Constituents of Japanese Peppermint Oil

Part III.[†] Isolation and Identification of Two New Constituents from "Shubi"

By Isao SAKATA and Takeshi HASHIZUME*

Laboratory of The Oriental Menthol Industry, Ltd., Okayama, Japan

*Department of Food Science and Technology, Kyoto University, Kyoto, Japan

Received June 29, 1970

Isopentyl isovalerate and anisic acid were first isolated and identified from the Japanese peppermint oil. The former compound possesses characteristic apple-like odor. α -Bourbonene, menthofurolactone, and β -caryophyllene epoxide were also isolated and identified from the oil of Shubi, a newly registered Japanese peppermint.

In spite of numerous investigations on the constituents of peppermint oil, neither compounds having apple-like odor nor aromatic alkoxy acid have been isolated. Currently, we have first isolated and identified isopentyl isovalerate and anisic acid from the low- and high-boiling fractions of the Japanese peppermint oil. (Japanese name, Shubi, Okako No. 6)

Although Lahey *et al.*¹⁾ previously have isolated the former from the oil of *Eucalyptus microcorys* F. Muell (tallow-wood), and Guenther *et al.*²⁾ the latter from the oil of fennel and castor,³⁾ the occurrence of both compounds in the peppermint oil have not been reported. We also have isolated and identified α -bourbonene and methofurolactone from this oil. This paper deals with the isolation and identification of the above four constituents.

EXPERIMENTAL SECTION

All the melting points were uncorrected. The infrared spectral data were obtained with a model EPI-G3 Hitachi infrared spectrometer. The PMR spectra were recorded on a Hitachi R-20 A spectrometer using tetramethylsilane as internal standard unless otherwise stated.

The fractionation and the isolation procedures of the constituents of the dementhorized oil were shown in Scheme 1.

Four kg of the dementhorized oil (sp. gr. at 25°, 0.9029; n_D^{20} , 1.4612; α_D^{20} , -21.40°; acidity, 0.99) was fractionally distilled in the manner described earlier*, and the fractions boiled at 90~95°C/20 mmHg (sp. gr. at 25°, 0.9231; n_D^{20} , 1.4601; $[\alpha]_D^{23}$, +16.16°; acidity, 3.83; yield, 48.2 g) and 115~120°C/10 mmHg (sp. gr. at 25°, 0.9230; n_D^{20} , 1.4650; $[\alpha]_D^{21}$, -38.94°; acidity, 2.44; yield, 173.0 g) were collected.

The low-boiling fraction was placed on the top of a column, 43.5×10.5 cm *i.d.*, packed with a mixture of silicic acid and celite 545 (1:1 by weight), and eluted in turn with *n*-hexane, *n*-hexane-benzene, benzene, benzene-ethyl acetate and ethyl acetate. The *n*-hexane eluate was collected and evaporated to give an oil. The oil was again fractionated by silicic acid column chromatography eluted with *n*-hexane, *n*-hexane-benzene, benzene and benzene-ethyl acetate.

The oil obtained from the *n*-hexane-benzene eluate was rechromatographed over silicic acid to give a

[†] For Part II, see T. Hashizume and I. Sakata, *Agr. Biol. Chem.*, **34**, 638 (1970).

This work was presented in part at the 14th Symposium of Terpenoid, Essential Oils and Aromatics Chemistry, Kitami, September 5, 1970.

1) H. Lahey, *Chemisches Zentralblatt*, **1**, 1280 (1940).

2) E. Guenther, "The Essential Oils," IV, 639 (1950).

3) E. Lederer, *C.A.*, **39**, 1448 (1945).

SCHEME I. Fractionation and Isolation of the Six Constituents from the Dementhorized Oil of Shubi.

colorless oil (I) in a yield of 0.5% from the dementhORIZED oil.

The benzene eluate was collected and evaporated to dryness. Upon recrystallizations from *n*-hexane, colorless needles (II) were obtained in a yield of 0.003% from the dementhORIZED oil.

The benzene-ethyl acetate eluate was treated in the same manner described the above and scaly crystals (III) were obtained upon recrystallizations from ethyl acetate in a yield of 0.05% from the dementhORIZED oil.

The high-boiling fraction was fractionated by using the column packed with silicic acid and celite 545 (1:2 by weight)[†], and eluted in turn with *n*-hexane, *n*-hexane-benzene, benzene, benzene-ethyl acetate and ethyl acetate. The *n*-hexane eluate was collected and evaporated to give an oil. The oil was again chromatographed over silicic acid,

The *n*-hexane eluate was collected and evaporated. The residual oil was rechromatographed with silicic acid to give an oil (IV) in a yield of 0.1% starting from the dementhORIZED oil.

