Secoiridoid Glycosides from *Gentiana scabra*

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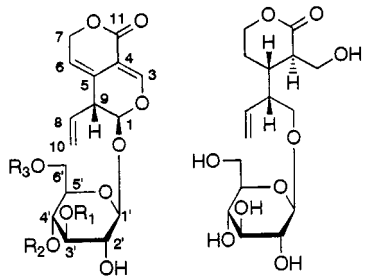
Two new secoiridoid glycosides, 4′-*O*-*β*-D-glucopyranosylgentiopicroside (**1**) and 6′-*O*-*β*-D-glucopyranosylgentiopicroside (**2**), have been isolated from the rhizomes and roots of *Gentiana scabra* together with three known compounds, olivieroside, 1-*O*-*β*-D-glucopyranosylamplexine, and benzyl alcohol *O*-α-L-arabinopyranosyl (1 → 6)-*β*-D-glucopyranoside. The structures of **1** and **2** were elucidated using chemical and physicochemical (MS and NMR) studies.

The rhizomes and roots of *Gentiana scabra* Bunge (Gentianaceae) are the crude drug Gentianae Scabrae Radix, used as a stomachic or stimulant of appetite in Japan.[1] The constituents of this crude drug have been previously investigated and shown to contain secoiridoid glucosides.[1−3] In this paper, we describe the isolation and structure elucidation of two new secoiridoid glycosides, 4′-*O*-*β*-D-glucopyranosylgentiopicroside (**1**) and 6′-*O*-*β*-D-glucopyranosylgentiopicroside (**2**), together with three known compounds from the rhizomes and roots of *G. scabra*. The known compounds were identified as olivieroside (**3**),[4] 1-*O*-*β*-D-glucopyranosylamplexine (**4**),[5] and benzyl alcohol *O*-α-L-arabinopyranosyl (1 → 6)-*β*-D-glucopyranoside,[6] respectively, by comparison of their spectroscopic data with those previously described in the literature. This is the first report of the latter of these compounds from *G. scabra*.



1 $R_1 = R_3 = H$, R_2 = Glc
2 $R_1 = R_2 = H$, R_3 = Glc
3 R_1 = Glc, $R_2 = R_3 = H$
5 $R_1 = R_2 = R_3 = H$

4

The positive-ion FABMS of compound **1** showed two quasimolecular ions at *m*/*z* 519 ([M + H]$^+$) and *m*/*z* 541 ([M + Na]$^+$). Acid hydrolysis of **1** with 5% HCl yielded glucose. The ^{1}H NMR spectrum of the aglycone part of **1** was essentially the same as that of gentiopicroside (**5**),[4] showing signals for a vinyl group, an acetal methine proton, and two trisubstituted double bonds. Two anomeric proton signals (*δ* 4.39 and 4.68) were recognized. The coupling constants of two anomeric protons indicated that the glycosyl linkages are of *β*-configuration. The ^{13}C NMR spectrum showed close similarity to that of **5**. However, a set of additional signals, corresponding to a terminal *β*-glucopyranosyl group, appeared at *δ* 62.5 (C-6″), 71.4 (C-4″), 75.0 (C-2″), 77.9 (C-3″), 78.2 (C-5″), and 105.0 (C-1″) in the ^{13}C NMR spectrum of **1**. The terminal *β*-glucopyranosyl group was involved in a glycosyl linkage at C-4′ of the inner *β*-glucopyranosyl group, because the signal due to C-4′ of the inner *β*-glucopyranosyl residue was markedly displaced downfield at *δ* 80.5 (+9.0 ppm), while the signals due to C-3′ and C-5′ were shifted upfield at *δ* 76.4 (−1.6 ppm) and 77.0 (−1.4 ppm), respectively, when comparing the ^{13}C NMR spectrum of **1** with that of **5**. This was confirmed by observation of a long-range correlation from the anomeric proton signal of the terminal *β*-glucopyranosyl group at *δ* 4.39 to C-4′ of the inner *β*-glucopyranosyl moiety in the HMBC spectrum. On the basis of this evidence, the structure of **1** was determined to be 4′-*O*-*β*-D-glucopyranosylgentiopicroside.

