

Experimental

Apparatus. *Gas Chromatography.* Preparative gas chromatography was carried out in a Varian 90-P gas chromatograph, fitted with a 10 ft-by-3/8 inch aluminum column packed with 20% Carbowax 20M or SF-96 on Chromosorb W. The flow rate of the helium carrier gas was 30—60 *ml*/min. The separated components were trapped in thin-walled glass capillaries. Analytical gas chromatography was performed in a Hitachi K-53 gas chromatograph, wherein two 45 m-by-0.25

1) A. Engler, "Syllabus der Pflanzenfamilien," Band II, Gebrüder Borntraeger, Berlin (1964), p. 263.

2) T. Sakai, K. Yoshihara and Y. Hirose, This Bulletin, **41**, 1945 (1968).

3) S. Kusumoto, A. Ohsuka, M. Kotake and T. Sakai, *ibid.*, **41**, 1950 (1968).

mm stainless steel capillary columns coated with HB-2000 and SF-96 were used.

Infrared Spectroscopy. The infrared spectra were taken as thin films between sodium chloride plates on a Hitachi EPI-G2 spectrometer equipped with a beam condenser.

Nuclear Magnetic Resonance Spectrometry. The NMR spectra were measured on a JEOL Model C-60 spectrometer at 60 MHz in carbon tetrachloride, using tetramethylsilane as the internal reference. The microcell technique was used.

Mass Spectrometry. The mass spectra were obtained on a Hitachi RMU-6 mass spectrometer. The operating conditions were as follows: ionization voltage, 80 eV; ion-accelerating voltage, 2000 V; temperatures of ionization chamber and sample injection block, 250 and 150°C respectively.

Preparation and Separation of the Volatile Oils. The volatile oil of the ripe fruits of Asakura-sanshoo which was used was the same as in a previous study.[2)] The volatile oils of the ripe fruits of Hana-sanshoo imported from Szechwan, Red China, and the ripe fruits of Fuyu-sanshoo cultivated at the Botanical Gardens of Osaka City University, Kisaichi, Osaka-fu,[*1] were obtained by steam distillation.

The volatile oil of Hana-sanshoo was fractionated by means of distillation and column chromatography. The volatile oil of Fuyu-sanshoo was divided into two fractions, hydrocarbons and oxygenated compounds, by silicagel column chromatography. Each fraction was further fractionated by preparative gas chromatography utilizing various columns.

Results and Discussion

Figure 1 shows the comparative capillary chromatograms of the ripe fruit oil of Asakura-sanshoo (top of Fig. 1), the ripe fruit oil of Hana-sanshoo (middle), and the ripe fruit oil of Fuyu-sanshoo (bottom). Each chromatogram was obtained by injecting 0.5—1.0 μl of the raw volatile oil into a HB-2000 capillary column. The column temperature was held initially at 80°C for 4 min; then it was programmed at 2°C per minute to 150°C, and run isothermally at 150°C until all the components had emerged. The nitrogen-carrier gas inlet pressure was 1.0 kg/cm^2. Attenuation was kept at 1 except otherwise indicated. The location of each component was determined by a manner reported previously.[2)]

The gas chromatogram of the Fuyu-sanshoo oil fairly well resembles that of the unripe fruit oil of the Asakura-sanshoo. Monoterpene hydrocarbon fractions of both oils contain almost the same components. A serious difference was, however, noted in that there were large amounts of citronellal and methyl cinnamate in the Asakura-sanshoo oil, while, on the contrary, there was only a small amount of citronellal and no methyl cinnamate in the Fuyu-sanshoo oil. A considerable amount of nerolidol was found in the Fuyu-sanshoo oil.

