Studies on the Essential Oils of the Genus *Mentha*

Part VI.[†] On the Components of the Essential Oils of *Mentha japonica* Makino^{††}

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Received March 25, 1970

The essential oil of *Mentha japonica* Makino (Japanese name, Himehakka) was found to consist mainly from *l*-menthone (50.8%), *d*-isomenthone (18.6%) and *d*-pulegone (12.6%) besides smaller amounts of α -pinene (0.4%), β -pinene (0.3%), limonene (0.3%), 3-octanone (3.6%), 1,8-cineole (0.8%), *p*-cymene (0.1%) 3-octyl acetate (0.8%), 3-octanol and a ketone (1.9%), 3-methylcyclohexanone (0.1%), 1-octen-3-ol (0.9%), menthyl acetate (1.8%), *l*-isopulegone (0.6%), menthol (0.4%), piperitone (2.6%), *trans*-pulegone oxide (0.4%), iso-piperitenone (0.4%), *cis*-pulegone oxide (0.4%), piperitenone (0.2%) and other compounds.

Some considerations to the relationships among *M. Pulegium*, *M. Gattefossei* and *M. japonica* have been done from viewpoint of the chemical systematics.

Mentha japonica (Miq.) Makino (= *Micromeria japonica* Miq.; *Satureia japonica* (Miq.) Matsum. et Kudo; *Micromeria Yezoensis* Miyabe et Tatewaki),¹⁾ (Japanese name, Himehakka) is a small, rather rare endemic mint growing usually in marshy places of Hon-shu and Hokkaido in Japan.

Many years ago, Murayama²⁾ reported that *M. japonica* collected from Ibaragi Prefecture, afforded the essential oil in 0.7% yield by steam distillation of dried herbs and the oil was composed from about 70% *l*-menthone with the small amounts of an alcohol and its ester.

Shimizu *et al.*³⁾ also reported that the oil of this plant (chromosome number $2n=48$) obtained from fresh herbs in 0.35% yield, contained *l*-menthone 80% and *d*-pulegone 16% besides other unidentified components by gas-liquid chromatography.

In this paper, the authors will describe the results of examination of the essential oils of *M. japonica* cultivated in the experimental garden at Ikeda-shi, Osaka Prefecture.

The oils were obtained in 0.21~0.30% yields by steam distillation of the fresh herbs. The characteristics of the oil were the abundant existence of *l*-menthone, *d*-isomenthone and *d*-pulegone. Besides these main components, the following eighteen minor components were also identified: α -pinene, β -pinene, limonene, 3-octanone, 1,8-cineole, *p*-cymene, 3-octyl acetate, 3-octanol, 3-methylcyclohexanone, 1-octen-3-ol, menthyl acetate, *l*-isopulegone, menthol, piperitone, *trans*-pulegone oxide, iso-

[†] Part V. S. Fujita and Y. Fujita, *Nippon Nōgei-kagaku Kaishi*, **44**, 293 (1970).

^{††} Presented at the 11th Symposium on the Chemistry of Terpenes, Essential Oils and Aromatics, Matsuyama, October, 1967.

1) J. Ohwi, "Flora of Japan," Shibundo, Tokyo, 1965, p. 1170.

2) Y. Murayama, *Nippon Kagaku Zasshi*, **32**, 1091 (1911); *Nippon Yakugaku Zasshi*, **31**, 783 (1911).

3) S. Shimizu, N. Ikeda, H. Ueda and K. Hoshino, *Koryo*, **67**, 17 (1962).

