

# Sesquiterpene Alcohols in the Essential Oils of *Mentha aquatica* L. and *Mentha piperita* L.\*

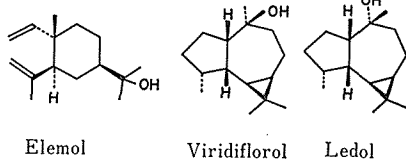
by Den-ei KARASAWA and SUMIO SHIMIZU

Laboratory of Analytical Chemistry, Fac. Agric., Shinshu Univ.

The essential oils originated from various species of *Mentha* generally contain predominantly the monoterpenes of following various types<sup>1)</sup>; acyclic (linalool, myrcene), monocyclic (menthol, menthone, pulegone, piperitone, carvone, piperitenone); bicyclic ( $\alpha$ -pinene,  $\beta$ -pinene, isopinocampone, borneol); oxide (piperitone oxide, piperitenone oxide, 1,2-epoxy-neomenthyl acetate).

Various complicated mixtures of these monoterpenes found in mint oils have been quickly examined by recent gas chromatography and contributed extremely to chemotaxonomical studies in the previous works<sup>2)3)4)5)</sup>.

In 1967, Vlahov *et al.*<sup>6)</sup> isolated twentythree sesquiterpene hydrocarbons of complicated structures from the Bulgarian peppermint oil. However, a few studies in the recent papers were related to oxygenated sesquiterpenes<sup>7)8)9)</sup>. From chemotaxonomical view, sesquiterpene hydrocarbons, distributed widely in various plants seemed scarcely specific to the oils of *Mentha*, while a few papers concerning sesquiterpene alcohols such as a cadinol isomer<sup>8)</sup> and elemol, were interesting.



In the preliminary paper in 1969<sup>4)11)</sup>, we reported the isolation of viridiflorol from an oil of *M. aquatica*\*\* firstly as a component of the mint oils. Viridiflorol, a tricyclic sesquiterpene alcohol containing cyclo-propane

ring, was found in the essential oils of following plants:<sup>12)</sup> *Melaleuca viridiflora*, *Himatadora baccata*, *Juniperus oxycedorus* and *Amorpha fructosa*. This terpene alcohol was also confirmed in the oil of Japanese *piperita* (Hokkaido, M-4 strain) in comparison with the authentic infrared spectrum of viridiflorol.<sup>14)</sup> Thereafter, Lawrence *et al* found in 1971<sup>9)</sup> ledol, an epimer of viridiflorol, as a component of an American peppermint oil (0.1%), while Nano *et al* reported the presence of viridiflorol in an Italian peppermint oil.<sup>10)</sup>

\* A part of this report was presented at the 13th Symposium on the Chemistry of Terpenes and Essential Oils and Aromatics of Japan held on Oct. 16th, 1969.

\*\* The origin of this strain of *Mentha aquatica* L, Istituto ed orto Botanico dell' Univ. di Roma, as reported previously in our paper [Agr. Biol. Chem., **30**, 200 (1966)].

