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Monoterpene Constituents of the Essential Oil of Mitsuba (*Cryptotaenia japonica* Hassk.)

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Mitsuba, *Cryptotaenia japonica* Hassk., which is an herb of the Umbelliferae family, is cultivated as a vegetable in Japan for its fresh flavor. The chemical constituents of the essential oil of mitsuba have been investigated by Hirao[1] in fairly long ago; he characterized the main component of the monoterpene fraction as a new terpene compound with a structure of 2-methylnonatriene, and he gave it the name "Cryptotaenene." However, its structure still has not been completely established.

The essential oil was obtained in a 0.013% yield by the steam distillation of the whole herb; it was then separated into a monoterpene fraction (26%) and a sesquiterpene fraction (40%).

A gas chromatogram of the monoterpene fraction is shown in Fig. 1.

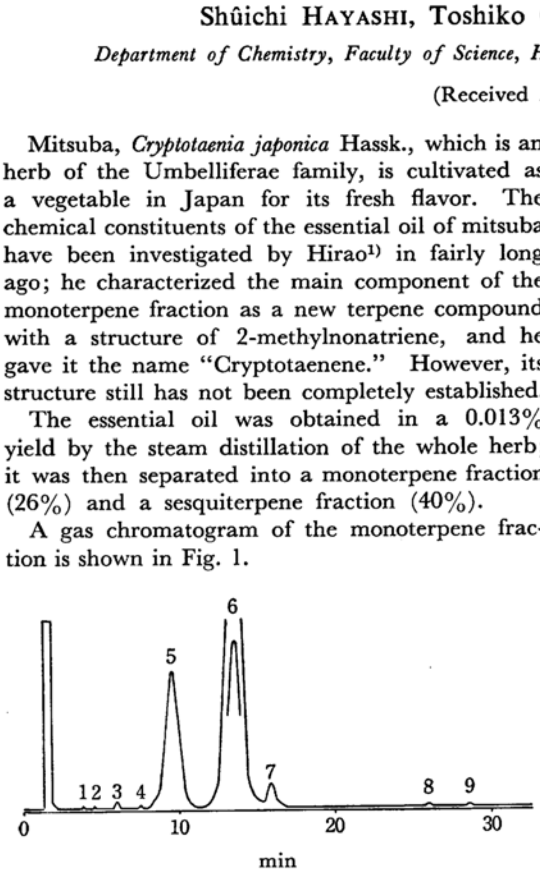

Fig. 1. Gas chromatogram of mitsuba oil at 65°C on Carbowax-6000-Diasolid L.

The main component of the monoterpene fraction, Peak 6 in Fig. 1, was isolated by preparative gas chromatography as a colorless, oily compound with a characteristic odor. The compound had a molecular formula of $C_{10}H_{16}$ (M^+, m/e 136). Its IR spectrum indicated the presence of a terminal methylene group (890—910 cm^{-1}) and a conjugated diene system (1596 cm^{-1}), and its NMR spectrum showed the presence of two allylic methyl groups (1.59 and 1.67 ppm, each 3H), a vinyl group attributable to an ABX-type split (6.36, 1H, q, $J=18$, 11; 5.19, 1H, q, $J=18$, 1.2; 5.00 ppm, 1H, q, $J=11$ and 1.2 cps), and a terminal methylene group (4.96 ppm, 2H, s). These IR and NMR spectra and its mass spectrum were in good agreement with those[2,3] of β-myrcene.

An adduct of this compound with maleic anhydride and its free acid were found to be identical with those of β-myrcene by a mixed-melting-point determination; moreover, a bromide which was obtained by the reduction of this monoterpene with sodium and alcohol, followed by bromination, showed a melting point corresponding to that of tetrabromide of dihydromyrcene. Thus, it is certain that the main component is β-myrcene.

The components of the 3, 5, and 7 peaks were isolated and identified as α-pinene, (+)-β-pinene, and (−)-limonene respectively on the basis of the measurement of the IR and the mass spectra; the minor components of the 4 and 8 peaks were also identified as camphene and *p*-cymene by means of gas-chromatographic comparison with authentic specimens. In addition, α-thujene and sabinene were found to be included in Peak 3 or 5 in the gas chromatogram of Carbowax-6000 (Fig. 1).

