Constituents of Japanese Peppermint Oil
Part II.† Isolation and Identification of (−)-β-Caryophyllene Epoxide from "Okako No. 6"

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In spite of numerous investigations on the constituents of peppermint oil, sesquiterpene epoxides have not been isolated.

The present authors have first isolated and identified (−)-β-caryophyllene epoxide from the high-boiling fraction of the oil of Japanese peppermint (Japanese name, Okako No. 6) which is a new variety* produced in Okayama in 1968. Although Treibs,1 Müller,2 and Naves et al.3 have previously isolated it from the oils of Eugenia caryophyllata T. and Lavandula officinalis C., respectively, the occurrence of this compound in the peppermint oil has not been reported. This communication deals with the isolation and identification of the epoxide.

Four kg of the oil (sp. gr. at 25°, 0.9029; nD°, 1.4612; αD, −21.40°; acidity, 0.99) was fractionally distilled under reduced pressure in the stream of nitrogen gas, and the fraction boiling at 115°–120°/10 mmHg (sp. gr. at 25°, 0.9230; nD°, 1.4650; αD, −38.94°; acidity, 2.44; yield, 173.0 g.) was collected. The high-boiling fraction was placed on the top of a column, 43.5 × 6.1-cm i.d., packed with a mixture of silicic acid (purchased from Mallinckrodt Co.) and celite 545 (1 : 2 by weight), and eluted with n-hexane, n-hexane-benzene, benzene, benzene-ethyl acetate, and ethyl acetate in turn at a flow rate of 1.0 ml/min. The n-hexane eluate was collected and evaporated to give an oil. This fraction was separated by column chromatography on silicic acid. The benzene eluate was collected and evaporated to give an oil. Crystallization of the oil and recrystallizations from methanol gave colorless prisms in a yield of 0.14% from the dementhorized oil.

This crystalline compound had mp 61–63°C, [α]D° = −54.0° (c=1.43, chloroform), with a molecular formula of C15H24O by elemental analysis and mass spectrometry (M+, 220). This has been identified as (−)-β-caryophyllene epoxide (I) on the basis of the following evidences.

The infrared spectrum in KBr pellet indicated the presence of terminal methylene (1625 and 890 cm⁻¹) and its overtone (1780 cm⁻¹), geminal dimethyl (1383 and 1366 cm⁻¹), epoxide ring (3030, 1255, 865 and 755 cm⁻¹) and the absence of hydroxyl and ketone groups.

In the pmr spectrum (in CDCl₃), two singlets at 0.99 and 1.00 ppm (6H) are ascribed to two methyl groups, singlet at 1.20 ppm (3H) and multiplet at a region of 2.65–3.00 ppm (1H) a methyl group and a proton attached to carbon bearing epoxide ring. Signals at 4.86 and 4.99 ppm (2H) showed the presence of terminal methylene. The ultraviolet ab-
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The compound I gave a single spot on thin-layer chromatogram in benzene with the Rf-value of 0.3, and was appeared as a bright purple spot on spraying with a 5% solution of vanillin in sulfuric acid and turned dark violet after one hour. The compound was treated with 2,4-dinitrophenylhydrazine under acidic ethanol conditions to give orange colored hydrazone, and the thiosulfate test was faintly positive. From the above observations, the compound I was inferred to be a sesquiterpene epoxide.

Authentic (-)-β-caryophyllene epoxide (II) was prepared by the monoperphthalic acid oxidation of (-)-β-caryophyllene which was isolated from peppermint oil. The compound I was compared directly with a sample of II. The two compounds had the same melting point, which showed no depression on admixture, and were identical in every respects examined.

Therefore, this new constituent isolated here from the peppermint oil must be (-)-β-caryophyllene epoxide.

![Mass Spectrum of I at 70eV.](image)
This was further substantiated by the mass spectral results\(^7\) as shown in Fig. 1 which was determined by a Hitachi S-4 mass spectrometer. The spectrum displays prominent peaks at \(m/e\) 205, 177, 69, 55, 43, and 41, which seem to be formed in the manner shown in Scheme 1.

It is noteworthy that \((-\)-\(\beta\)-caryophyllene epoxide was found to react with an acidic solution of 2,4-dinitrophenylhydrazine. This reaction seems to occur in a way that the carbonyl compound probably results from rearrangement during acid-catalyzed hydration of the epoxide ring.\(^4\)

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