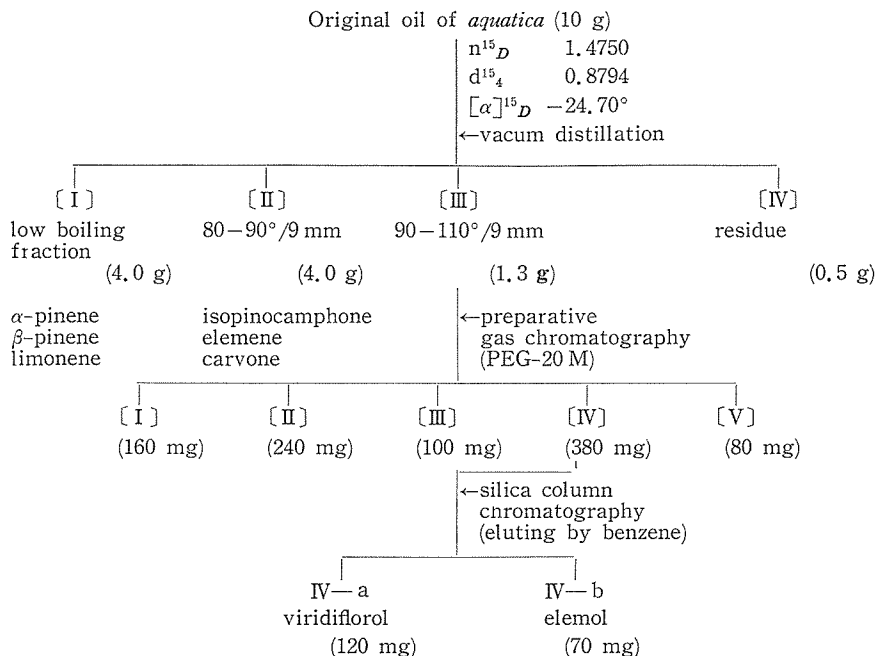


Fig. 1. A Scheme for Fractional Procedure of *aquatica* oil

Since viridiflorol and ledol show the very similiar infrared absorption spectra, we re-examined whether the sesquiterpene in the *Genus Mentha* is a mixture of viridiflorol and ledol or not. In this paper we wish to report that 1.2 % of viridiflorol has been isolated from an *aquatica* oil as shown in Fig. 1 and 0.15 % from a Japanese piperita oil (M-4, Hokkaido). The IR spectrum of our terpene was not identical with that of ledol isolated from a carqueja oil (essential oil of *Baecharis genistelloides*) by Naves, as shown in Fig. 2.

An *aquatica* oil (10 g) was fractionated according to the procedure described in Fig. 1; colorless needle, (120 mg). It melted at 70°-72° C after crystallization from hexane:  $[\alpha]_D^{15} + 4.81^\circ$ . This terpene was assigned to be a sesquiterpene alcohol with cyclo-propane ring from the following spectral data; -OH ( $\nu$  3350  $\text{cm}^{-1}$ ,  $\delta_{\text{TMS}}^{\text{CCl}_4}$  1.38 ppm, 1H), cyclopropane ring ( $\nu$  3000  $\text{cm}^{-1}$ ,  $\delta$  0.0 - 0.4 ppm). Mass spectrum of this alcohol showed  $m/e$  204, due to  $M^+ - 18$  ( $\text{H}_2\text{O}$ ). This indicated that  $M^+$  is  $m/e$  222, corresponding to  $\text{C}_{15}\text{H}_{26}\text{O}$ . Finally this terpene has been established to be viridiflorol by comparing the IR of our sample with those reported previously<sup>14</sup>.

The content of viridiflorol in the oils of various *Mentha*, which was estimated by gas chromatography, is shown in Table 1. A gas chromatogram of the *aquatica* oil containing iso-pinocampnone as the major constituent reported previously<sup>4</sup> is shown in Fig. 3, indicating that the compound of peak 22 corresponds to viridi-

