BULLETIN OF THE CHEMICAL SOCIETY OY JAPAN VOL. 41 1950—1953 (1968)

Constituents of Leaf Oil from Japanese Pepper

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(Received February 20, 1968)

The steam-volatile constituents from leaves of Japanese pepper were separated by column chromatography and GLC, and their chemical structures were studied by IR, NMR, and mass spectrometry. The volatile oil was found to be composed of about 100 components; those identified were as follows: 15 hydrocarbons (α- and β-pinene, camphene, sabinene, myrcene, α- and β-phellandrene, limonene, *p*-cymene, terpinolene, α-copaene, caryophyllene, humulene, α-muurolene, and γ-cadinene), 16 alcohols (2-penten-1-ol, *n*-hexanol, 3-hexen-1-ol, *trans*- and *cis*-sabinene hydrate, *cis*-linalool oxide, linalool, isopulegol, neoisopulegol, terpinen-4-ol, α- and δ-terpineol, limonen-4-ol, benzyl alcohol, β-phenylethyl alcohol, and nerolidol), 2 aldehydes (2-hexenal and citronellal), 1 ketone (cryptone), 7 esters (isobutyl caproate, linalyl acetate, citronellyl acetate, terpinyl acetate, geranyl acetate, methyl salicylate, and methyl cinnamate) and 2 ethers (1,8-cineol and methyl chavicol).

The fruits and the young leaves of Japanese pepper (*Xanthoxylum piperitum* DC. (Japanese name, "Sanshoo"), and a variety of it, *X. piperitum var. inerme* Makino ("Asakura-sanshoo")) are widely used as spice in our country because of their characteristic odor and burning taste.

The essential oil of the fruits has been studied by several investigators, and recently almost all the components of the oil were identified by one of the present authors, Tsutomu Sakai.[1] The components responsible for its pungent taste have been shown to be α- and β-sanshool by Crombie and Tayler.[2] However, the constitution of the leaves has not yet been studied. Therefore, the study of the composition of the volatile oil isolated from the young leaves of "Asakura-sanshoo"

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

[2] L. Crombie and J. L. Tayler, *J. Chem. Soc.* **1957**, 2760.

was undertaken.

Experimental

Extraction and Steam Distillation. 1) Twenty-six kilograms of fresh young leaves of *X. piperitum* DC. *var. inerme* Makino (collected at the beginning of June in Kyoto Prefecture) were extracted three times with redistilled petroleum ether (bp below 50 ℃) at room temperature. The residue obtained by the evaporation of the solvent was steam-distilled under reduced pressure (30—40 mmHg). The distillate was saturated with sodium chloride and extracted with petroleum ether. The solvent was evaporated after the removal of acids to give a colorless oil (about 37 g).

2) Twelve kilograms of the leaves (see above) were extracted with ether instead of petroleum ether. About 11 g of a pale yellow oil was obtained.

Silica-gel Chromatography of the Essential Oil. The essential oils obtained in 1) and 2) were combined and placed in a column packed with 300 g of silica-gel (Merck 0.05—0.2 mm). This mixture was eluted first with petroleum ether, and then with petroleum ether containing various concentrations of ether (from 2 to 50%). The fractions obtained are summarized in Table 1.

TABLE 1. FRACTIONS OF THE SILICA GEL CHROMATOGRAPHY

Fraction	Wt, g	Constituent
A	26.0	Hydrocarbons
B	7.0	Ethers and esters
C	1.9	Ethers, aldehydes, esters and alcohols
D	0.4	Ethers, aldehydes, esters and alcohols
E	1.4	Alcohols
F	1.0	Alcohols
G	0.2	Alcohols
H	0.3	Alcohols

The Isolation of the Components by Preparative Gas-liquid Chromatography (GLC). Each fraction obtained by silica-gel chromatography was distilled under reduced pressure, and the components of the distillate were isolated by preparative GLC under suitable conditions. A Shimadzu GC-2B-type gas chromatograph with a copper spiral column (4 mm in diameter, 5 m in length) was used in the preparation. Glass capillaries were employed as receivers, and hydrogen, as the carrier gas. The conditions employed are shown in Table 2.