The benzene eluate obtained above was treated in the same manner described earlier[†] and colorless prisms (V) was obtained in a yield of 0.14% from the dementhORIZED oil.

The another benzene eluate was chromatographed on silicic acid column. The *n*-hexane-ethyl acetate eluate was collected and evaporated to dryness. The residual solid (35 mg) was recrystallized from benzene yielding colorless needles (VI). Yield, 0.006% based on the original oil.

Colorless oil I had bp 190~193°C; sp. gr. at 18°, 0.855; n_D^{19} , 1.415, with a molecular formula of $C_{10}H_{20}O_2$ by mass spectrometry (M^+ , 172). The infrared spectrum showed absorptions at 1720 and a region of 1300~1100 cm^{-1} (presumably that of ester), 1380 and 1370 cm^{-1} (isopropyl group) and the absence of hydroxyl group and double bond. The PMR spectrum (in $CDCl_3$) showed the sharp and intense two doublets at 0.92 and 0.95 ppm (12H, d., $J=6$ Hz) ascribed to two isopropyl groups, signals at a region of 2.10~2.25 (2H) and 3.75~4.10 ppm (2H) double two protons attached to carbons bearing ester group. The saponification of I in the usual way afforded an acid and an alcohol. The acid and the alcohol were characterized and identified as isovaleric acid and isopentyl alcohol by means of TLC, GC and IR, respectively. Finally, I was identified with authentic isopentyl isovalerate by TLC, GC and IR.

Colorless needles II showed mp 144~145°C and remained unidentified.

Scaly crystals III with a molecular formula of $C_{10}H_{14}O_3$ was identified as menthofurolactone by TLC, IR, UV, and PMR. Further III was derived to the ester and confirmed. Very recently, this constituent⁴⁾ has been isolated from peppermint oil by M. Ito, T. Onogaki and their coworkers. Colorless oil IV was identified as α -bourbonene from the IR spectrum.⁵⁾ Colorless prisms V was identified as β -caryophyllene epoxide as described in earlier paper.[†]

Colorless needles VI is mp 182~183°C with a molecular formula of $C_8H_8O_3$. In the infrared spectrum of VI, the presence of para-substituted phenyl group (a region of 2100~1700, 1610, 1580, 1520, 1430 and 1170 cm^{-1}), ether group (1260 and 1030 cm^{-1}) and carboxyl group (3000, 1690 and 930 cm^{-1}) was indicated. In the PMR spectrum (in d_6 -acetone), the signal at 3.88 ppm (3H, s) was ascribed to $-O-CH_3$ and two doublets at 7.03 and 8.08 ppm (4H, d.d., $J=9$ Hz) were ascribed to two paired protons in benzene ring. Thus, VI was identified as anisic acid by TLC, IR,

I

unidentified.

II

III

IV

V

VI

4) M. Ito, S. Sagawa, K. Abe and T. Onogaki, *The Memoirs of the Kitami Kogyo University*, 2, No. 4, 585 (1969).

5) T. Nishida, M. Yazima and T. Onogaki, *Abstr. Papers, The 12th Symposium of Terpene, Essential Oil and Aromatics, Hamamatsu, October*, p. 134 (1968).

FIG. 1. A Typical Gas Chromatogram of the DementORIZED Oil of the Shubi.

Gas chromatographic conditions.

Golay column, 45 m $\times$ 0.25-mm *i.d.*, was coated with HB-2000. The injector temperature was 210°C, the column temperature was programed from 100° to 160°C (program rate 2°/min). Flow rate of carrier gas (nitrogen) was 1.0 kg/cm² (inlet pressure).

Peaks identified from their retention times are as follows: 2. α -pinene, 5. β -pinene, 12. limonene, 13. 1,8-cineol, 22. isopentyl isovalerate, 23. 3-octanol, 30. L-menthone, 31. menthofuran, 32. isomenthone, 35. *cis*- β , γ -hexenyl isovalerate, 42. neomenthyl acetate? 44. neomenthol, 46. 1-menthyl acetate, 49. L-menthol, 51. pulegone, 60. caryophyllene, 61. piperitone epoxide, 62. piperitone, 87. caryophyllene epoxide.

PMR⁶) and the melting point upon admixing with authentic sample.

6) N. S. Bhacca, L. F. Johnson and J. N. Shoolery, "NMR Spectra Catalog," No. 1, 196, Varian associates, Palo Alto, California (1962).

Acknowledgments. We wish to thank Professor T. Mitsui and his associates for performing microanalyses and for measuring PMR and IR spectra. Thanks are also due to Professor M. Ito for valuable suggestions.