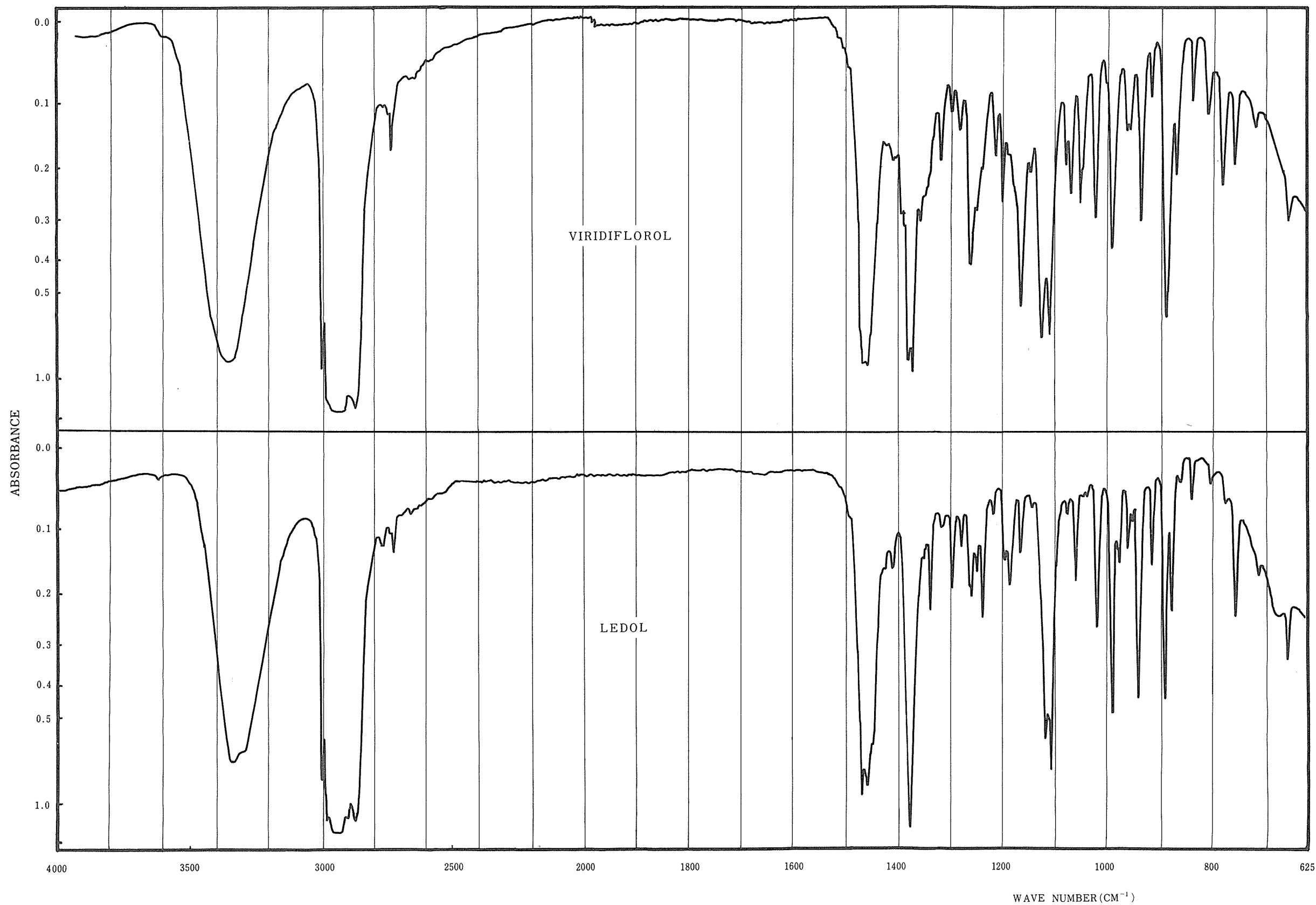


Fig. 2 Infrared Spectra of Viridiflorol and Ledol

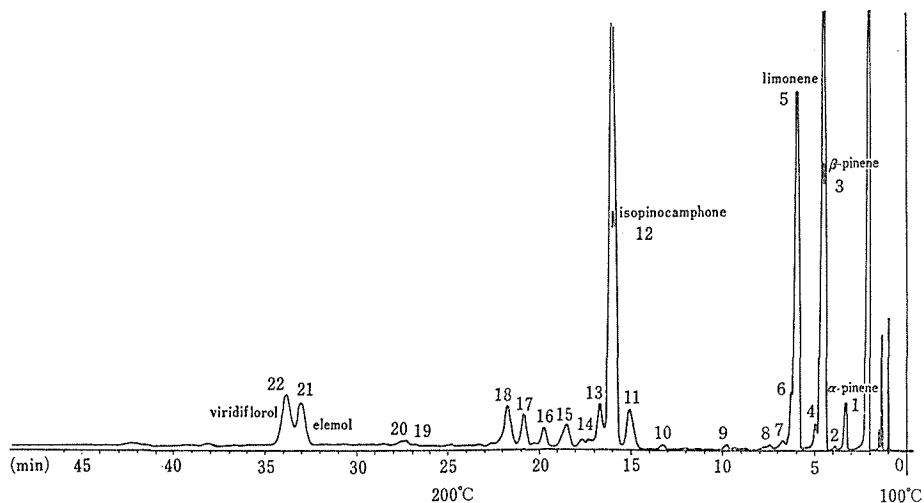


Fig. 3. Temperature programmed gaschromatogram of *M. aquatica* oil (column : PEG. 20 M.)

