Polyacetylene Glycosides from *Gymnaster koraiensis*

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Two polyacetylene glycosides, gymnasterkoreasides A and B, were isolated from the roots of *Gymnaster koraiensis*. Their structures were elucidated to be (3*R*)-8-decene-4,6-diyne-1,3-diol 1-*O*-β-D-glucopyraside and (3*R*)-8-decene-4,6-diyne-1,3-diol 1-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyraside on the basis of spectroscopic analysis including COSY, HMQC, and HMBC experiments, as well as chemical methods, which confirmed the determination of a chiral center by the modified Mosher's method.

Key words *Gymnaster koraiensis*; polyacetylene glycoside; modified Mosher's method

Gymnaster koraiensis (NAKAI) KITAMURA (Compositae) is an endemic species in Korea. Earlier and more recently, we isolated eight polyacetylenes from a CH_2Cl_2-soluble fraction of the roots of this plant, which showed significant cytotoxicity against L1210 tumor cells with ED_{50} values of 0.12—3.28 μg/ml.[1)] As part of our continuing research to find pharmacologically active constituents from *G. koraiensis*, we have isolated two new polyacetyelene glycosides, called gymnasterkoreasides A (**1**) and B (**2**), from a BuOH-soluble fraction of the roots of this plant. This paper describes the isolation and structure elucidation of two new C_{10}-acetylenic glycosides isolated from *G. koraiensis*.

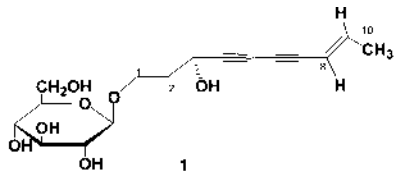
Results and Discussion

The BuOH-soluble fraction of 80% ethanol extract of the roots of *G. koraiensis* was subjected to repeated column chromatography on silica gel, Sephadex LH-20, and preparative HPLC to give two compounds (**1**, **2**) (Chart 1).

Gymnasterkoreaside A (**1**), bright yellow oil, has the molecular formula $C_{16}H_{22}O_7$ based on high-resolution fast atom bombardment mass spectrometry (HR-FAB-MS) with a molecular ion peak at *m/z* 349.1260 $[M+Na]^+$. The UV spectrum showed typical absorptions at λ_{max} 239, 252, 266, and 281 nm for an ene-diyne chromophore.[2)] The IR spectrum of **1** showed the presence of a conjugated triple bond (2245 cm^{-1}), a conjugated double bond (1660 cm^{-1}), and one or more hydroxyl groups (3350 cm^{-1}). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed signals for a methyl at δ 1.80 (dd, *J*=6.8, 1.8 Hz), an oxymethylene at δ 4.64 (t, *J*=6.7 Hz), an anomeric proton at δ 4.26 (d, *J*=7.8 Hz), and two olefinic protons at δ 6.31 (dq, *J*=15.8, 6.8 Hz) and 5.57 (dq, *J*=15.8, 1.8 Hz) (Table 1). The ^{13}C-NMR measurements, aided by distortionless enhancement by polarization transfer (DEPT) and ^{1}H-detected multiple quantum coherence (HMQC) spectra, revealed the presence of one methyl at δ 18.9, two olefinic carbons at δ 145.1 and 110.5, four quaternary carbons at δ 83.7, 78.1, 72.4, and 69.7, and an anomeric carbon at δ 104.5.

Analysis of the ^{1}H–^{1}H shift correlation spectroscopy (COSY), HMQC, and heteronuclear multiple-bond correlations (HMBC) spectra of **1** allowed its structural fragments to be determined. In the COSY spectrum, two terminal spin systems began with a methyl at δ 1.80 and hydroxymethylene at δ 3.73 and 3.98, respectively. The former proton coupled to an olefinic proton (δ 6.31) that was further coupled to an other olefinic proton (δ 5.57). The latter protons correlated to methylene protons (δ 1.96) and an oxymethylene (δ 4.64), indicating the end of the spin system (Chart 1). In addition, partial structures were linked in the HMBC experiments. Long-range correlations among δ_H 4.64 (H-3) and δ_C 83.7 (C-4)/69.7 (C-5), δ_H 5.57 (H-8) and δ_C 72.4 (C-6), and δ_H 6.31 (H-9) and δ_C 78.1 (C-7) confirmed that a conjugated diyne was connected to C-3 and C-8. The C-8,9 double bond was found to be *E*, as evidenced by their vicinal coupling constant ($J_{8,9}$=15.8 Hz). From these data, the aglycone was determined to be 8-decene-4,6-diyne-1,3-diol.