TABLE 2. CONDITIONS OF PREPARATIVE GAS CHROMATOGRAPHY

	Stationary phase	Column temp. ℃	H_2 Flow rate ml/min
a)	PEG 6000	120	20
b)	PEG 20 M	160	25
c)	PEG 20 M	170	35
d)	PEG 20 M	175	25
e)	DEGSPE*	120	20
f)	DEGSPE	140	20
g)	DEGSPE	160	25
h)	DEGSPE	170	30

* Diethyleneglycol succinate polyester

The Identification of the Isolated Components. The isolated components were identified by means of IR, NMR,[*1] and mass spectrometry, and by a direct comparison of the retention times in an analytical GLC with those of authentic samples. The analytical GLC was carried out using a Hitachi Perkin-Elmer Model F-6D gas chromatograph provided with a flame-ionization detecter. Stainless-steel capillary columns (0.25 mm in diameter and 45 m in length) were coated with HB-2000 and FS-45. The column temperature was programmed from 70 to 150℃, or was held isothermally at 80, 110, and 130℃. The nitrogen-carrier gas-inlet pressure was 1.0 kg/cm^2.

Synthesis of *trans*- and *cis*-Sabinene Hydrate. A mixture of sabinene (1.05 g), potassium permanganate (0.21 g), potassium periodate (7.35 g), and potassium carbonate (0.52 g) in 200 ml of water was stirred continuously for 39 hr at room temperature. After the manganese dioxide precipitated had been removed by filtration, the aqueous layer was extracted with ether; this ether extract was then washed with water, dried, and concentraed under reduced pressure to give 0.6 g of a colorless oil; ν_{max}^{film} 1720 cm^{-1}. The oil was found by its gas chromatogram to be composed of almost pure sabinaketone.

A solution of this product in 3 ml of absolute ether was added, drop by drop, to a solution of methylmagnesium bromide (which had been prepared from 0.21 g of magnesium) in 10 ml of absolute ether; the mixture was refluxed for 40 min with occasional shaking, and then, after cooling, the excess reagent was decomposed with crushed ice. The product was isolated by steam distillation and by the extraction of the distillate with ether. *trans*- and *cis*-Sabinene hydrate were isolated from this crude product (0.53 g) by preparative GLC (PEG 20 M, 170℃); the *trans*-isomer, mp 59—60℃ (Lit.[4]) 60—61℃), and the *cis*-isomer, mp 36—37.5 ℃ (Lit.[4]) 38—39℃).

Results and Discussion

Since the essential oils prepared from fresh leaves of "Asakura-sanshoo" by extraction with either ether or petroleum ether were found through their gas chromatograms to be composed of almost identical substances, the

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[*1] NMR spectra were measured in carbon tetrachloride using TMS as internal reference at 60 Mc on a JEOL model C-60 spectrometer.

3) B. M. Mitzner and S. Lemberg, *Am. Perf. & Cosm.*, **81**, 25 March, 37 May (1966).

4) J. W. Daly, E. C. Green and R. H. Eastman, *J. Am. Chem. Soc.*, **80**, 6330 (1958).

