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Isolation of *n*-Butyl-β-D-fructopyranoside from *Gastrodia elata* Blume

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Abstract – In the course of the continual work on the fresh tubers of *Gastrodia elata*, a fructoside constituent was isolated from the *n*-butanol soluble fraction prepared from the methanol extract. The structure of this compound was identified as *n*-butyl-β-D-fructopyranoside from various spectroscopic data including ^{1}H- and ^{13}C-NMR, HMQC, HMBC, and FABMS.
Keywords – *Gastrodia elata*, *n*-butyl-β-D-fructopyranoside

Introduction

Gastrodia elata Blume (Orchidaseae) has been considered as one of the most important herbal medicines and used for the treatment of headaches, migraines, dizziness, epilepsy, rheumatism, neuralgia, paralysis, and other neuralgic and nervous disorders in oriental traditional or folk medicine (Bensky *et al.*, 2004; Tang and Eisenbrand, 1992). Phytochemical studies of this plant revealed the presence of several phenolic compounds including 4-hydroxybenzaldehyde, 4-hydroxybenzylmethylether, 4-hydroxybenzylalcohol, 4,4'-dihydroxy-dibenzylether, 4,4'-dihydroxydiphenyl methane, 4,4'-dihydroxybenzyl sulfoxide, 4,4'-dihydroxybenzyl sulfone, 4-[4'-(4"-hydroxybenzyloxy) benzyloxy]benzyl methyl ether, 3-*O*-(4'-hydroxybenzyl)-β-sitosterol, gastrodin, parisin and gastrol (Zhou *et al.*, 1980; Taguchi *et al.*, 1981; Noda *et al.*, 1995; Lin *et al.*, 1996; Yun-Choi and Pyo, 1997; Yun-Choi *et al.*, 1998; Hayashi *et al.*, 2002, Pyo *et al.*, 2004) and two furan type compounds including cirsiumaldehyde and 5-hydroxymethyl-2-furancarboxadehyde (Yun-Choi *et al.*, 1997, Pyo *et al.*, 2004). In this paper, we report the isolation of a fructoside component, *n*-Butyl-β-D-fructopyranoside, from the *n*-butanol soluble fraction prepared from the methanol extract of fresh *G elata*.

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Experimental

Instruments – Melting points were determined on a Mitamura-Riken melting point apparatus and were uncorrected. IR spectra were recorded on a Jasco FT/IR-5300 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were taken at 600 MHz and 150 MHz respectively on a Bruker Advanced 600 Spectrometer with tetramethylsilane as the internal standard. FABMS were taken on a VG 70-VSEQ (VG Analytical) Mass Spectrometer in *m*-nitrobenzyl-alcohol (NBA) matrix in the positive ion mode. $[\alpha]_D$ was obtained with Rudolph Research Autopolarimeter.

Plant materials – Fresh tubers of *G elata* were purchased from Korean Agricultural Development Farm in Seoul in March 1998. They were identified by Prof. Hyung Joon Chi, Natural Products Research Institute, Seoul National University. The voucher specimens (NAPRI 980320-475) were deposited at the Herbarium, Natural products Research Institute, Seoul National University.

Extraction and isolation – Fresh tubers of *G elata* (20 kg) were sliced and percolated with MeOH for several weeks at room temperature. The MeOH extract, after removal of the solvent, was partitioned between $CHCl_3$ and H_2O. The aqueous layer was extracted with EtOAc and then with BuOH to obtain BuOH soluble fraction (112 g). The BuOH fraction (20 g) was chromatographed on a silica gel (1.5 kg) column eluted with $CHCl_3$-MeOH-H_2O (90 : 9 : 1) yielding 136 mg of colorless prismatic crystalline compound.

***n*-Butyl-β-D-fructopyranoside** – Colorless prisms from MeOH-EtOAc; mp : 156-158 °C, $[\alpha]_D$-150.0 (*c*, 1.18 in

MeOH); IR $\nu_{max}cm^{-1}$(KBr): 3439 (OH), 2955 (aliphatic CH), 1057 (glycosidic C-O); Positive HR-FABMS *m/z*: 259.1165 $[M+Na]^+$; calc for $C_{10}H_{20}O_6Na$: 259.1158, ^{1}H-NMR (CD_3OD, 600 MHz): δ 3.90 (1H, d, *J* = 10.0 Hz, H-3), 3.84-3.83 (1H, m, H-5), 3.77 (1H, dd, *J* = 10.0, 3.2 Hz, H-4), 3.75 (1H, dd, *J* = 12.3, 1.2 Hz, H-6a), 3.74 (1H, d, *J* = 11.3 Hz, H-1a), 3.69 (1H, d, *J* = 11.3 Hz, H-1b), 3.65 (1H, dd, *J* = 12.3, 1.8 Hz, H-6b), 3.51 (1H, td, *J* = 9.1, 7.0 Hz, H-1'a), 3.49 (1H, td, *J* = 9.1, 6.5 Hz, H-1'b), 1.57-1.54 (2H, m, H-2'), 1.40 (2H, tq, *J* = 15.5, 7.4 Hz, H-3'), 0.93 (3H, t, *J* = 7.4 Hz, H-4'); ^{13}C-NMR (CD_3OD, 150 MHz): δ 101.59 (C-2), 71.55 (C-4), 71.07 (C-5), 70.62 (C-3), 65.15 (C-6), 63.48 (C-1), 61.63 (C-1'), 33.30 (C-2'), 20.49 (C-3'), 14.32 (C-4').