TABLE I. THE COMPARISON OF THE ESSENTIAL OIL COMPONENTS OF *M. Pulegium*, *M. Gattefossei* AND *M. japonica* (Sample III)

Peak No.	$t_R^{a)}$	Components	<i>M. Pulegium</i> (%)	<i>M. Gattefossei</i> (%)	<i>M. japonica</i> (%)
1	1.5	α -Pinene	0.1	0.5	0.4
2	2.1	β -Pinene	0.1	0.4	0.3
3	2.6	Limonene	0.1	3.4	0.3
4	2.8	3-Octanone	0.4	—	3.6
4 ₁	3.0	1,8-Cineole	—	—	0.8
5	3.2	<i>p</i> -Cymene	—	—	0.1
5 ₁	3.6	3-Octyl acetate	0.2	—	0.8
6	4.0	3-Octanol	2.5	0.8	1.9 ^{b)}
7	4.6	3-Methylcyclohexanone	3.2	0.6	0.1
8	4.8	1-Octen-3-ol	1.2	0.2	0.9
8 ₁	5.7	Unidentified	—	0.2	0.4
9	6.9	<i>l</i> -Menthone	26.4	10.2	50.8
10	7.6	<i>d</i> -Isomenthone	3.2	1.6	18.6
10 ₁	8.0	Menthyl acetate	—	—	1.8
11	10.4	<i>l</i> -Isopulegone	2.0	2.7	0.6
11 ₁	11.5	Menthol	trace	—	0.4
12	13.2	<i>d</i> -Pulegone	50.5	68.4	12.6
13	17.0	Piperitone	1.8	1.5	2.6
14	23.5	<i>trans</i> -Pulegone oxide	1.4	1.2	0.4
14 ₁	25.0	Isopiperitenone	—	—	0.4
15	30.5	Unidentified ketone	5.5	4.8	1.6
16	33.5	<i>cis</i> -Pulegone oxide	1.4	3.5	0.4
17	35.0	Piperitenone	—	—	0.2

^{a)} PEG 6000 (30%), 1.5 m $\times$ 0.5 cm column, at 170°C, 80 ml/min H₂.

^{b)} Contains another unidentified ketone.

piperitenone, *cis*-pulegone oxide and piperitenone.

The total contents of *l*-menthone and *d*-isomenthone amounted to 69.4% of the oil. This value is nearly equal to the *l*-menthone contents 70% of Murayama and 80% of Shimizu *et al.* The *l*-menthone of Murayama and Shimizu *et al.* seem to contain small amounts of *d*-isomenthone. The specific optical rotation of the separated menthone fraction of Murayama ($[\alpha]_D -8.23^\circ$) is much lower than that of pure *l*-menthone ($[\alpha]_D -29^\circ$),⁴⁾ and shows the fraction contained *d*-isomenthone about 17%. But it is difficult to discuss the accurate

amounts of these two compounds as *l*-menthone isomerize easily to *d*-isomenthone by heating or even spontaneously.

Next, the authors compared the oil of *M. japonica* with those of *M. Pulegium* and *M. Gattefossei* reported previously.⁵⁾ As shown in Table I, the total amounts of *l*-menthone, *d*-isomenthone and *d*-pulegone in the oils were 80.1% for *M. Pulegium*, 80.2% for *M. Gattefossei* and 83.0% for *M. japonica* respectively. The minor components of the oil of *M. japonica* also resembled to those of *M. Pulegium* and *M. Gattefossei*. Though the amount of limonene in the oil of *M. japonica* is 0.3%, the each

4) E. Guenther, "The Ess. Oils," Vol. II, D. Van Nostrand Co., New York, 1949, p. 392.

5) Y. Fujita and S. Fujita, *Nippon Kagaku Zasshi*, **88**, 767 (1967).

FIG. 1. The Schematic Mode of Formation of the Essential Oil *in vivo*.

content of *l*-menthone, *d*-isomenthone, menthol and menthyl acetate is the highest among the three. On the other hand, the content of *d*-pulegone is the lowest. Viewing from the mode of formation of the essential oil components shown in Fig. 1, these differences may indicate that *M. japonica* is in the highest rank of evolution among these three allied species.

EXPERIMENTAL

Materials. Some rhizomes of *M. japonica* (probably from Nagano Prefecture) got from Kyoto Herbal Garden, Research and Development Division, Takeda Chemical Industries Ltd. at September 16, 1949, were cultivated in the experimental garden at Ikeda-shi, Osaka.

