Short Communication

The First Naturally Occurring 3-Octulose, D-Gluco-L-glycero-3-octulose, as the Main Constituent of Laurus nobilis Flush†

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In the course of our new application of 13C-NMR spectroscopy to the instant classification and evaluation of tea, we discovered a new inositol glycoside, 2-O-β-(l-arabinopyranosyl)-myo-inositol, amounting about 0.8% of made tea (Japanese green tea, black tea, etc.). The inositol glycoside was also found in a hot water extract of the fresh flush of Camellia sasanqua, which is closely related to the tea plant C. sinensis var. sinensis, by the characteristic 13C-NMR signals, suggesting its physiological importance in carbohydrate metabolism in the genus Camellia. We focused our research interest on the application of this analytical method to extracts of the fresh flush of various plants.

Fresh flush (up to the third leaf) harvested from June to July in 1988 was immediately steamed for 8 min to stop enzymic activities. Each sample (6 g) was finely cut and extracted with 4.0 ml of boiling water for 10 min. The extract was filtered or centrifuged to give a clear extract, which was subjected to 13C-NMR measurement in the same manner as previously reported. The spectra of several plant extracts were unexpectedly very characteristic and interesting. Especially the spectrum of the laurel Laurus nobilis seemed to be that of a nearly pure compound (Fig. 1), although the signals could not be assigned to any known compound. The isolation and characterization of this compound were attempted while being guided by the characteristic 13C-NMR signals.

Fresh buds and young leaves (160 g) of L. nobilis plucked in September 1988 were finely cut and extracted twice with 500 ml of boiling water for 10 min. The combined extracts were extracted with ethyl acetate. The signals in question were found in the spectrum of the residual aqueous solution.

The aqueous solution was concentrated and passed through an activated carbon column (ϕ 2.0 × 37 cm, H2O) and elution was done by distilled water. Four fractions were obtained according to the TLC analysis (silica gel G, EtOAc–MeOH–AcOH–H2O = 12 : 3 : 3 : 2). The fractions showing the signals in question were combined and repeatedly purified by Toyopearl HW-40SF column (H2O, ϕ 2.0 × 100 cm) chromatography to yield about 2 g (ca. 1.3% of the fresh flush) of compound A (1) as colorless crystals (from MeOH): 170 ~ 171°C; [α]D 20 +55.7° (c = 1.0, H2O). Anal.

Fig. 1. 13C FT-NMR Spectra of Hot-water Extracts of the Laurel L. nobilis (Lower) and Compound A (1, Upper).

Spectra were measured at 50°C by an external standard method, inserting a sealed tube containing 3-(trimethylsilyl)propionic acid-d4 sodium salt (TSP-d4) in D2O in a 10 mmφ cell (JEOL FX-90Q FT-NMR spectrometer; 22.5 MHz; spectral width, 5000 Hz; 45° pulse; recycle time, 1 sec.).

† 13C-NMR Study of Plant Extracts. Part I.
Found: C, 39.65; H, 6.83. Calcd. for C₈H₁₆O₈: C, 40.00; H, 6.71%. ¹³C-NMR δ (D₂O, TSP-d₄, 22.5 MHz) 63.3 (t), 63.8 (t), 71.9 (d), 73.4 (d), 74.8 (d), 75.2 (d), 76.5 (d), 101.1 (s); ¹H-NMR δ (D₂O, TSP-d₄, 400 MHz) 3.44 (1H, dd, 9.2, 9.5), 3.65~3.82 (6H), 3.84 (1H, dd, 7.6, 4.0), 3.93 (1H, dd, 11, 4.0).

Eight oxygenated carbon signals (δ 63 ~ 101) including a tertiary anomeric carbon signal (δ 101.1) of 1 reminded us of an unusual saccharide. The base peak at m/z 241 (M+H) in the FD-MS spectrum as well as the analytical data gave the molecular formula C₈H₁₆O₈ (MW 240), suggesting an octulose.

Acetylation with acetic anhydride and pyridine gave a hexaacetate (2) as fine needles (from CHCl₃-hexane): mp 111.5-112°C; [α]D +68° (c=0.95, CHCl₃); ¹³C-NMR (Table I); ¹H-NMR δ (benzene-d₆, acetone-d₆=3.4:1) 1.73 (3H, s), 1.77 (6H, s), 1.79, 1.86 1.90 (3H each, s), 4.14 (H-8, dd, 12.1, 2.2), 4.30 (H-8', dd, 12.1, 4.8), 4.40 (H-1, dd, 12.1, 8.2), 4.42 (H-7, m), 4.92 (H-1', dd, 12.1, 2.7), 5.36 (H-6, t, 10.0), 5.53 (H-4, d, 10.0), 5.56 (H-2, dd, 8.2, 2.7), 5.83 (H-5, t, 10.0), 5.32 (OH, s).

A comprehensive 400 MHz ¹H-NMR analysis of 2 clarified its relative stereostructure. The large vicinal coupling constants (J = 10 Hz) of four sequential protons (H-4 to H-7) indicate that 2 took a hexopyranose form. The presence of three isolated spin system (H-1, 1' and 2) with a large geminal coupling constant (J₁,₁, = 12.1) and a hydroxyl proton (δ 5.32, s) imply that 2 was a 3-keto-octose, e.g., 3-octulose, hexaacetate.

No 3-octulose has been found in nature, but d-glucol-L-glycero-3-octulose has been obtained in a low yield as one of the condensation products of d-erythrose.⁴) The ¹³C-NMR spectral and optical rotational data of 2 coincided well with those of this synthetic material (Table I). The structure of compound A (1) was, therefore, concluded to be d-glucol-L-glycero-3-octulose.

This report is, to our knowledge, the first one on the isolation of naturally occurring 3-octulose (1). Only two kinds of octuloses have been isolated so far, each being 2-octuloses: d-glycero-L-galacto-2-octulose from Persea gratissima,⁵¹ Primula officinalis⁵ and Cannabis sativa,⁶ and d-glycero-D-manno-2-octulose from P. gratissima⁵ and Sedum spectabile,⁵ and Fabiana imbricata.⁶ They are all minor constituents.

On the contrary, so much accumulation of 1 in the flush of L. nobilis (Fig. 1) is rather surprising. Even in matured leaves, 1 seemed to be one of the main constituents.⁵³ These facts suggest its important physiological role in the plant, which remains to be clarified. Our new application of NMR spectroscopy suggests its usefulness not only for classification and qualification of liquid type foods like vinegar,⁸ etc., but also as a survey for new and useful chemical constituents among plant metabolites.

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References
3) K. Sakata, H. Hagiwara, A. Yagi and K. Ina, in
preparation for publication.