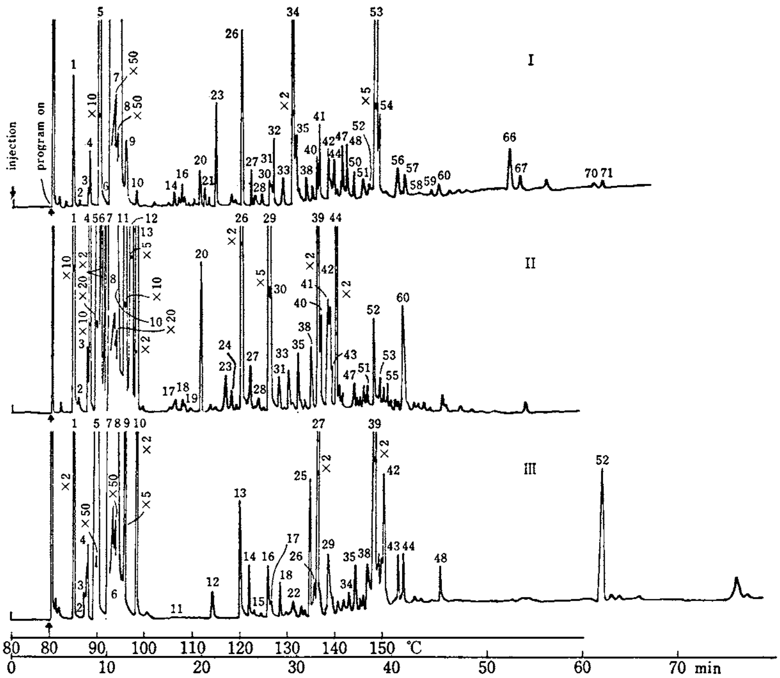

Fig. 1. Gas chromatograms of the genus *Zanthoxylum* I. Asakura-sanshoo oil, II. Hana-sanshoo oil, III. Fuyu-sanshoo oil 45 m×0.25 mm HB-2000 column; temperature isothermal at 80°C for 4 min then programmed at 2°C per minute to 150°C and run isothermal thereafter; sample 0.5—1.0 μl; range 1×1 except as indiated.

[*1] The authors thank Botanical Garden of Osaka City University for supplying ripe fruits of Fuyu-sanshoo.

TABLE 1. CONSTITUENTS OF VOLATILE OILS FROM ASAKURA-SANSHOO, HANA-SANSHOO, AND FUYU-SANSHOO

Identified component	Kovats' indices		Peak No. in Fig. 1		
	HB-2000	SF-96	Asakura-sanshoo	Hana-sanshoo	Fuyu-sanshoo
α-Pinene	994	948	1	1	1
Camphene	1025	959	2	2	2
β-Pinene	1060	987	3	3	3
Sabinene	1066	980	4	4	4
Myrcene	1095	987	5	5	5
α-Phellandrene	1105	1007	6	6	6
α-Terpinene	1118	1019		7	
Limonene	1135	1034	7	8	7
1,8-Cineol	1137	1035	7	9	
β-Phellandrene	1145	1034	8	10	8
Ocimene	1156	1089		11	
p-Cymene	1172	1025	9	12	9
γ-Terpinene	1172	1058		12	
Terpinolene	1203	1024	10	13	10
Isobutyl isovalerate	1223	1094	11		
Isobutyl caproate	1274	1142	14		
Alloocimene	1276	1121	14	17	
Perillene	1290	1100		18	
β-Terpinen-3,4-oxide	1307	1048	16		
p-Isopropenyltoluene	1309	1092	17	19	
cis-Linalool oxide	1335	1100		20	
trans-Limonen-1,2-oxide	1341	1146	20		
cis-Limonen-1,2-oxide	1346	1146	21		
Citronellal	1352	1146	23	22	12
Linalool	1376	1101	26	26	13
2-*p*-Menthen-1-ol	1396	—	27	27	14
Isopulegol	1424	1162	28	28	15
Terpinolen-4,8-oxide	1432	1218	28		
trans-2,8-Menthadien-1-ol	—	—	28		
Linalyl acetate	1437	1246	30	29	16
cis-2,8-*p*-Menthadien-1-ol	1438	—	30		
Terpinene-4-ol	1449	1191	31	30	16
Dihydrocarvone	1444	1211	32		
α-Copaene	1455	1389	32	30	17
Cryptone	1501	1200	34	33	22
Methyl chavicol	1498	1200	35	34	
Limonen-4-ol	1501	1071	38	37	24
β-Elemene	1515	1403	39	38	25
α-Terpineol	1516	1204	40	39	26
β-Caryophyllene	1530	1425	41	40	27
Carvone	1531	1246	42		
Piperitone	1548	1260	42	41	29
Citronellyl acetate	1540	1342	43	42	30
Phellandral	1555	1246	44	43	31
Terpinyl acetate	1558	1348	47	44	33
Cuminaldehyde	1562	1271	48	45	34
Perillaldehyde	1586	1280	49		
Humulene	1583	1457	50	47	35
1(7),8-*p*-Menthadien-*trans*-2-ol	—	—	51		
Citronellol	1594	1348	51		
γ-Muurolene	1600	1475	52	51	38
Geranyl acetate	1605	1370	53	52	39
trans-Carveol	1617	1246	54	53	40