The positive-ion FABMS of **2** showed two quasimolecular ions at *m*/*z* 519 ([M + H]$^+$) and *m*/*z* 541 ([M + Na]$^+$). Acid hydrolysis of **2** with 5% HCl yielded glucose. The ^{1}H and ^{13}C NMR spectra resembled those of **5**, except for the presence of an additional *β*-glucopyranosyl group. This *β*-glucopyranosyl group was involved in a glycosyl linkage at C-6′ of the inner *β*-glucopyranosyl group, because the signal due to C-6′ of the inner *β*-glucopyranosyl residue was markedly downfield shifted at *δ* 70.0 (+7.2 ppm), when comparing the ^{13}C NMR spectrum of **2** with that of **5**. This was confirmed by the observation of a long-range correlation from the anomeric proton signal of the terminal *β*-glucopyranosyl group at *δ* 4.37 to C-6′ of the inner *β*-glucopyranosyl moiety in the HMBC spectrum. These results indicated that the structure of **2** was 6′-*O*-*β*-D-glucopyranosylgentiopicroside.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 600 spectrometer at 600 and 150 MHz, respectively, with tetramethylsilane as internal standard. Optical rotations were determined using a JASCO DIP-360 digital polarimeter. UV spectra were recorded with a Beckman DU-64 spectrophotometer. FABMS (positive-ion mode) were recorded on a JEOL JMS-DX 303 mass spectrometer, using a glycerin matrix. Column chromatography was carried out on Kieselgel 60 (230−400 mesh, Merck). HPLC was carried out on a Tosoh HPLC system (pump, CCPS; detector, RI-8020). GC was carried out on a Shimadzu GC-7A gas chromatograph.

Plant Material. The dried rhizomes and roots of *Gentiana scabra* (from Jilin, China) were purchased from Uchida Wakanyaku Co., Ltd., Tokyo, Japan, in 1999. A voucher specimen (No. 8) is deposited in the laboratory of M. Kikuchi.

Extraction and Isolation. The dried rhizomes and roots of *G. scabra* (1.5 kg) were extracted with MeOH at room temperature. The MeOH extract (160.0 g) was successively

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extracted with $CHCl_3$, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble fraction was concentrated under reduced pressure to afford a residue (23.7 g). A part of this residue (11.4 g) was chromatographed on a silica gel column using $CHCl_3$−MeOH−H_2O (30:10:1), and the eluate was separated into 53 fractions. Fraction 22 was purified by preparative HPLC [column, TSKgel ODS-120T (7.8 mm i.d. × 30 cm); column temperature, 40 °C; mobile phase, MeOH−H_2O (1:3); flow rate, 1.5 mL/min; UV detector, 270 nm] to give **4** (7.6 mg). Fraction 24 was purified by preparative HPLC [column, TSKgel ODS-120T (7.8 mm i.d. × 30 cm); column temperature, 40 °C; mobile phase, MeOH−H_2O (1:3); flow rate, 1.5 mL/min; UV detector, 270 nm] to give **3** (0.5 mg) and **5** (0.2 mg). Fraction 34 was purified by preparative HPLC [column, TSKgel ODS-120T (7.8 mm i.d. × 30 cm); column temperature, 40 °C; mobile phase, MeOH−H_2O (1:3); flow rate, 1.5 mL/min; UV detector, 260 nm] to give **1** (1.1 mg). Fraction 35 was purified by preparative HPLC [column, TSKgel ODS-120T (7.8 mm i.d. × 30 cm); column temperature, 40 °C; mobile phase, MeOH−H_2O (1:3); flow rate, 1.5 mL/min; RI detector] to give **2** (13.6 mg).