The presence of glucose in **1** was confirmed by the signals of anomeric proton at δ 4.26 in the ^{1}H-NMR spectrum and six carbon signals at δ 104.54, 75.06, 78.09, 71.56, 77.90, and 62.70 in the ^{13}C-NMR spectrum.[3)] The anomeric configuration of the glucose moiety was determined to be β on the basis of the J_{H-H} value (7.8 Hz) of the anomeric proton in the ^{1}H-NMR spectrum. Acid hydrolysis of **1** with 4 N HCl–diox-



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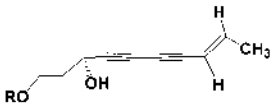

1a: R= H
1b: R= (*R*)-MTPA
1c: R= (*S*)-MTPA

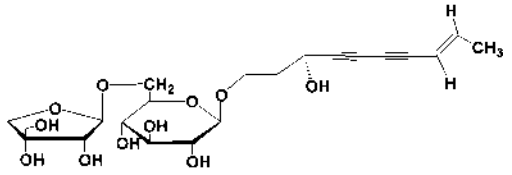

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Chart 1. Structures of Compounds Isolated from the Roots of *G. koraiensis*

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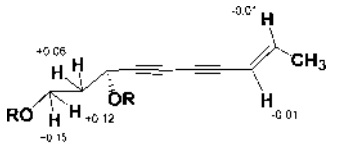

1b: R= (*R*)-MTPA
1c: R= (*S*)-MTPA

Fig. 1. Chemical Shift Difference for the (*S*)-MTPA Ester (**1c**) and (*R*)-MTPA Ester (**1b**) in ppm

ane produced a sugar component, which was determined to be D-glucose by GLC of its pertrimethylsilated L-cysteine methyl ester derivative.[4] The linkage of glucose was determined by HMBC, which showed a correlation between the signals at δ_H 3.73/3.98 (H_2-1 of the aglycone) and δ_C 104.54 (Glc-C-1′), indicating glycosylation at C-1.

Determination of the absolute configuration at C-3 of **1** was examined with the modified Mosher's method.[5—7] Compound **1a**, obtained by enzyme hydrolysis of **1**, was treated with (+)- and (−)-α-methoxy-α-trifluoromethylphenylacetate (MTPA) chlorides in the presence of 4-dimethylaminopyridine (DMPA) to give (*R*)- and (*S*)-MTPA esters (**1b**, **c**). In the ^{1}H-NMR spectrum of (*S*)-MTPA ester (**1c**), proton signals assigned to H_2-1 and H_2-2 were observed at a lower field than those in the (*R*)-MTPA ester (**1b**), while signals due to H-8 and H-9 in the former ester were shifted to a higher field than those in the latter ester (Fig. 1). Therefore the absolute configuration at C-3 was concluded to be 3*R*. On the basis of the above findings, gymnasterkoreaside A (**1**) was determined to be (3*R*)-8-decene-4,6-diyne-1,3-diol 1-*O*-β-D-glucopyranoside.

Gymnasterkoreaside B (**2**) was isolated as optically active oil, $[\alpha]_D$ −78°, and its molecular formula was established to be $C_{21}H_{30}O_{11}$ by HR-FAB-MS. On acid hydrolysis, **2** yielded glucose and apiose. Its UV spectrum was similar to that of **1**, indicating the presence of a conjugated diyne. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **1**, except for the signals of sugar components. In its ^{1}H-NMR spectrum, **2** exhibited signals for two anomeric protons at δ 4.25 (d, J=7.7 Hz) and 4.99 (d, J=2.3 Hz). Detailed analysis of the 2D-NMR (COSY, HMQC, and HMBC) revealed that its aglycone was the same as that of **1**. Long-range correlation between the anomeric proton at δ 4.99 and the carbon signal at δ 68.49 (C-6′) of the glucose observed in the HMBC spectrum suggested the second sugar moiety to be an ether-linked one at C-6′. This was confirmed by comparing the ^{13}C-NMR spectrum of **2** with that of **1**, in which a downfield shift of the C-6′ (5.8 ppm) signal due to glucose moiety was observed. The ether-linked sugar was determined to be D-apiose by acid hydrolysis of **2** followed by GLC analysis of its sugar derivative (see Experimental). By comparing the NMR spectra of **2** with those of **1** and analysis of its 2D-NMR, the ^{1}H- and ^{13}C-NMR signals were assigned as shown in Table 1. The structure of **2** therefore was confirmed to be (3*R*)-8-decene-4,6-diyne-1,3-diol 1-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of **1** and **2** in MeOH-d_4