Sample I. The transplanted plants sprouted at April 6, 1950 and on August 22, the material (8.0 kg) under the beginning of flowering stage (the average length of herb: 30 cm, weight: 8.0 g, number of branchlet: 12, leaves with small amounts of inflorescences 43.5% and branchlets 56.5%) was subjected to steam distillation. The distilled oil was extracted with ether, and dried over anhydrous sodium sulfate. The oil (24.0 g) obtained in 0.30% yield, has the following properties: $d_4^{30}=0.9184$, $n_D^{30}=1.4692$, $\alpha_D^{15}=+3.25^\circ$.

Sample II. On November 4, 1954, the material (39.2 kg) under the fruiting stage (the average length of herb: 45 cm, weight: 7.2 g, number of branchlet: 10, leaves with inflorescences 49% and branchlets 51%) was distilled with steam. The oil (83.3 g) obtained in 0.21% yield, has the following properties: $d_4^{30}=0.9199$, $n_D^{30}=1.4660$, $\alpha_D^{15}=-1.20^\circ$.

Sample III. On September 4, 1961, the material (6.8 kg) under the beginning of flowering stage (the average length of herb: 42 cm, weight: 3.7 g, number of branchlet: 8, leaves 48.5%, inflorescences 11.0% and branchlets 40.5%) was subjected to steam distillation. The oil (19.0 g) obtained in 0.28% yield, has the following properties: $d_4^{30}=0.9197$, $n_D^{30}=1.4627$, $\alpha_D^{15}=+10.00^\circ$.

Gas-liquid chromatographic analysis. The gas-liquid chromatography (GLC) was carried out by Shimadzu Model GC-1B equipment, with a thermal conductivity detector. A 150 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 III) was shown in Fig. 2. The percentages of the constituents of this oil were calculated from the area of each peak (*cf.* Table I).

Fractional distillation of the oil. The total oil

FIG. 2. Gas Chromatogram of the Essential Oil of *M. japonica* (Sample III). PEG 6000 (30%), 170°C, 80 ml/min H₂.

TABLE II. THE RESULTS OF THE FRACTIONAL DISTILLATION

Fraction	bp, °C (20mmHg) ^{a)}	Yield (ml)	d_4^{30}	n_D^{30}	α_D^{15} (°)	bp, °C (758 mmHg) ^{a)}
I	53~ 57	0.8	0.8453	1.4336	-18.10	164
II	57~ 63	1.0	0.8434	1.4380	-18.20	172
III	63~ 65	1.0	—	1.4376	—	—
IV	65~ 68	2.0	0.8469	1.4322	-6.70	179
V	68~ 84	2.5	—	1.4450	—	—
VI	84~ 86	3.0	0.8879	1.4466	-1.30	204
VII	86~ 88	2.5	—	1.4472	—	—
VIII	88~ 91	7.5	—	1.4478	—	—
IX	91~ 94	15.0	0.8931	1.4488	+2.90	207
X	94~ 96	20.0	—	1.4506	—	—
XI	96~100	15.0	—	1.4572	—	—
XII	100~106	8.0	0.9319	1.4700	+30.40	218
XIII	106~	4.5	1.0373	1.4748	+27.40	222

^{a)} The thermometer uncorrected.

(Samples I, II and III) was redistilled with steam. The colorless oil obtained had the following properties $d_4^{30}=0.8879$, $n_D^{30}=1.4540$, $\alpha_D^{15}=+8.80^\circ$. The oil was fractionated by distillation under reduced pressure in the current of nitrogen with Widmer column and thirteen fractions were obtained as shown in Table II.

Identification of individual component. Identifications of the components were performed, comparing to the essential oil of *M. Pulegium*.⁵¹ Furthermore, the main components were isolated by the preparative GLC using PEG 6000 column and identified by the comparison of IR spectra with those of authentic samples.

For the minor components, three kinds of column (PEG 6000 (30%), Silicone DC-550 (30%) and Apiezone-L (30%)) were used in order to get the accurate results in case of need.

α -Pinene, β -pinene and limonene. The retention times of peaks 1, 2 and 3 were identical with those of authentic α -pinene, β -pinene and limonene.

