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The Determination of Components of Essential Oil of *Eriobotrya japonica*

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For the investigation of biologically active substances contained in the leaves of *Eriobotrya japonica* (Japanese name, BIWA), the components of essential oils were examined by gas chromatography.

The oils were obtained in the yields of 4.5×10^{-2}—10.8×10^{-2}% from the fresh materials and they were found to be composed from α-pinene, camphene, β-pinene, β-myrcene, *p*-cymene, *cis*-β, γ-hexenol, *trans*-linalooloxide, *cis*-linalooloxide, linalool, camphor, α-ylangene, β-farnesene, unidentified sesquiterpene hydrocarbon (S. H. C.), α-farnesene, the mixture of nerol and S. H. C., the mixture of geraniol and S. H. C., *cis*-nerolidol, *trans*-nerolidol, elemol, α-cadinol, *trans*, *cis*-farnesol, *trans*, *trans*-farnesol and other unidentified compounds. Nerolidol and farnesol were found to be main components of the essential oils.

The leaves of *Eriobotrya japonica* LINDL. (Japanese name, BIWA) have been used as a folkmedicin (diuretic, peptic, refrigerant, etc.) in Japan. However, little work on the components of the leaves has been reported in the literature.[2] In order to get the biologically active substances in the leaves of this plant, the authors undertook an investigation on the components of essential oils.

In this paper a popular sort of *E. japonica* (Cult. "Tanaka"), was used as the material. Essential oils obtained in 4.5×10^{-2}—10.8×10^{-2}% yield from various parts of fresh leaves were subjected to gas-liquid chromatography to separate nerolidol (60.6—73.8%), farnesol (6.8—15.6%) and many other minor components, which showed no great difference in contents among samples. As mentioned above, nerolidol and farnesol are main components of the essential oil, and in *E. japonica*, is noted as it contains nerolidol and farnesol in a high content 72.9—83.3%[3].

When another material (2.0 kg) obtained in Nara Prefecture was subjected to steam distillation, the distilled oil in every 2 *l* of the distillate, was extracted with ether successively. From the first distillate, 35 mg of the oil was obtained in 1.7×10^{-3}% yield, containing nerolidol 29.2% at comparatively lower content and no farnesol. The quantity of distilled oil increased in proportion to the continual time of steam distillation and came up maximum (143 mg/2 *l*) in the neighborhood of 30 *l* of total distillate, showing nerolidol content in 65.0% rate. Afterward, however, the quantity decreased gradually.

This fact supported that nerolidol does not exist in the essential oil as free state but as aglycone of conjugated compounds and when the distillation progressed, the conjugated compounds were gradually hydrolyzed to nerolidol. It is the reason why the fragrance of nerolidol was not perceived in the fresh leaves. The state of outflow on nerolidol by fractional steam distillation was shown in Fig. 2.

Nerolidol (*trans*-form) in the essential oils was separated as the eluted part on alumina-column chromatography (neutral activated alumina) and its purity was analyzed as 92.2% by gasliquied chromatography. The oil obtained has following properties: d_4^{28} 0.8958, n_D^{25} 1.4782, α_D^{25}—1.05°, $[\alpha]_D^{25}$—1.68° (in 5%—EtOH solution). The value of optical rotation of this oil was very small in comparison with that of nerolidol

1) Location: *Karasuma-Imadegawa, Kamikyo-ku, Kyoto.*
2) Kurssanow, Planta **1932**, 15, 4, Heft 752 (Spear).
3) Y. R. Naves, *Helv.* 1947, **30**, 278; Y. P. Kathpalia und S. Dutt, *ref. Chem. Absts.* 1953, **47**, 273.

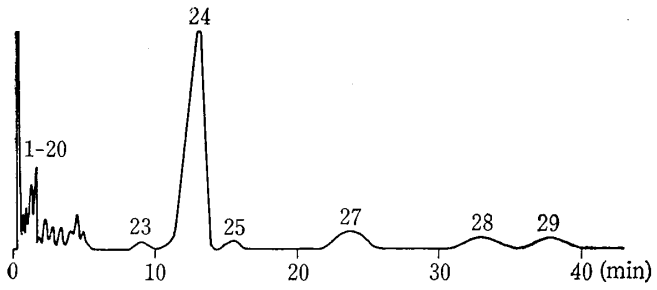

Fig. 1. Gas-liquid Chromatogram of the Essential Oil of *E. japonica* (Sample II)
condition : column PEG 6000 (30%) on Celite 545, 2 m×0.5 cmϕ, 175°C, 71.5 m*l*/min H_2