Results and Discussion

Present compound, mp 156-158 °C, $[\alpha]_D$-150.0, was obtained as colorless prisms upon recrystallization from MeOH-EtOAc. The IR spectrum exhibited the characteristic absorption of hydroxyl groups (3439 cm^{-1}). The molecular formula of this compound was determined as $C_{10}H_{20}O_6$ from HR-FABMS (*m/z*: 259.1165 $[M+Na]^+$). The ^{13}C-NMR and ^{1}H-NMR spectra taken in CD_3OD suggested the presence of *n*-butyl ether moiety. Three methylene carbons at δ 61.63 (C-1'), 33.30 (C-2'), 20.49 (C-3') and one terminal methyl carbon at δ 14.32 (C-4') were observed. The corresponding hydrogen peaks (HMQC) were shown at δ 3.51 (1H, td, *J* = 9.1, 7.0 Hz, H-1'a), 3.49 (1H, td, *J* = 9.1, 6.5 Hz, H-1'b), 1.57-1.54 (2H, m, H-2'), 1.40 (2H, tq, *J* = 15.5, 7.4 Hz, H-3') and 0.93 (3H, t, *J* = 7.4 Hz, H-4'). The remaining six carbon peaks at δ 101.59, 71.55, 71.07, 70.62, 65.15 and 63.48 indicated the presence of a six carbon saccharoside β-D-fructopyranoside moiety (Breitmaier and Voelter, 1987). The ^{1}H-NMR spectrum also confirmed the β-D-fructopyranoside moiety. The doublet signals at δ 3.90 (1H, *J* = 10.0 Hz) and the signals at δ 3.77 (1H, dd, *J* = 10.0, 3.0 Hz) are indicative of the presence of two adjacent axial protons of H-3 and H-4. Two double-doublet signals of H-6 at δ 3.75 and 3.65, with *J* = 12.3, 1.8 Hz and *J* = 12.3, 1.2 Hz respectively, suggested that H-5 is present at equatorial position. The analysis of HMBC data also supported the above assignments. On the basis of above spectral data and with comparing with the literature spectral values (Zhang *et al.*, 1996), this compound was identified as a *n*-butyl-β-D-fructopyranoside (Fig. 1). The ^{13}C-NMR spectrum was also taken in DMSO-d_6, which was used as the NMR solvent by Zhang *et al.* to confirm the structure.

Previously, enzymatic formation of methyl-β-D-fructofuranoside and ethyl-β-D-fructofuranoside were reported from aqueous methanol and ethanol extracts of Japanese persimmon due to the invertase in the fruits (Hirai *et al.*, 1986). Ethyl-α-D-fructofuranoside, which was obtained from ethanol extracts of Zizyphi Fructus, was also considered as an artifact since it was only isolated from the ethanol extract but not from the water extract of this plant (Yagi *et al.*, 1981). However, the isolation of *n*-butyl-D-fructofuranoside was not reported by Yagi *et al.* although *n*-butanol was used during the process. *n*-Butyl-β-D-fructopyranoside has been reported from *Diospyros kaki* (Matsuura and Inuma, 1978), *Cynomorium songaricum* (Zhang *et al.*, 1996) and *Smilax bockii* (Xu *et al.*, 2005). Xu *et al.* used *n*-butanol during the solvent fractionation of aqueous ethanol extract of *S. bockii,* as we did during our processing of *G. elata.* However, Zhang *et al.* extracted *C. songaricum* with EtOAc and *n*-butanol was not employed during the whole process of isolation of *n*-butyl-β-D-fructopyranoside, hence *n*-butyl-β-D-fructopyranoside could not be produced as an artifact during the isolation process. Therefore, the present compound, *n*-butyl-β-D-fructopyranoside, isolated from *G. elata* is considered as a genuine component rather than an artifact, although the possibility of being an artifact formed during the isolation process could not be totally excluded.

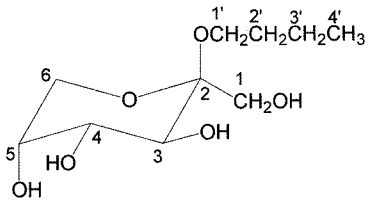

Fig. 1. The structure of *n*-Butyl-β-D-fructopyranoside.

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