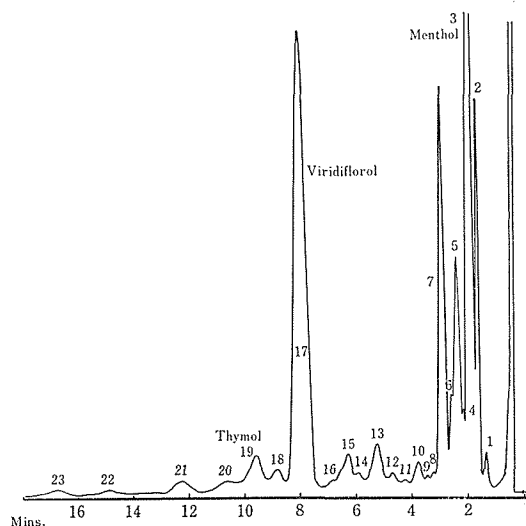


Fig. 4. A Gas chromatogram of a High-Boilong Fraction of Peppermint Oil (PEG-20M, 185°)

florol. The compound of peak 21 was separated from the fraction after the elute containing viridiflorol : crystals of mp. 54° C.,  $[\alpha]^{15}_D - 6.25^\circ$ . It was determined (-) -elemol from the following spectral data ; MS., Parent peak, m/e 222 (very weak intensity), m/e 204 was attributable to  $M^+ - H_2O$ ; ( $M^+$ , corresponding to  $C_{15}H_{26}O$ ). From IR spectrum it was assumed a sesquiterpene alcohol with terminal

Table 1 Content of Viridiflorol in Oils of *Mentha* by Gas chromatography\*

		$[\alpha]^{15}_D$	$n^{15}_D$	viridiflorol (%)** w/v
<i>Peppermint oil</i>				
M-4, Hokkaido, Japan		-23.46°	1.4572	0.28
Willamette, USA***		-27.50°	1.4580	0.16
Madras, USA***		-24.32°	1.4570	0.16
Yakima, USA***		-23.20°	1.4590	0.12
<i>Spearmint oil</i>				
Yakima, USA***		-48.50°	1.4885	less than 0.01
<i>arvensis mint oil</i>				
Okayama, Japan		-25.60°	1.4610	less than 0.01
<i>aquatica oil</i>	Japan	-24.70°	1.4750	7.0

\* Column of PEG-20M (3mm × 3m) maintained at 180°C. Carrier gas, Nitrogen, 10ml/min., Detector, FID, att,  $1 \times 10^2$ .

\*\* Calculated by using the internal standard solution containing a weighted amount of viridiflorol.

\*\*\* These oils were given to one of the authors on the observation trip in 1966.

double bonds. Its characteristic NMR spectrum including the signals due to terminal methylenes were identical with those of elemol<sup>1)</sup>.

Furthermore, the high boiling fraction of an American peppermint oil imported in about 1968 was examined by the same procedure shown in Fig. 1. The compound of peak 17 in Fig. 4 was isolated and confirmed to be viridiflorol, mp. 70-72°C, while the compound corresponding to peak 16 with very small area was assumed to be elemol, by GC-MS of the fraction which was obtained by fractional distillation and column chromatography.

From Table 1 it is clear that an oil of *aquatica* contained 7% of viridiflorol and *piperita* oils of the various origins contained more than 0.1% of viridiflorol, while *arvensis* and spearmint oils contained less than 0.01%. The results seem significant from genetical point of view, since *Mentha piperita* (2n = 72) has been assumed to be originated from the crossing between *M. spicata* L. (2n = 48) and *M. aquatica* L. (2n = 96)<sup>16)</sup>.

