Isolation and Identification of *l*-Menthyl- β -D-glucoside from Shubi[†]

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Received February 3, 1975

Previously, we have reported the isolations and identifications of several minor constituents from the oil of Japanese peppermint (*Mentha arvensis* var. *piperascens* Hol., Japanese name, Shubi).¹⁾ Further extensive study on the constituents in the methanol extracts of the leaves resulted in the isolation of a new monoterpene glucoside, *l*-menthyl- β -D-glucoside. The glucoside was found to be the first plant constituent, although Francis *et al.*²⁾ have isolated monoterpene glucosides such as citronellol, geraniol and nerol glucoside from the flowers of the Hybrid Tea rose "Lady Seton" and *Rosa damascena*, cv. *versicolora*. This communication deals with the isolation and identification of the glucoside.

Four kilograms of the leaves were extracted twice with 10 kg of methanol. The methanol extracts were collected and concentrated. The concentrate was then extracted with ether for 46 hr employing a continuous liquid-liquid extractor. The ether extract was dried over anhydrous magnesium sulfate and the solvent was evaporated to give a viscous oil (70 g). The oil was then fractionated in turn with 5% Na₂CO₃ and 1% NaOH aq. solutions in the usual way to give a neutral fraction (36.4 g).

The neutral fraction was placed on the top of a column (50×6 cm *i.d.*), packed with a mixture of silicic acid and celite 545 (1:1 w/w), and the column was eluted with *n*-hexane, *n*-hexane-benzene, benzene, benzene-ethyl acetate, ethyl acetate and acetone, successively. The acetone eluate (543 mg) was rechromatographed over silicic acid with ethyl acetate to give a colorless solid. Acetylation of the solid with pyridine-acetic anhydride afforded a mixture of acetates. The mixture was fractionated through silicic acid column with *n*-hexane-ethyl acetate to yield 115 mg of a solid. Recrystallizations of the solid from ethanol-water gave a crystalline compound having mp 130°C.

Saponification of the compound with methanolic ammonium gave a new constituent (I) as colorless crystals (identified as the colorless solid by means of tlc, 72mg, 0.0018% from the leaves), mp 75°C, $[\alpha]_D^{20}$ -91.37° (c =0.924, CHCl₃) and a molecular formula of C₁₆H₃₀O₆·H₂O by elemental analysis (Found: C, 57.17; H, 9.57%. Calcd. for M.W., 336.43: C, 57.12; H, 9.59%).

I showed reddish purple—dark blue color reaction with vanillin/H₂SO₄ spray reagent³⁾ on a tlc plate. The IR spectrum (KBr pellet) indicated the presence of hydroxyl groups (3400 and 1100~1000 cm⁻¹), and an isopropyl group (1387 and 1370 cm⁻¹). In the pmr spectrum (in deuterated pyridine) the sharp and intense three doublets at δ 0.84, 0.90 (3H each, J =7 Hz), and δ 0.86 (3H, J =6 Hz) were ascribed to an isopropyl and a methyl group, respectively. A doublet at δ 4.85 (1H, J =8 Hz) was assigned to an anomeric proton of a sugar moiety⁴⁾ and broad signals at δ 6.3~7.4 which disappeared on shaking with D₂O were due to four hydroxyl groups. From the above observations, I was estimated to be a menthol glycoside.

Acetylation of I with pyridine-acetic anhydride yielded a tetraacetate (II), mp 130°C, $[\alpha]_D^{25}$ -59.9° (c =1.028, CHCl₃) and a molecular formula of C₂₄H₃₈O₁₀ by elemental analysis (Found: C, 59.44; H, 7.89%. Calcd. for M.W., 486.56: C, 59.25; H, 7.87%).

In the IR spectrum (KBr pellet) of II, the absence of hydroxyl groups and the presence of ester groups were indicated. A region of δ 1.98~2.04 (12H, four singlets) in the pmr spectrum (CDCl₃) were ascribed to four O-acetyl groups, and a sextet at δ 3.39 (1H, J =10 and 5 Hz) to a proton attached to a carbon (on the menthane skeleton) bearing an ether oxygen. Signals at δ 3.67 (1H, octet, J =10, 5 and 3 Hz), δ 4.13 and 4.15 (2H, two doublets, J =5 and 3 Hz), δ 5.03 (1H, quartet, J =10 and 8 Hz), and δ 4.92 and 5.23 (2H, two triplets, J =10 Hz) were ascribable to six protons attached to C₅, C₆, C₂, C₃ and C₄ of a tetraacetyl glucoside, respectively. A characteristic signal due to the β -anomeric proton of glucose appeared at δ 4.56 (1H, doublet, J =8 Hz) with little shift, indicating that the anomeric carbon is participated in the glucosidic linkage. In addition, the spectrum indicated that I is composed of one mole of menthol and glucose.

The enzymatic hydrolysis of I in the usual way (emulsin, pH 5.25) afforded an alcohol and a sugar which were identified as menthol and glucose by means of tlc, ppc, gc and IR, respectively.

An authentic sample of *l*-menthyl-tetra-O-acetyl- β -D-glucoside was prepared from *l*-menthol and acetobromoglucose by Königs-Knorr synthesis,⁵⁾ and an authentic sample of *l*-menthyl- β -D-glucoside was obtained by saponification of the synthetic *l*-menthyl-tetra-O-acetyl- β -D-glucoside with sodium methylate. The two pairs of compounds had the same melting point, which showed no depression on admixture, and were identical

[†] Constituents of Japanese Peppermint. Part VI. This work was presented in part at the 18th Symposium of Terpenoid, Essential Oils and Aromatics Chemistry, Chiba, September 28, 1974.

in every respects examined. The new constituent I was, thus, inferred to be *l*-menthyl- β -D-glucoside.

Acknowledgements. We are grateful to Dr. T. Hashizume, to Dr. K. Koshimizu and to Dr. H. Ohigashi for valuable suggestions.

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