	1		**2**	
	^{1}H[a)]	^{13}C[b)]	^{1}H[a)]	^{13}C[b)]
1a	3.73 (dd, 10.1, 6.3)	66.8	3.73 (dd, 6.6, 4.0)	67.0
1b	3.98 (dd, 10.1, 6.3)		3.95 (dd, 6.6, 4.0)	
2	1.96 (m)	38.9	1.96 (m)	39.0
3	4.64 (t, 6.7)	60.2	4.64 (t, 6.7)	60.2
4		83.7		83.7
5		69.7		69.7
6		72.4		72.0
7		78.1		78.1
8	5.57 (qd, 15.8, 1.8)	110.5	5.58 (qd, 15.8, 1.7)	110.5
9	6.31 (qd, 15.8, 6.8)	145.1	6.33 (qd, 15.8, 6.9)	145.1
10	1.80 (dd, 6.8, 1.8)	18.9	1.81 (dd, 6.9, 1.7)	18.8
glc-1′	4.26 (d, 7.8)	104.5	4.25 (d, 7.7)	104.6
-2′	3.16 (dd, 8.9, 7.8)	75.1	3.16 (dd, 8.9, 7.9)	75.1
-3′	3.34 (t, 8.9)	78.9	3.32 (t, 8.9)	78.0
-4′	3.29 (t, 8.9)	71.6	3.28 (t, 8.9)	71.6
-5′	3.26 (m)	77.9	3.39 (m)	76.8
-6′a	3.67 (dd, 12.4, 5.8)	62.7	3.62 (dd, 11.6, 5.9)	68.5
-6′b	3.98 (dd, 12.4, 5.8)		3.98 (dd, 11.6, 5.8)	
api-1″			4.99 (d, 2.3)	111.0
-2″			3.90 (d, 2.3)	78.0
-3″				80.6
-4″a			3.76 (d, 9.7)	75.0
-4″b			3.96 (d, 9.7)	
-5″			3.60 (s)	65.7

a) 300 MHz. *b*) 75 MHz.

Experimental

Optical rotations were measured with a JASCO DIP-370 Digital polarimeter. UV spectra were recorded on a Milton Roy Spectronic 3000 spectrophotometer. IR spectra were determined on an IR Report-100 spectrophotometer (JASCO). FAB-MS spectra were measured on an Autospec Mass spectrometer (Micromass). NMR spectra were recorded on a Bruker NMR DRX300, 600 spectrometer, with the chemical shift being represented in parts per million with tetramethylsilane as an internal standard. Preparative HPLC was performed on Shimadzu LC-10AD pump, CTO-10A column oven, and SPD-10AV UV-detector. GC-MS was carried out on a Shimadzu GC-17A and JEOL Automass system II mass detector.

Plants Materials Roots of *G. koraiensis* (NAKAI) KITAMURA were collected in May 1997 at Gurae, Chunnam province, Korea. A voucher specimen (CNU96003) is deposited in the herbarium of the College of Pharmacy, Chungnam National University, Taejon, Korea.