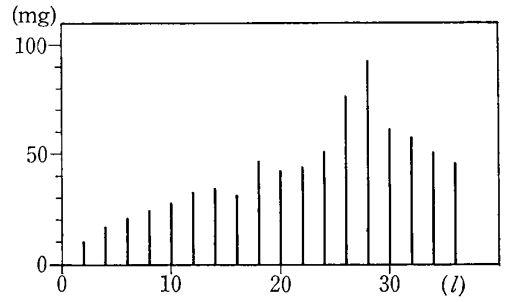

Fig. 2. The State of Outflow on Nerolidol by Fractional Steam Distillation

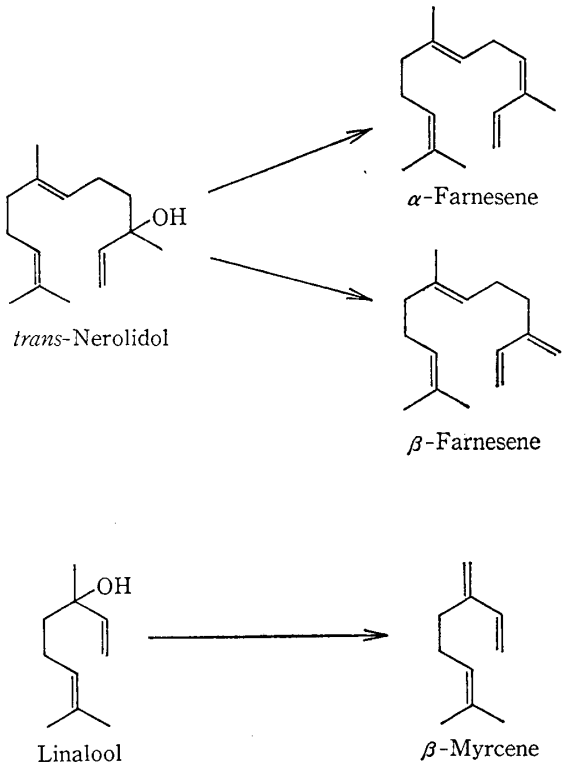

Fig. 3. The Scheme of Dehydration of *trans*-Nerolidol and Linalool

separated from other essential oil ($[\alpha]_D$+15.5°).[4] This phenomenon may owe to the facts that during distillation the conjugated compound is hydrolyzed to nerolidol and at the same time racemization of nerolidol or reversible reaction between nerolidol and farnesol occurs. This speculation was supported from two facts that α-farnesene and β-farnesene, which are dehydrolyzed products of nerolidol, and β-myrcene, which is dehydrolyzed product of linalool, are found as components of the essential oil of *E. japonica*. The relationship of each components mentioned above are shown in Fig. 3.

Experimental

Materials

Seven materials were gathered at various parts and they were subjected to steam distillation. The distilled oils were extracted with ether, and dried over anhydrous sodium sulfate. The solvent was evaporated to leave the oils. The sampling condition of the leaves of *E. japonica* and the physical properties of the

4) E. Guenther, "Essential Oil," D. Von Nostrand Co., New York, 1949, p. 260.

oils were shown in TABEL I.

TABLE I. The Sampling Condition of the Leaves of *E. japonica* and the Physical Properties of the Oils

Sample	I	II	III	IV	V	VI	VII
Date of sampling	70. 5. 6	70. 5. 21	70. 6. 4	70. 7. 10	71. 6. 9	71. 6. 30	71. 7. 17
Habitat	Shizuoka Prefecture	Kyoto City	Kagawa Prefecture	Kyoto City	Kyoto City	Kyoto City	Kyoto City
Material (kg)	2. 8	1. 2	1. 3	4. 3	1. 1	2. 5	3. 6
Obtained Oil (g)	2. 8	1. 3	1. 0	3. 3	0. 5	1. 6	2. 8
Yield (%) $\times 10^2$	10. 0	10. 8	7. 7	7. 7	4. 5	6. 4	7. 8
d_4^{30}	0. 8796	0. 8932	—	0. 8824	—	0. 8970	0. 8513
n_D^{25}	1. 4858	1. 4821	1. 4838	1. 4813	1. 4833	1. 4897	1. 4862
Remark	fresh leaves	fresh leaves					

Gas-liquid chromatographic analysis

The gas-liquid chromatography (GLC) was carried out by Yanagimoto Model GCG-3 equipment, with a thermal conductivity detector. A 200 cm length of 0.5 cm inside diameter stainless steel column was packed with PEG 6000 (30%) on celite 545 (100 mesh) and hydrogen was used as a carrier gas.

The gas chromatogram of the essential oil (Sample II) was shown in Fig. 1. The percentage of the constituents of these oils were caluculated from the area of each peaks. Components of the essential oils of *E. japonica* were shown in TABLE II.

Separation and identification of the individual components

The essential oil (Sample II) was divided into hydrocarbons and oxygenated compounds by alumina-column chromatography (neutral activated alumina) using *n*-pentane and ether as the eluents. Main compounds were isolated by preparative GLC using a PEG 6000 column and were identified by a comparison of the IR spectra and retention times (Rt.) with those of authentic samples. Furthermore, some components were examined by mass spectra and minor components were identified by Rt's. only, using two kinds of columns (PEG 6000 and SE-30). The results are shown in TABLE II. In the remarks column in TABLE II. Rt. means the components were identified or determined by the comparison of retention times of GLC with those of authentic samples respectively. Similarly IR means the compounds were identified by the comparison of IR spectra with those of authentic samples and MS means the compounds were determined by mass spectra of those peaks respectively. The details of determination of some compounds are described below.

