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Comparative Biochemical and Chemo-taxonomical Studies of the Essential Oils of *Magnolia salicifolia* Maxim. I[1,2]

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The essential oils of *Magnolia salicifolia* Maxim. gathered at Mt. Ohira and its vicinity, Kawanishi-shi, Hyogo Prefecture have been examined. The characteristics of the shoot oils are abundant existence of methylchavicol (13.5—22.5%), safrole (17.0—24.6%), and methyleugenol (21.0—43.5%).

Magnolia salicifolia Maxim. (Japanese name, "Tamushiba") of the Magnoliaceae, grows widely in Japan on Honshu and Kyushu,[4] and has several variations in the essential oil components and the shape of leaves.

From the view-point of chemical systematics, comparative studies of the essential oils of the materials gathered from various localities in Japan have been performed.

In this paper, the results of the examination of the essential oils obtained from the plants gathered at Mt. Ohira and its neighborhood, Kawanishi-shi, Hyogo Prefecture will be reported firstly.

Experimental

Materials——The materials were gathered at Mt. Ohira (270 m above the sea level) and Yanagi-dani (about 200 m above the sea level) in Kawanishi-shi, Hyogo Prefecture.

Shoots——Sample II: The material was gathered from some trees near the top of Mt. Ohira on June 29, 1966. The average length of a shoot: 70 cm; its weight: 37 g; the sample consisted of 51% leaves and 49% branchlets.

Sample IV: The material was gathered from a tree (5 cmϕ) at a place 170 m above the sea level of the same mountain on November 2, 1967. The average length of a shoot: 35 cm; its weight: 13 g, it was consisted of 69% leaves and 31% branchlets.

Sample V: The material was gathered from a tree (4 cmϕ) at Yanagi-dani on November 5, 1967. The average length of a shoot: 35 cm; its weight 5.7 g, it was consisted of 70% leaves and 30% branchlets.

Leaves——Sample III_A: The material was gathered from some trees at the place about 170 m height of Mt. Ohira on November 1, 1966. The average length of a shoot: 55 cm; its weight: 27 g, it was consisted of 67% leaves, plus a small amount of buds, and 33% branchlets. The leaves of these shoots were used as the material.

Branchlets and Trunk——Sample I: The material was gathered from a tree at a place about 170 m height of Mt. Ohira on April 6, 1966. The tree was in the last stage of blooming and had not any leaf.

Sample III_B: The branchlets of the sample III.

Sample VI_A: The material was gathered from a tree (7 cmϕ) at Yanagi-dani on April 9, 1968. The tree was under blooming and the branchlets less than 1 cmϕ were used.

Sample VI_B: The trunk bigger than 1 cmϕ of the sample VI.

Flower——Sample VI_C: The flowers collected from the tree of the sample VI.

Sample VII: The flowers collected from some trees of the same place with the sample VI on April 9, 1968.

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1) This forms "Miscellaneous Contributions to the Essential Oils of the Plants from Various Territories. XXXI." Part XXX: S. Fujita and Y. Fujita, *Yakugaku Zasshi*, **92**, 1285 (1972).
2) A part of this work was presented at the 11th Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics of the Chemical Society of Japan, Matsuyama, October, 1967.
3) Location: *a*) *Ikebiraki-cho, Nishinomiya-shi, Hyogo*; *b*) *Midorigaoka, Ikeda-shi, Osaka.*
4) J. Ohwi, "Flora of Japan," Revised ed., Shibundo, Tokyo, 1965, p. 649.
5) S. Fujita and Y. Fujita, *Yakugaku Zasshi*, **90**, 1514 (1970).

Isolation of the Essential Oils——The fresh materials cut in small pieces were subjected to steam distillation on every next day. The distilled oils were extracted with ether and dried over anhydrous sodium sulfate. After the distillation of the ether, essential oils were obtained.

The weight of fresh materials used, the oils obtained, the yields of oil to the fresh material, and the physical properties of each oil were shown in Table I.