### Experimental

The infrared spectral data were obtained with a model EPI-G<sub>8</sub> Hitachi spectrometer and a Perkin-Elmer spectrometer. The NMR spectra were recorded on a Hitachi R-24 spectrometer using tetramethyl-silane as a internal standard in CCl<sub>4</sub>. Gas chromatographical measurements were carried out by a JEOL, JGC-810 with a column (PEG-20 M, 3 m length) maintained at 180°C. GC-MS were taken on a Hitachi RMU-6E spectrometer with a capillary column (PEG-20M, 0.28 mm × 90 m).

All melting points were uncorrected.

*Constituents of an oil of aquatica* (1967) :

According to the procedure shown in Fig. 1, 120 mg of needle was isolated from an original oil; mp. 70-72° C (from hexane),  $[\alpha]_D^{15} + 4.81^\circ$ ; MS., m/e (relative intensities %), 44 (100), 55 (33), 69 (36), 81 (27), 93 (23), 109 (26), 121 (11), 135 (7), 147 (5), 161 (12), 189 (8), 204 (6%,  $M^+ - 18$ ); IR.,  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>, 3380 (hydroxyl), 3000 (cyclopropane ring), 2950, 2920, 1460, 1380, 1270, 1130, 1120, 900.; NMR.,  $\delta_{\text{TMS}}^{\text{CCl}_4}$  ppm. 0.0 - 0.4 (protons attached to cyclopropane ring), 0.95 (d, J = 6 Hz, -CH<sub>3</sub>), 0.99 (s, 3H, -CH<sub>3</sub>), 1.01 (s, 3H, -CH<sub>3</sub>), 1.10 (s, 3H, Methyl attached to carbon having hydroxyl), 1.65 (m., 1H in hydroxyl). Since the IR spectrum of this compound was superimpossible on that of viridiflorol<sup>14)</sup>, but not on that of ledol, isolated by Dr. Naves, it was determined to be (+) -viridiflorol. Accompanied with viridiflorol, elemol corresponding to peak 21 in Fig. 3 was also separated by preparative gas chromatography; Crystals of mp. 53-54° C.,  $[\alpha]_D^{15} - 6.24^\circ$ , yield, 70 mg. IR.,  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>, 3380 (hydroxyl), 3080 (C = CH<sub>2</sub>), 1636 (C = C), 1465, 1440, 1383, 910, 892, 885 (last two absorption, C = CH<sub>2</sub>); NMR.,  $\delta_{\text{TMS}}^{\text{CDCl}_3}$  ppm. 1.13 (tertiary hydroxyl attached to carbon having two methyl), 1.70 (s., 3H. = C - CH<sub>3</sub>), 4.53 and 4.73 (m., 2H, -C = CH<sub>2</sub>), 4.80, 4.83 5.719 (3H, in ABX-type). The IR and NMR spectra of this compound were compared with the authentic spectrum<sup>14)</sup> and determined to be elemol.

The content of viridiflorol, elemol and other terpenes in this *aquatica* oil was calculated, on the basis of relative peak area in the gas chromatogram (Fig. 3) and was shown in parentheses as following; peak 1,  $\alpha$ -pinene (1.2), peak 2, camphene (0.1), peak 3,  $\beta$ -pinene (21.2), peak 5, limonene, (15.6), peak 6, cineol (trace), peak 12, isopinocampone (27.8), peak 13, elemene (3.5), peak 18, carvone (4.2), peak 21, elemol (4.1) and viridiflorol corresponding to peak 22 (7.0).