3-Octanone and 1,8-cineole. The peak 4 was isolated from the fraction I by the preparative GLC. IR spectrum shows the presence of carbonyl group (1715 cm⁻¹). This compound gave 2,4-dinitrophenylhy-

drazone mp 69°C,⁶⁾ reddish yellow needles after recrystallization from aqueous ethanol, and the melting point was not depressed on admixing with that of 3-octanone prepared from 1-octen-3-ol.^{7,8)} IR spectrum of this derivative also coincided with that of synthesized one.

When the isolated oil as peak 4 was again gas chromatographed with Silicone DC-550 column, it showed a small 4₁ peak together with the main peak of 3-octanone. This peak was proved to be 1,8-cineole by the comparison with authentic sample in GLC.

3-Octanol and 1-octen-3-ol. The component peak 7 was isolated from the fraction IV by the preparative GLC, and identified as 1-octen-3-ol by the comparison with IR spectrum and retention time of GLC of authentic sample.^{7,9)}

The isolated peak 6 contains 3-octanol, but IR spectrum shows the presence of larger amount of an unidentified ketone (1710 cm⁻¹).

3-Octyl acetate and p-cymene. The component peak 5₁ was identified as 3-octyl acetate by the comparison of retention time with that of authentic sample. And moreover, when the original oil was saponified, peak 5₁ was disappeared, and the area of peak 6 (3-octanol) was increased.

The minor component peak 5 was identified as *p*-cymene by the retention time.

3-Methylcyclohexanone. The component peak 7 was isolated from the fraction IV and identified as 3-methylcyclohexanone by the comparison of IR spectrum with that of authentic sample. But the authors think this compound will be the secondary decomposition product of pulegone.

6) S. Shimizu, N. Ikeda, H. Ueda and K. Hoshino, *Nippon Nôgeikagaku Kaishi*, **36**, 913 (1962).

7) T. Ueda, *Nippon Kagaku Zasshi*, **83**, 338 (1962).

8) Y. Fujita, T. Ueda and S. Fujita, *ibid.*, **85**, 892 (1964).

9) G. Hoffmann, *J. Am. Oil Chemists. Soc.*, **39**, 439 (1962).

Menthone, isomenthone and pulegone. The major components (peaks 9, 10 and 12) were isolated from the fraction X and identified as menthone, isomenthone and pulegone by IR spectra¹⁰⁾ respectively.

Menthyl acetate, isopulegone and menthol. The minor components (peaks 10₁, 11 and 11₁) were identified as menthyl acetate, isopulegone and menthol by the comparison of the retention times of GLC with those of authentic samples using two kinds of column.

Piperitone. The compound peak 13 was isolated from the fraction XIII and identified as piperitone by IR spectrum.¹⁰⁾

trans- and cis-Pulegone oxide, isopiperitenone and piperitenone. Although no attempt was made to isolate these compounds from the fraction XIII owing to their scarcity, the peaks 14 and 16 were identified as *trans*- and *cis*-pulegone oxide by the comparison of retention times with those of synthesized samples prepared from pulegone.¹¹⁾

And the peaks 14₁ and 17 were also identified as isopiperitenone and piperitenone by the comparison of the retention times with those of synthesized samples prepared from limonene.¹²⁾

The peaks 8 and 15. These were not yet identified.

Acknowledgement. The authors wish to thank Kyoto Herbal Garden, Research and Development Division, Takeda Chemical Industries Ltd. for generous gift of some rhizomes of this plant.

10) M. Horák, O. Motl, J. Pliva and F. Šorm, "Die Terpene," II, Akademie-Verlag, Berlin, 1963, M. 24, 25, 28 and 30.

11) W. Treibs, *Ber.*, **66**, 1492 (1933); W. Reusch and C. K. Johnson, *J. Org. Chem.*, **28**, 2557 (1963); C. Djerassi, N. Klyne, T. Norin, G. Ohloff and E. Klein, *Tetrahedron*, **21**, 163 (1965).

12) K. Fujita, *Nippon Kagaku Zasshi*, **78**, 1112 (1957).