Table I. Properties of the Materials and the Essential Oils of these *M. salicifolia*

Sample	II	IV	V	III_A	I	III_B	VI_A	VI_B	VI_C	VII
The part distilled	Shoots (Leaves and branchlets)			Leaves	Branchlets and trunk	Branchlets		Trunk	Flowers	
Weight of fresh materials (kg)	2.6	1.9	1.2	3.1	10.8	1.8	1.5	12.9	575g	1460g
Weight of the oil obtained (g)	25.3	12.2	8.3	29.0	7.0	8.5	10.0	9.5	2.5	6.0
Yield (%)	0.97	0.64	0.69	0.94	0.07	0.47	0.67	0.07	0.44	0.41
d_4^{30}	1.0165	0.9899	0.9653	0.9988	0.8630	0.8435	0.8759	0.8554	0.8625	0.8769
n_D^{30}	1.5156	1.5232	1.5137	1.5200	1.4805	1.4833	1.4760	1.4666	1.4804	1.4802
$\alpha_D^{(}$(°C)	(27)	(19)	(20)	(27)	(27)	(27)	(18)	(18)	(16)	(16)
	−0.55	−0.50	−1.50	+0.35	−2.35	−2.80	−5.45	−10.75	−1.15	+0.35
A.V.	6.0	1.1	2.0	4.0	5.5	2.0	1.7	1.2	1.0	1.5
E.V.	15.0	16.7	11.6	12.5	3.0	3.7	1.8	3.2	15.7	15.3

The Analysis of the Oils by Gas-Liquid Chromatography——The gas-liquid chromatography (GLC) was carried out by Shimadzu GC-1B equipment with a thermal conductivity detector. A 150×0.5 cm stainless steel column was packed with PEG 6000 (30%) or Silicone DC-550 (30%) on Celite 545 (100 mesh), and hydrogen was used as the carrier gas.

The Fractional Distillation of the Neutral Oil——The total oil of Samples II, IV, V, and III_A was washed with 5% potassium hydroxide solution, and the neutral oil was fractionated by distillation under reduced pressure in the current of nitrogen with Widmer column into twelve fractions as shown in Table II.

Table II. Results of Fractional Distillation of the Neutral Oil (Samples II, IV, V, and III_A)

Fraction	bp 18 mmHg (°C)	Dist. (ml)	d_4^{30}	n_D^{30}	α_D^{22} (°)
I	53— 63	1.5	0.8871	1.4588	+0.55
II	63— 77	1.0	0.8952	1.4620	+2.80
III	77— 97	1.5	0.9476	1.4906	+4.05
IV	97—100	4.0	0.9661	1.5118	−1.40
V	100—105	6.0	0.9867	1.5171	−1.45
VI	105—111	4.0	1.0195	1.5200	−0.75
VII	111—114	4.0	1.0460	1.5243	0.00
VIII	114—125	7.5	1.0465	1.5265	0.00
IX	125—127	4.5	1.0337	1.5278	0.00
X	127—128	8.5	1.0315	1.5274	0.00
XI	128—	1.5	1.0319	1.5256	0.00
XII	Residual oil	1.5 (g)	—	1.5223	—

All the temperatures were uncorrected.

Separation and Identification of the Individual Component——The each fraction was further divided into hydrocarbons and oxygenated compounds by alumina-column chromatography. The main components were isolated by preparative GLC and were identified by a comparison of infrared spectra and retention times (t_R.) with those of authentic samples. Some minor components were identified by t_{RS}. only using PEG 6000 and Silicone DC-550 columns.

The Results of Identification of the Main Components——Methylchavicol: Peak 18 (Table III.) (IR cm^{-1}: 1610, 1510, 1300, 1245, 1175, 1035, 990, 910, and 810) was isolated from fraction IV (85.5% to the fraction) and identified as 1-allyl-4-methoxybenzene by a comparison of the IR spectrum and t_R. with those of a sample isolated from *Agastache rugosa* O. Kuntze.[5]

Table III. Compositions of the Essential Oils of these *M. salicifolia* (%)