*Isolation of (+) -viridiflorol from a Japanese peppermint oil (M-4 Hokkaido)*

The Hokkaido Experimental Station for Agriculture, M-4 strain of *M. piperita* was cultivated in the Farm of Faculty of Agriculture, Shinshu University and the essential oil was obtained from the herbs harvested on Aug. 20th, 1968,  $[\alpha]_D^{25} - 23.46^\circ$ ,  $n_D^{25} 1.4572$ . From 100 g of the original oil, 1.0 g of a high boiling fraction (bp. 160-170° C/7 mm Hg) was separated. It was chromatographed over a column packed with 40 g of alumina (Merck); a hexane-ether (4 : 1) eluting fraction (300 mg) was then rechromatographed over silica gel to yield colorless needle (150 mg) from a benzene eluting fraction; mp. 70-72° C.,  $[\alpha]_D^{25} + 2.35^\circ$ . Its IR spectrum was found completely identical with the authentic spectrum of viridiflorol, but not with ledol as shown in Fig. 2.

*Viridiflorol and elemol in the high boiling fraction, an American peppermint oil.*

The high-boiling fraction of peppermint oil used in this work was a tar-like

residue obtained by re-distillation with steam of an original oil imported from USA in about 1968. This tar-like oil (60 g) was subjected to fractional distillation and the fraction of bp. 94-99°/2 mm Hg., containing dominantly viridiflorol was obtained; yield 6.8 g. This fraction (2.6 g) was fractionated by silicic acid gel column chromatography eluted in turn with hexane, benzene and ethyl acetate. Benzene fraction was divided into every 200 ml elute and the sixth fraction yielded crystals of mp. 70-72° C after recrystallization from hexane; yield 150 mg. This substance was identified as viridiflorol spectroscopically and confirmed by comparing its retention time with that of the sample isolated from the *aquatica* oil. GC-MS analysis of the sixth benzene fraction indicated that peak 16 in the gas chromatogram of original high-boiling oil (Fig. 4) was due to elemol, and peak 17, due to viridiflorol, by comparison their fragmentation patterns with those of elemol and viridiflorol respectively.

#### Acknowledgments

We wish to thank Dr. Y. R. Naves for measuring IR spectra of ledol and viridiflorol in comparison with that of our sample. Thanks are also due to Mr. A. Hirakata, Ogawa Perfumery Co. for measurement of GC-MS and Miss. S. Himejima for her technical assistance. This work was supported by the grant from the Ministry of Education of Japan.

#### Summary

Viridiflorol, mp. 70-72° C,  $[\alpha]^{15}_D + 4.81^\circ$ , a sesquiterpene alcohol of allo-aromadendrene skeleton, has been isolated from the essential oils of a strain of *Mentha aquatica* and *Mentha piperita*. By comparing the IR spectrum of our sample with that of ledol isolated by Dr. Naves from a carqueja oil, it was confirmed that our viridiflorol isolated from the *Mentha* oils did not contain ledol, an epimer of viridiflorol, though ledol had been reported in an American peppermint oil.

The content of viridiflorol and other terpenes in our *aquatica* oil was estimated by gas chromatography using a column (PEG-20M). The results were shown as follows: viridiflorol (7.0%),  $\beta$ -pinene (21.2), limonene (15.6), isopinocampone (27.8), elemene (3.5), carvone (4.2), and elemol (4.1). Furthermore, viridiflorol in the following oils of the *Genus Mentha* was estimated by the internal standard method: a Japanese strain of *M. piperita* (M-4), 0.28%; three American peppermint oils, 0.16-0.12%; an American spearmint oil, less than 0.01% and a Japanese *arvensis* oil (de-mentholized oil), less than 0.01%.

These results are seemed to be noteworthy for chemotaxonomical and genetical studies, since it has been suggested by cyto-genetical method that *piperita* was

originated from the crossing between *aquatica* and *spicata*.