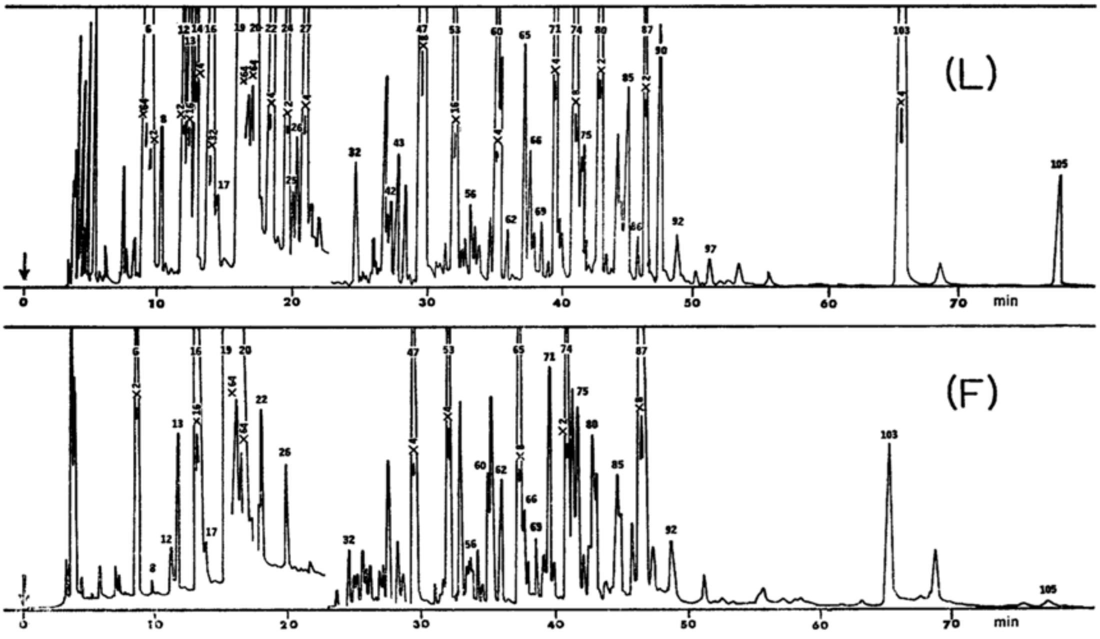

Fig. 1. Analytical gas liquid chromatograms of the essential oils.
(L) The essential oil of the leaves
(F) The essential oil of the fruits

two oils were combined for further study. Figure 1 shows the gas chromatograms of the whole essential oil obtained from young leaves by ether extraction and the whole volatile oil of the fruits.[1] These chromatograms were obtained by injecting 1.0 μl of each oil into an HB-2000 capillary column. The column temperature was initially held at 70°C for 3 min, then programmed at 2.5°C/min to 150°C and ran isothermally at 150°C until all the components had emerged. Table 3 lists the components, which were identified by means of spectral studies.

The compounds responsible for the characteristic odor of Japanese pepper, however, were not obtained in this instance, just as in the earlier work.[1] Therefore, the natural odor may be attributed to a mixed effect of many components.

Limonene and β-phellandrene were the main constituents of the monoterpene hydrocarbons, accounting for about 60% of the total volatile oil.

The major components of the oxygenated compounds were 1,8-cineol, citronellal, linalool, and methyl cinnamate, followed by terpinen-4-ol, α-terpineol, terpinyl acetate, and geranyl acetate.

Although citronellal could be isolated directly from the essential oil by preparative GLC, it disappeared completely after the oil had been subjected to silica-gel column chromatography, and the amounts of isopulegol and neoisopulegol were more than those found to be present in the original oil. Therefore, these alcohols may partly be formed as a result of the isomerization of citronellal on the silica-gel column.

One compound, which was masked by the peak (53) due to linalool in Fig. 1, was isolated from fractions E and F by preparative GLC. Its IR spectrum showed the presence of the tertiary hydroxyl group (ν_{max}^{film} 3370, 1135 cm^{-1}), the isopropyl group (1380, 1364 cm^{-1}), and the methylene group in a cyclopropane ring (3050 cm^{-1}). Its NMR spectrum also suggested the presence of an isopropyl group ($\delta=0.88$, 0.93 ppm, each 3H d, $J=6$ cps), two protons of a cyclopropane ring ($\delta=0.23$ ppm, 1 H dd, $J=9$, 5.3 cps and $\delta=0.70$ ppm, 1H dd, $J=4.5$, 5.3 cps), and a methyl group on a carbon atom bearing an oxygene function ($\delta=1.28$ ppm, 3H s). No sign of a double bond was found in either of the spectra. The mass spectrum showed a molecular-ion peak at m/e 154 ($C_{10}H_{18}O$, 4%) and a base peak at m/e 93. Furthermore, its mass spectrum lower than m/e 136 (M–H_2O) was similar in every respect to that of sabinene. These results suggest that this alcohol is sabinene hydrate (4-thujanol). In order to confirm this, *cis*- and