Peak No.	t_R.[a] (min)	Component	Sample II	IV	V	III_A Leaves	Methods of identification
			Shoots (leaves and branchlets)				
1	1.0	α-pinene	0.7	0.2	0.7	0.4	t_R.
2	1.2	camphene	0.2	0.1	0.3	0.1	t_R.
3	1.6	β-pinene	1.0	0.4	2.0	0.3	IR, t_R.
4	1.8	limonene	2.3	3.0	0.1	0.6	IR, t_R.
5	2.0	1,8-cineole	4.9	2.0	18.7	2.3	IR, t_R.
6	2.2	*p*-cymene	0.9	0.3	0.8	0.2	IR, t_R.
7	2.4	β-methylheptenone	—	—	0.1	—	
8	2.8	*cis*-alloocimene	0.5	0.2	1.0	0.5	UV
9	3.4	unidentified	0.2	—	—	—	
10	4.1	*trans*-linalooloxide	0.3	0.1	0.2	0.1	t_R.
11	4.3	*d*-fenchone	0.5	—	0.8	0.4	IR, t_R.
12	4.8	*cis*-linalooloxide	0.3	0.1	0.2	0.1	t_R.
13	5.2	*l*-linalool	0.5	0.3	0.6	0.4	IR, t_R.
14	6.5	*l*-camphor	1.0	0.2	0.9	0.2	IR, t_R.
15	6.6	unidentified	0.1	—	0.3	—	
16	7.3	*l*-terpinen-4-ol	1.1	0.5	1.4	0.2	IR, t_R.
17	8.3	caryophyllene	0.1	0.8	0.5	0.2	IR, t_R.
18	9.6	methylchavicol	**22.5**	**18.0**	**13.5**	**19.0**	IR, t_R.
19	9.8	*l*-α-terpineol	0.4	0.2	1.6	0.3	IR, t_R.
20	10.0	citral-b	1.8	3.6	3.8	1.8	IR, t_R.
21	11.6	citral-a	2.8	6.2	5.5	2.9	IR, t_R.
22	13.0	citroenellol	0.1	—	0.2	0.1	t_R.
23	14.0	nerol	0.1	0.1	0.9	0.2	t_R.
24	16.0	geraniol	—	0.2	0.6	—	t_R.
25	16.4	*trans*-anethole	1.6	1.3	1.2	1.5	IR, t_R.
26	19.8	safrole	**24.6**	**17.0**	**21.5**	**21.0**	IR, t_R.
27	30.2	methyleugenol	**29.4**	**42.0**	**21.0**	**43.5**	IR, t_R.
28	35.0	*p*-anisaldehyde	0.1	—	—	0.1	t_R.
29	37.5	*cis*-methylisoeugenol	—	0.2	—	—	t_R.
30	50.2	eugenol	0.3	0.1	0.2	0.5	IR, t_R.
31	52.0	*trans*-methylisoeugenol	1.3	2.3	1.2	2.0	IR, t_R.
32	65.0	neric acid	0.1	0.1	0.1	—	IR, t_R.
33	68.0	myristicine	0.1	0.1	0.1	0.2	IR
34	80.0	geranic acid	0.2	0.1	0.1	—	IR, t_R.

a) t_R.: Retention time, PEG 6000 (30%), 160°, 80 ml/min H_2.

Safrole: Peak 26 (IR cm^{-1}: 2775, 1640, 1500, 1485, 1445, 1245, 1190, 1040, 990, 920, and 810) was isolated from fraction VII (67.0% to the fraction) and identified as 1-allyl-3,4-methylenedioxy benzene by a comparison of the infrared (IR) spectrum and t_R. with those of authentic sample.

Methyleugenol: Peak 27 (IR cm^{-1}: 1590, 1510, 1260, 1235, 1155, 1140, 1030, 990, 910, and 805) was isolated from fraction X (95.5% to the fraction) and identified as 1-allyl-3,4-dimethoxybenzene by a comparison of the IR spectrum and t_R. with those of a synthetic sample prepared from eugenol.

Citral-b and Citral-a: Peaks 20 and 21 were isolated from fractions IV, V, and VI with Girard's reagent P.

l-Linalool, *l*-Terpinen-4-ol, and *l*-α-Terpineol: Peak 13 (92.5% purity: $[\alpha]_D^{15}$ −10.3°, c=2.9 in EtOH), peak 16 (86.0% purity: $[\alpha]_D^{15}$ −13.0°, c=5.3 in EtOH), peak 19 (85.0% purity: $[\alpha]_D^{15}$ −38.8°, c=4.9 in EtOH) were isolated from the fraction (bp. 110—122°/30 mmHg) of the distillation of oil of the samples I, III_B, VI_A, and VI_B, and identified as linalool terpinen-4-ol, and α-terpineol respectively by the IR spectra[6] and t_Rs.

d-Fenchone and *l*-Camphor: Peak 11 (98.0% purity: $[\alpha]_D^{20}$ +49.3°, c=7.0 in EtOH), and peak 14 (mp. 172°, $[\alpha]_D^{20}$ −21.7°, c=5.3 in EtOH) were isolated from the same fraction and identified by a comparison of the IR spectra and t_Rs.

6) B.M. Mitzner, E.T. Theimer, and S.K. Freeman, *Appl. Spectrosc.*, **19**, 169 (1965).