4′-*O*-*β*-D-Glucopyranosylgentiopicroside (1): amorphous powder; $[\alpha]^{24}_D$ −78.9° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 253 (3.79), 268 (3.83) nm; ^{1}H NMR (CD_3OD) δ 7.44 (1H, br s, H-3), 5.75 (1H, ddd, J = 17.2, 10.3, 7.0 Hz, H-8), 5.62 (1H, m, H-6), 5.62 (1H, d, J = 2.9 Hz, H-1), 5.23 (1H, ddd, J = 17.2, 1.5, 1.5 Hz, H-10b), 5.20 (1H, dd, J = 10.3, 1.1 Hz, H-10a), 5.07 (1H, dddd, J = 17.6, 2.6, 1.5, 1.1 Hz, H-7b), 4.99 (1H, dd, J = 17.6, 3.3 Hz, H-7a), 4.68 (1H, d, J = 8.1 Hz, H-1′), 4.39 (1H, d, J = 8.1 Hz, H-1″), 3.93 (1H, dd, J = 12.1, 2.2 Hz, H-6′b), 3.87 (1H, dd, J = 11.7, 2.2 Hz, H-6″b), 3.84 (1H, dd, J = 12.1, 4.4 Hz, H-6′a), 3.65 (1H, dd, J = 11.7, 5.9 Hz, H-6″a), 3.55 (1H, dd, J = 9.2, 8.8 Hz, H-4′), 3.51 (1H, dd, J = 8.8, 8.8 Hz, H-3′), 3.45 (1H, ddd, J = 9.2, 4.4, 2.2 Hz, H-5′), 3.30−3.34 (4H, m, H-9, H-3″, H-4″, H-5″), 3.221 (1H, dd, J = 8.8, 8.1 Hz, H-2′), 3.216 (1H, dd, J = 8.8, 8.1 Hz, H-2″); ^{13}C NMR (CD_3OD) δ 166.3 (s, C-11), 150.6 (d, C-3), 135.0 (d, C-8), 127.0 (s, C-5), 118.6 (t, C-10), 117.3 (d, C-6), 105.0 (d, C-1″), 104.6 (s, C-4), 100.1 (d, C-1′), 98.6 (d, C-1), 80.5 (d, C-4′), 78.2 (d, C-5″), 77.9 (d, C-3″), 77.0 (d, C-5′), 76.4 (d, C-3′), 75.0 (d, C-2″), 74.3 (d, C-2′), 71.4 (d, C-4″), 70.9 (t, C-7), 62.5 (t, C-6″), 61.8 (t, C-6′), 46.7 (d, C-9); FABMS (positive-ion mode) *m*/*z* 519 $[M + H]^+$, 541 $[M + Na]^+$.

6′-*O*-*β*-D-Glucopyranosylgentiopicroside (2): amorphous powder; $[\alpha]^{26}_D$ −111.7° (*c* 1.36, MeOH); UV (MeOH) λ_{max} (log ϵ) 252 (3.91), 270 (3.95) nm; ^{1}H NMR (CD_3OD) δ 7.45 (1H, br s, H-3), 5.76 (1H, ddd, J = 17.2, 10.3, 7.0 Hz, H-8), 5.64 (1H, d, J = 2.9 Hz, H-1), 5.61 (1H, m, H-6), 5.24 (1H, ddd, J = 17.2, 1.5, 1.5 Hz, H-10b), 5.22 (1H, ddd, J = 10.3, 1.5, 1.1 Hz, H-10a), 5.07 (1H, dddd, J = 17.6, 2.6, 1.5, 1.1 Hz, H-7b), 5.00 (1H, dd, J = 17.6, 2.2, 1.1 Hz, H-7a), 4.66 (1H, d, J = 8.1 Hz, H-1′), 4.37 (1H, d, J = 7.7 Hz, H-1″), 4.17 (1H, dd, J = 11.7, 2.2 Hz, H-6′b), 3.87 (1H, dd, J = 11.7, 2.2 Hz, H-6″b), 3.76 (1H, dd, J = 11.7, 6.2 Hz, H-6′a), 3.66 (1H, dd, J = 11.7, 5.5 Hz, H-6″a), 3.52 (1H, ddd, J = 9.2, 6.2, 2.2 Hz, H-5′), 3.26−3.36 (6H, m, H-9, H-3′, H-3″, H-4′, H-4″, H-5″), 3.21 (1H, dd, J = 8.8, 7.7 Hz, H-2″), 3.16 (1H, dd, J = 8.4, 8.1 Hz, H-2′); ^{13}C NMR (CD_3OD) δ 166.4 (s, C-11), 150.8 (d, C-3), 135.0 (d, C-8), 127.1 (s, C-5), 118.8 (t, C-10), 117.2 (d, C-6), 105.1 (d, C-1″), 105.0 (s, C-4), 100.5 (d, C-1′), 98.8 (d, C-1), 78.1 (d, C-3′, C-5″), 77.9 (d, C-3″), 77.5 (d, C-5′), 75.1 (d, C-2″), 74.5 (d, C-2′), 71.6 (d, C-4′), 71.4 (d, C-4″), 70.9 (t, C-7), 70.0 (t, C-6′), 62.8 (t, C-6″), 46.7 (d, C-9); FABMS (positive-ion mode) *m*/*z* 519 $[M + H]^+$, 541 $[M + Na]^+$.

Acid Hydrolysis of 1 and 2. Acid hydrolysis of each of compounds **1** and **2** with 5% HCl yielded D-glucose. The TMSi derivative of the sugar was identified as a D-glucose derivative by GC analysis by comparing with that of a standard sample. GC conditions: column, SE-52 (2.0 mm i.d. × 3 m); column temperature, 160 °C; carrier gas, N_2; flow rate, 25 mL/min; detector, FID. D-Glucose, t_R 31.8 and 52.1 min.

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