The relative composition of this essential oil was calculated to be as shown in Table 1 from the peak areas in the gas chromatogram as measured by Carbowax-6000 at 65°C. The main components of the essential oil of *Cryptotaenia japonica* were β-myrcene and (+)-β-pinene.

Hirao's *cyrptotaenene*, which was separated by

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[1] N. Hirao, *Kogyo Kagaku Zasshi (J. Chem. Soc. Japan, Ind. Chem. Sect.)*, **29**, 48 (1926).

[2] G. Ohloff, J. Seibl and E. sz. Kovâts, *Ann. Chem.*, **675**, 83 (1964); B. M. Mitzner, E. T. Theimer, L. Steinbach and J. Walt, *J. Org. Chem.*, **30**, 646 (1965).

[3] A. F. Thomas and B. Willhalm, *Helv. Chim. Acta*, **47**, 475 (1964); R. Ryhage and E. von Sydow, *Acta Chem. Scand.*, **17**, 2025 (1963).

Table 1. Monoterpene hydrocarbons of the essential oil of *Cryptotaenia japonica*

No. of peak in Fig. 1	Compound identified	Retention time*		Relative content (%)
		Apiezon L	β,β'-Hydroxydipropionitrile	
1	unidentified	—	—	trace
2	unidentified	—	—	trace
3	α-pinene	7.80	5.20	1.5
	α-thujene	6.90	5.95	trace
4	camphene	8.95	7.15	0.1
5	(+)-β-pinene	10.50	8.80	34.9
	sabinene	9.35	11.65	trace
6	β-myrcene	9.10	15.10	56.9
7	(−)-limonene	14.30	15.10	56.9
8	*p*-cymene	13.35	35.70	trace
9	unidentified	—	—	trace

* Operating condition. Apiezon L: 110°C, N_2 17 m*l*/min; β,β'-hydroxydipropionitrile: 60°C, N_2 15 m*l*/min.

fractional distillation only and which was characterized as a new terpene hydrocarbon on the basis of the difference of its physical properties from those of β-myrcene, seems to be β-myrcene contaminated with a small amount of (+)-β-pinene; this conclusion is based on the closeness of the boiling points of β-myrcene and β-pinene. This view is also supported by a comparison of the physical constants of cryptotaenene, bp 67—68°C/15 mmHg, $[\alpha]_D^{19.8}$ +2.66°, d_4^{25} 0.8128, n_D^{25} 1.47476, with those of β-myrcene and β-pinene.

Experimental

For the measurement of the gas chromatograms, a Shimadzu GC-2B apparatus equipped with a hydrogen-flame-ionization detector was used in combination with three kinds of separation columns (4 mm × 3 m): Carbowax-6000 on Diasolid L (3%), Apiezon L on Gas-Chrom P (4.6%), and β,β'-hydroxydipropionitrile on Diasolid H (10%). Preparative gas chromatography was carried out by using Carbowax-6000 on Celite (20%) (8 mm × 3 m). The NMR spectrum was measured in a $CDCl_3$ solution.

Steam Distillation of the Essential Oil. Mitsuba (1323 kg) cultivated in the suburbs of Hiroshima City was harvested, together with its roots, in May, 1966, by which time it had grown to a height of 40—50 cm. It was air-dried in the shade for several hours and then distilled with steam. In addition to an oil (I) (104 g, d_4^{25} 0.8637, $[\alpha]_D^{25}$ +52.44°, n_D^{25} 1.4888) floating upon the distilled water, an oil (II) (68 g, d_4^{25} 1.1101, n_D^{25} 1.4831) was obtained by extracting the distilled water with ether and chloroform. In the present experiment, the oil (I) (100 g) was fractionated into thirty-four fractions *in vacuo*; the monoterpene fractions (Fr. 1—11), bp 82—140°C/50 mmHg, totaled 26.46 g, and the sesquiterpene fractions (Fr. 12—34), bp 84.5—168.0°C/3 mmHg, 40.30 g.