### References

- 1) S. Shimizu, *Chemistry* (Japan), **22**, 67 (1967) *Chemistry and Biology*, (Japan) **12**, 10 (1974)
- 2) S. Shimizu, N. Ikeda, H. Ueda and K. Hoshino, *The Koryo*, **67**, 17 (1962)
- 3) S. Shimizu, D. Karasawa and N. Ikeda, *Agr. Biol. Chem.*, **26**, 543 (1962)
- 4) S. Shimizu, D. Karasawa and N. Ikeda *J. Fac. Agr. Shinshu Univ.*, Vol. **6**, 67 (1969)
- 5) S. Shimizu, S. Udo and T. Sacco, *Allionia* (Italy), **13**, 215 (1967)
- 6) R. Vlahov, M. Holub and V. Herout, *Coll. Czec. Chem. Commun.*, **32**, 887 (1967), *Chem. Abst.* **67**, 113, 328 (1967)
- 7) M. I. Goryaev, F. S. Sharipova and L. K. Tikhonova, *Izv. Akad. Navk. Kaz. SSR. Ser. Khim.*, **17** (6), 73 (1967); *Chem. Abst.* **68**, 81373 (1968)
- 8) J. Katsuhara, H. Yamasaki, K. Hashimoto and M. Kobayashi, *Kogyo Kagaku Zasshi*, **69**, 1170 (1966), **70**, 343 (1967) *Proceedings of Symposium on Chem. Terpene, Essent. oil and Aromatics of Japan*, **10**, 75 (1966); **11** 160 (1967)
- 9) B. M. Lawrence, J. W. Hogg and S. J. Terhune, *Flavour Industry*, **3** (9) 467 (1972), 5th International Congress on Essential Oils held in Sao Paulo, Brazil, Oct, (1971)
- 10) G. M. Nano, A. Fundaro, R. Calvino and P. Cavella, *Essenze Deriv. Agrum* **42** (3), 325 (1972), *Chem. Abst.* **78**, 151546 (1973)
- 11) D. Karasawa and S. Shimizu, *Proceedings of 13th Symposium on Chem. Terpenz Essential oil and Aromatics, held at Kagoshima Univ.* Oct. 16th. (1969), pp.131.
- 12) K. Gildemeister : "Atherischen Ole" Ed. **III b**. (Akadem. Verlag, Berlin) pp. 314 (1962)
- 13) Y. R. Naves, *Helv. Chim. Acta*, **42**, 1996 (1959)
- 14) J. Pilva, M. Horak, V. Herout and F. Sorm, "Die Terpenz" Teil **I** Sesquiterpene, (Akadem. Verlag Berlin 1960) pp. 224.
- 15) Japan Perfumery Flav. Assoc. "Spectral Atlas of Terpenz and Related Compounds" (Hirokawa, Tokyo, 1973)
- 16) S. Udo, S. Shimizu and N. Ikeda, *Sci. Rep. Fac. Agr. Okayama Univ.*, **20** 1 (1962)



## アクアチカハツカ油およびペパーミント油中の セスキテルペンアルコール

唐沢伝英・清水純夫

信州大学農学部 分析化学研究室

### 摘 要

アクアチカハツカ油とペパーミント油から、アロアロマデレドレン骨核を有するセスキテルペンアルコールであるビリディフロロール  $C_{15}H_{26}O$ 、旋光度  $+4.2^\circ$ 、融点  $70\sim 72^\circ C$  を分離した。このビリディフロロールの異性体であるレドールが米国ペパーミント油から分離されたことが報告されたが、我々のビリディフロロールの赤外スペクトルは、ナーブ博士が分離したレドールの赤外スペクトルと比較し全く異なり、レドールの存在は認められなかった。

我々のアクアチカハツカ油は既報の様にイソピノカンホン (27.8%) 主成分以外は次の様であった。 $\alpha$ -ピネン (1.2)  $\beta$ -ピネン (21.2) リモネン (15.6), エレメン (3.5), カルボン (4.2), エレモール (4.1)。

日本産および米国産のペパーミント油中のビリディフロロールの含量は、0.26~0.12%であったが、日本ハツカ油およびスペアミント油の本セスキテルペンアルコールとの含量は0.01%以下であった。

これらのことから、ビリディフロロールはハツカ属植物精油の化学的分類的および遺伝学的研究に注目すべきものと考えられる。

正 誤 表

頁		行	誤	正
21	目次	4	1 植物種の大別	1 食物種の大別
26		10	1 植物種の大別	1 食物種の大別
94		15	<b>Experimenral</b>	<b>Experimental</b>