Table IV. Compositions of the Essential Oils of these *M. salicifolia* (%)

Peak No.	Component	Sample	I	III_B	VI_A	VI_B	VI_C	VII
			Branchlets and trunk	Branchlets		Trunk	Flowers	
1	α-pinene		0.7	1.3	1.6	0.9	0.4	0.4
2	camphene		0.5	0.6	0.2	0.4	0.1	0.3
3	β-pinene		1.0	1.6	5.6	3.2	1.3	1.3
4	limonene		0.1	0.1	0.4	0.4	0.8	0.9
5	1,8-cineole		**23.6**	**16.5**	**30.6**	**59.4**	2.7	0.6
6	*p*-cymene		7.4	2.5	2.1	3.9	0.6	0.2
8	*cis*-alloocimene		1.2	1.3	0.6	0.2	1.2	1.5
9	unidentified		—	0.3	0.2	0.1	0.3	0.2
10	*trans*-linalooloxide		0.3	0.5	0.2	0.1	0.3	0.2
11	*d*-fenchone		0.6	0.8	0.1	—	0.5	0.7
12	*cis*-linalooloxide		0.2	0.5	0.2	0.1	0.3	0.2
13	*l*-linalool		1.8	1.4	0.9	0.7	1.2	1.1
14	*l*-camphor		3.3	1.5	0.8	1.9	1.0	1.8
15	unidentified		—	—	0.2	—	0.5	0.4
16	*l*-terpinen-4-ol		6.1	2.5	4.2	7.5	1.7	1.7
17	caryophyllene		0.5	0.1	0.4	0.3	0.4	0.7
18	methylchavicol		1.5	0.8	2.0	0.1	2.0	2.7
19	*l*-α-terpineol		6.4	2.0	5.9	9.8	2.0	3.5
20	citral-b		**10.0**	**16.0**	**13.1**	2.8	**25.3**	**24.2**
21	citral-a		**20.0**	**36.0**	**24.5**	4.2	**48.4**	**48.6**
22	citronellol		0.1	0.1	—	—	—	—
23	nerol		1.1	0.9	1.4	1.4	0.9	1.3
24	geraniol		2.1	1.2	2.0	1.4	2.6	1.7
25	*trans*-anethole		—	—	0.1	—	—	—
26	safrole		1.4	2.5	0.6	0.1	1.2	1.6
27	methyleugenol		6.6	6.2	1.1	0.4	0.9	1.9
28	*p*-anisaldehyde		—	0.1	—	—	—	—
29	*cis*-methylisoeugenol		0.2	—	—	—	—	0.1
30	eugenol		0.4	0.1	0.1	0.5	1.0	0.8
31	*trans*-methylisoeugenol		1.0	0.7	—	—	0.5	0.5
32	neric acid		0.7	0.5	—	—	—	—
34	geranic acid		1.0	1.0	—	—	—	—

Acidic Part——From the separated acidic part of the oil, eugenol (peak 30), guaiacol, phenol, *o*-cresol, *p*-cresol, neric acid[7] (peak 32), and geranic acid[7] (peak 34) were also identified by a study of IR spectra and Rts.

Result and Discussion

The yields of the essential oils of these *M. salicifolia* were 0.64—0.97% to the fresh shoots (samples II, IV, and V), 0.94% to the fresh leaves (sample III_A), 0.47—0.67% to the branchlets (samples III_B and VI_A), 0.07% to the trunks (samples I and VI_B), and 0.41—0.44% to the fresh flowers (samples VI_C and VII).

Table III and Table IV show the compositions of the oils of shoots, leaves, branchlets, trunks, and flowers respectively.

From these results, the oil of shoots (mainly composed from the leaves) is characterized by the abundant occurrence of methylchavicol (13.5—22.5%), safrole (17.0—24.6%), and methyleugenol (21.0—43.5%) together with small amounts of *trans*-anethole, *cis*-methylisoeugenol, eugenol, *trans*-methylisoeugenol, myristicine. The total amounts of these phenol-ethers reached to 59.7—88.5% of the oils.

7) F.W. Semmler, *Ber.*, 23, 3556 (1890); K. Bernhauer and R. Forster, *J. prakt. Chem.*, **147**, 199 (1937).

On the other hand, the charactristics of the oils from branchlets and trunks are the abundant existence of 1,8-cineole (16.5—59.4%), citral-b (2.8—16.0%), and citral-a (4.2—36.0%). And the oil of flowers is also characterized by the existence of large amounts of citral-b (24.2—25.3%) and citral-a (48.4—48.6%) with very small amount of phenolethers.