α-Pinene and α-Thujene (Peak 3). The hydrocarbon, $C_{10}H_{16}$ (M^+, *m/e* 136), was isolated by the preparative gas chromatography of Fr. 2 (4.1 g, $[\alpha]_D^{18}$ +13.5°, 9% content of α-pinene). Its IR and mass spectra were in good agreement with those of α-pinene;[3,4] its identity was also confirmed by gas-chromatographic comparison with an authentic specimen, a comparison which was carried out by the use of three separation columns.

When the isolated hydrocarbon was re-gaschromatographed with Apiezon L or β,β'-hydroxydipropionitrile, it showed a small peak in addition to the main peak of α-pinene (*cf.* the third and fourth columns in Table 1). This small peak was assigned to α-thujene on the basis of a comparison with an authentic specimen.

(+)-β-Pinene and Sabinene (Peak 5). The hydrocarbon, $C_{10}H_{16}$ (M^+, *m/e* 136), was isolated from Fr. 2. Its IR and mass spectra and gas chromatogram were in good agreement with those of β-pinene.[3,4] In gas chromatography using Apiezon L or β,β'-hydroxydipropinonitrile, this isolated hydrocarbon exhibited a small peak in addition to the main peak of β-pinene; this small peak was assigned to sabinene by using an authentic specimen.

The β-pinene in the essential oil was classified as *d*-form on the basis of the positive optical rotation of Fr. 3 containing 66% of β-pinene ($[\alpha]_D^{18}$ +14.7°).

β-Myrcene (Peak 6). The component was separated from Fr. 7 (3.7 g, $[\alpha]_D^{18}$ −1.4°). Its IR ν_{max}: 3080, 1792, 1633, 1596, 1378, 1104, 989, 902, 890 and 820 cm^{-1}. Its mass spectrum: *m/e* 136 (M^+, 3.9%), 93 (82.8), 69 (64.5) and 41 (100).

Maleic Anhydride Adduct and Its Dibasic Acid. To 2.5 g of Fr. 6 containing 85% of Peak 6, 1.7 g of maleic anhydride was added; the mixture, after having been allowed to stand for two days at room temperature in a tightly-corked tube, was distilled to obtain a maleic anhydride adduct (bp 158—164°C/2 mmHg, 2.86 g), mp 28—30°C.[5] Its IR ν_{max}: 1835 and 1772 cm^{-1}. This adduct, after being dissolved in ether, was treated with 3% aqueous solution of sodium hydroxide in order to convert it into free acid (1.24 g), which was obtained as white needles, mp 122—123°C, in recrystallization from acetone. No depression was observed in a mixed-

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4) L. Friedmann and A. P. Wolf, *J. Am. Chem. Soc.*, **80**, 2424 (1958).

5) L. Ruzick, *Helv. Chim. Acta*, **19**, 423 (1936).

melting-point determination with an authentic specimen; mp 122—123°C.[5]

Tetrabromodihydromyrcene. 2.0 g of Fr. 7 containing 96% of Peak 6 was reduced with sodium (1.3 g) in absolute ethanol (15 m*l*) to obtain an oily product (bp 83—84°C/5 mmHg, 1.79 g). Its IR $\nu_{\max}$: 1660 and 823 cm^{-1}. The bromination of the product furnished a light yellow mass which showed a mp of 91.0—91.5°C[6] after being recrystallized from ethanol and ether. Its IR $\nu_{\max}$: 1370 and 1363 cm^{-1}.

(−)-Limonene (Peak 7). The hydrocarbon was isolated as an unreacted oil in the maleic anhydride-treatment of Fr. 10 (0.6 g, $[\alpha]_D^{18}$ −58.56°), which was contaminated with 10% of *β*-myrcene. It was gas-chromatographically identical with limonene; its IR and mass spectra also coincided with those of limonene.[3,4]

Camphene and *p*-Cymene. Although no attempt was made to isolate these compounds because of their minor components, the 4 and 8 peaks were identified as camphene and *p*-cymene respectively by comparison with authentic specimens in gas chromatography using the three separation columns.

The authors are deeply indebted to Professor Shô Itô of Tohoku University for his kind measurement of the NMR spectrum.

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[6] F. W. Semmler, *Ber.*, **34**, 3126 (1901).