TABLE 1. (Continued)

Identified component	Kovats' indices		Peak No. in Fig. 1		
	HB-2000	SF-96	Asakura-sanshoo	Hana-sanshoo	Fuyu-sanshoo
α-Muurolene	1628	1495	55	55	42
cis-Carveol	1633	1255	55		
Geraniol	1653	—	56		
δ-Cadinene	1655	1515	57	60	44
γ-Cadinene	1656	1436	57	60	44
Piperitenone	1676	1216	58		
Calamenene	1693	1513	59	64	48
α-Calacorene	1745	1531	60	66	
Methyl cinnamate	1766	1394	66		
Cuminalcohol	1798	1322	67		
β-Caryophyllene alcohol	1828	1540	70		
Nerolidol	1842	1597	71		52

The gas chromatograms show that the Hana-sanshoo oil is very rich in monoterpene hydrocarbons, we noticed several compounds, such as γ-terpinene, α-terpinene, ocimene, alloocimene, and perillene, which were not found in the oils of the other two species. A large amount of linalool oxide was also found in the Hana-sanshoo oil. It is a remarkable difference that citronellal, methyl cinnamate, and nerolidol were not found in this oil.

Table 1 lists the components, which were confirmed by a comparison of their Kovats' indices and their infrared, nuclear magnetic resonance, and mass spectra with those of authentic samples and the literature data. Kovats' indices were determined under isothermal conditions on 45-m capillary columns (α-pinene through perillene, HB-2000 column at 80°C, 1.0 kg/cm^2 N_2 : SF-96 column at 100°C, 0.5 kg/cm^2 N_2; β-terpinen-3,4-oxide through nerolidol, HB-2000, 130°C, 1.0 kg/cm^2 : SF-96, 130°C, 0.5 kg/cm^2).

Obviously, the young leaves and the unripe fruits of Asakura-sanshoo are most fragrant, and their essential oils contained the largest amount of citronellal. A parallelism is observed between the fragrance and the content of citronellal in a downward order from the ripe fruit of the Asakura-sanshoo through the ripe fruit of the Fuyu-sanshoo and Hana-sanshoo oils. The latter had a rather disagreeable odor because of the large amounts of myrcene and ocimene which replaced the citronellal.

As far as was tested, no identified component has been estimated to be essential to the characteristic flavor of the Sanshoo oil. The essential oil of Sanshoo loses its characteristic odor when stored for a week at room temperature in a tightly-sealed tube. The gas chromatogram of this deteriorated oil shows the disappearance of the peak of β-phellandrene and a decrease in the amounts of citronellal, myrcene, and terpinolene. On the other hand, the amounts of isopulegol, *cis*-2,8-*p*-menthadien-1-ol, dihydrocarvone, limonen-4-ol, carvone and piperiton, geranyl acetate, *trans*- and *cis*-carveols, cuminalcohol, β-caryophyllene alcohol, and, especially, cryptone increased.

Therefore, it may be concluded that the fragrance of the genus *Zanthoxylum* consists mainly in the characteristic odor of citronellal and depends on its content.