TABLE 3. COMPOUNDS IDENTIFIED FROM LEAF OIL OF JAPANESE PEPPER

Peak No. in Fig. 1.	Compound	Fr. in Table 1.	Conditions in Table 2
6	α-Pinene	A	a
8	Camphene*	A	
12	β-Pinene*	A	
13	Sabinene	A	a
14	2-Hexenal**	C	g, e
16	Myrcene	A	a
17	α-Phellandrene*	A	f, g
19	1, 8-Cineol	B, C	f
19	Limonene	A	a
20	β-Phellandrene	A	a
22	*p*-Cymene	A	a
24	2-Penten-1-ol**	F, G	b
25	*n*-Hexanol**	F, G	b
26	Terpinolene*	A	
27	3-Hexen-1-ol**	F, G	b, d
32	Isobutyl caproate*		
42	*cis*-Linalool oxide**	F	d
43	*trans*-Sabinene hydrate**	E	d
47	Citronellal		
53	Linalool	E	d
53	*cis*-Sabinene hydrate**	E, F	d
56	Isopulegol	C, D, E, F	d
56	Neoisopulegol	C, D	g
60	Linalyl acetate	B	d
60	Terpinen-4-ol	C, D, E	d
62	α-Copaene	A	c
65	Cryptone*		
66	Methyl chavicol*		
69	Limonen-4-ol	E	d
70(?)	δ-Terpineol[3]	F	d
71	α-Terpineol	F	d
74	Caryophyllene	A	c
75	Citronellyl acetate	B	d
80	Terpinyl acetate	B	d
85	Humulene	A	c
86	Benzyl alcohol**	G, H	g
	Methyl salicylate**	B	d
87	Geranyl acetate	B	d
90(?)	β-Phenyl ethyl alcohol**	G, H	g
92	α-Muurolene	A	c
97	γ-Cadinene	A	c
103	Methyl cinnamate	C, D	h
105	Nerolidol		h

* These were not isolated but identified by analytical GLC in comparison with the retention times of the components of fruits.

** These were not isolated from the fruit oil.

trans-sabinene hydrate were synthesized according to the method of Daly.[4] The alcohol thus isolated was found to be identical with the synthetic *cis*-isomer with respect to the IR, NMR, and GLC spectra. On the other hand, the *trans*-isomer was isolated in an impure state, as was indicated by an analytical gas chromatogram.

The IR spectrum of peak (24) in Fig. 1 indicates the presence of the primary hydroxyl group (ν_{max}^{film} 3350, 1040, 1000 cm^{-1}) and the double bond (1650 cm^{-1}). Its mass spectrum showed a molecular-ion peak at *m/e* 86 ($C_5H_{10}O$, 9%), a base peak at *m/e* 57, and strong peaks at *m/e* 68 ($M-H_2O$, 16%), 44 (33%), 41 (41%), and 29 (38%). These results suggest that this alcohol is a primary pentenol possessing no terminal methylene group. There are four possible structures consistent with the data given above, 2-methyl-2-buten-1-ol, 3-methyl-2-buten-1-ol, 3-penten-1-ol, and 2-penten-1-ol. The isolated alcohol was found not to be identical with the former two when examined by IR and mass spectra. It was considered to be the last one because of the fact that its mass spectrum showed a strong base peak at *m/e* 57, suggesting the presence of the $-CH=CH-CH_2OH$ group. The configuration of the double bond is probably the cis form, judging from its IR spectrum.

In addition to the compounds listed in Table 3, several esters, monoterpenic alcohols, and sesquiterpenic hydrocarbons were isolated, but their structures could not be deduced because of the small quantities present.

It is apparent from Fig. 1 that the main components of the leaves mentioned above are also present in high concentrations in the oil obtained from the fruit. However, their relative quantities varied considerably from case to case. For instance, cryptone, a relatively large amount of which was found in the fruit oil, was shown to exist in a much smaller amount in the leaf oil. Other carbonyl compounds, such as carvone, piperitenone, phellandral, and cuminaldehyde, isolated from fruit oil were not isolated from the leaf oil. In the leaf oil, on the other hand, caryophyllene was found in greater quantities than in the fruit oil.

Moreover, the aliphatic compounds, such as 2-hexenal (leaf aldehyde), 2-penten-1-ol, *n*-hexanol, and *cis*-3-hexen-1-ol (leaf alcohol), had the characteristic odor of the leaves.

It is still impossible at the present time to show the differences between the leaf and fruit oil, since they were not collected in the same localities and seasons and since different methods were used to obtain the oils.

The authors are grateful to Dr. Yoshio Hirose of the Institute of Food Chemistry for his suggestions and helpful discussions during the course of this work.