Sesquiterpene Hydrocarbon (*S. H. C.*) The component peak 18 was determined as S. H. C., because it was eluted as hydrocarbon part on column chromatography and gave M. W. 204 ($C_{15}H_{24}$) on mass spectrum.

Nerol, S. H. C. and Geraniol S. H. C. The component peaks 20 and 21 were assigned to nerol and geraniol, judging from the coinsidence of Rt's. of GLC with those of authentic samples and occurance of OH peaks (3500 cm^{-1}) in their IR spectra. While parent peaks 204 ($C_{15}H_{24}$) were given in each mass spectrum. So the component peaks 20 and 21 consist of S. H. C. including nerol and geraniol in each parts.

trans-Nerolidol The component peak 24 was isolated by the preparative GLC, and identified as *trans*-nerolidol by the comparison of IR spectrum and Rt. of GLC with that of authentic sample. This *trans*-nerolidol (purity: 92.2% by GLC, sample II) has the following properties: d_4^{28} 0.8958, n_D^{25} 1.4728, $\alpha_D^{25} -1.05°$, $[\alpha]_D^{25} -1.68°$ (in 5% −EtOH solution).

TABLE II. Components of the Essential Oils of the Leaves of *E. japonica* (%)[a]

Peak No.	component	I	II	III	IV	V	VI	VII	Remark
1	?	+[b]	+	+	2.7	1.6	0.5	1.0	
2	α-Pinene	—	—	—	+	—	—	—	Rt.[d]
3	Camphene	0.9	0.2	0.4	+	+	0.3	+	Rt.
4	β-Pinene	0.7	0.3	0.3	+	0.1	0.3	+	Rt.
5	β-Myrcene	—	—	0.2	0.1	+	0.1	+	Rt.
6	?	0.3	0.2	0.2	0.1	+	0.1	0.1	
7	?	—	—	—	—	—	0.1	—	
8	*p*-Cymene	0.4	1.6	0.3	0.4	0.1	0.1	0.1	Rt.
9	cis-β, γ-Hexenol	0.5	1.9	0.9	1.2	1.7	2.3	0.3	Rt.
10	?	—	—	—	—	0.2	0.2	0.1	
11	?	—	—	—	—	—	0.8	0.3	
12	*trans*-Linalooloxide	0.1	—	0.2	—	0.8	0.4	0.3	Rt.
13	*cis*-Linalooloxide	0.1	0.1	0.5	1.4	0.5	0.2	0.6	Rt.
14	Linalool	0.8	0.8	1.7	0.4	1.5	2.4	1.3	Rt., IR[e]
15	Camphor	—	—	—	0.1	0.5	0.4	0.2	Rt.
16	α-Ylangene (?)	1.3	1.2	1.6	0.3	0.4	0.4	0.6	Rt.
17	β-Farnesene	2.2	1.3	2.0	0.4	1.6	1.2	1.0	Rt., MS[f]
18	S. H. C.[c]	0.5	1.2	0.7	0.1	0.5	0.5	0.2	MS
19	α-Farnesene	4.0	3.1	3.3	1.5	2.0	2.6	1.5	Rt., MS, IR
20	Nerol, S. H. C.	2.0	0.8	1.4	0.3	0.7	0.7	0.4	Rt., MS
21	Geraniol, S. H. C.	—	—	+	+	0.4	1.0	0.1	Rt., MS
22	?	—	—	—	—	+	0.5	+	
23	*cis*-Nerolidol (?)	+	0.5	+	+	0.4	+	+	Rt., IR
24	*trans*-Nerolidol	68.2	64.2	60.6	73.8	65.2	62.8	65.0	Rt., MS, IR
25	Elemol (?)	+	1.8	2.5	—	1.4	+	—	Rt.
26	?	+	+	+	—	—	—	—	
27	α-Cadinol	10.9	10.9	10.6	7.4	4.8	11.2	11.5	Rt., MS, IR
28	*trans*, *cis*-Farnesol	4.4	5.3	5.1	6.3	6.3	2.7	3.7	Rt., MS, IR
29	*trans*, *trans*-Farnesol	2.4	4.1	7.2	9.3	9.3	7.6	11.1	Rt., MS, IR

a) Percentage of the components were calculated from GLC of the each oil after steam distillation of the fresh materials.
b) + : trace
c) S. H. C. : unidentified sesquiterpene hydrocarbon
d) Rt. : retention time
e) IR : infrared spectrum
f) MS : mass spectrum

Elemol The component peak 25 was determined as elemol, because its Rt. was coincident with that of authentic sample and it was eluted as oxygenated part on alumina-column chromatography.

trans, cis-Farnesol and trans, trans-Farnesol The component peaks 28 and 29 were isolated by the preparative GLC, and identified each other as farnesol by the comparison of IR spectra, and supported as farnesol by mass spectra. Moreover, the components of peaks 28 and 29 were determined each as *trans, cis*-farnesol and *trans, trans*-farnesol by the Rt's of GLC of synthesized farnesol.

The peaks 1, 6, 7, 10, 11, 22 and 26 have not yet been identified.

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