Extraction and Isolation The dried root (4.8 kg) was extracted with 80% aqueous EtOH (700 g). The EtOH extract was suspended in H_2O and extracted with CH_2Cl_2 and BuOH successively to give the CH_2Cl_2-soluble fraction (140 g) and BuOH-soluble fraction (123 g). The BuOH-soluble fraction was chromatographed on a silica gel column with a stepwise gradient of $CHCl_3$ and MeOH as eluent to give eight fractions (Fr. 1—8). Repeated column chromatography of Fr. 6 on silica gel ($CHCl_3$/MeOH/H_2O, 5 : 1 : 0 : 0.5) and RP-C_{18} column chromatography (58% aq. MeOH) afforded **1** (160 mg). Column chromatography of Fr. 7 using silica gel ($CHCl_3$/MeOH/H_2O, 5 : 1 : 0.5) and Sephadex LH-20 (100% MeOH), followed by preparative HPLC on RP-C18 (250×10 mm, 45% aq. MeOH, flow rate 1.5 ml/min) gave **2** (20 mg, retention time 17.9 min).

Compound **1**: Bright yellow oil. $[\alpha]_D^{20}$ −28° (*c*=1, MeOH); UV λ_{max} nm (MeOH, log ε): 239 (0.92), 252 (1.17), 266 (1.32), 281 (1.21); IR (KBr) ν_{max} cm^{-1}: 3350 (OH), 2930, 2245 (C≡C), 1660 (C=C), 1080 (C–O); HR-FAB-MS *m/z*: 349.1260 $[M+Na]^+$ (Calcd for $[C_{16}H_{22}O_7+Na]^+$ 349.1264); ^{1}H- and ^{13}C-NMR spectral data: see Table 1.

Compound **2**: Bright yellow oil. $[\alpha]_D^{20}$ −78° (*c*=1, MeOH); UV λ_{max} nm (MeOH, log ε): 239 (0.12), 252 (0.39), 266 (0.54), 281 (0.47); IR (KBr) ν_{max} cm^{-1}: 3380 (OH), 2345 (C≡C), 1650 (C=C), 1060 (C–O); HR-FAB-MS *m/z*: 481.1682 $[M+Na]^+$ (Calcd for $[C_{21}H_{30}O_{11}+Na]^+$ 481.1686); ^{1}H- and ^{13}C-NMR spectral data: see Table 1.

Enzymatic Hydrolysis of 1 Naringinase (200 mg) was added to a suspension of **1** (30 mg) in 50 mM acetate buffer (pH 5.5) and the mixture was stirred at 37 °C for 5 h. The reaction mixture was extracted with EtOAc (10 ml×3) and the organic layer was evaporated to dryness. The residue was chromatographed on silica gel eluted with hexane–acetone (3 : 1) to give 8-decene-4,6-diyne-1,3-diol (**1a**, 8 mg) as bright yellow oil. ^{1}H-NMR ($CHCl_3$): δ 6.30 (1H, dq, J=15.8, 6.9 Hz, H-2), 5.50 (1H, dq, J=15.8, 1.7 Hz, H-3),

4.68 (1H, t, J=6.3 Hz, H-8), 3.79 (2H, t, J=5.6 Hz, H-10), 1.94 (2H, m, H-9), 1.77 (3H, dd, J=6.9, 1.7 Hz, H-1).

(*R*)-MTPA Ester of 1a (+)-MTPA chloride (15 mg) and DMAP (10 mg) in pyridine (0.2 ml) was added to a solution of **1a** (2.0 mg) in CCl_4 (0.2 ml). After stirring at room temperature for 12 h, the mixture was poured into water (10 ml) and extracted with $CHCl_3$ (10 ml×2). The $CHCl_3$ extract was concentrated *in vacuo* and purified with preparative thin-layer chromatography (hexane/acetone, 5 : 1) to give an (*R*)-MTPA ester (**1b**, 1.5 mg) as a colorless oil. ^{1}H-NMR ($CHCl_3$): δ 6.38 (1H, m, H-9), 5.58 (1H, t, J=6.9 Hz, H-3), 5.52 (1H, ddd, J=15.8, 1.8, 0.7 Hz, H-8), 4.46 (1H, m, H-1a), 4.35 (1H, m, H-1b), 3.51 (3H, d, J=1.2 Hz, MTPA–OC$\underline{H}_3$), 3.52 (3H, d, J=1.0 Hz, MTPA–OC$\underline{H}_3$), 2.26 (2H, m, H-2), 1.84 (3H, dd, J=6.9, 1.8 Hz, H-10).

(*S*)-MTPA Ester of 1a (−)-MTPA chloride (15 mg) and DMAP (10 mg) in pyridine (0.2 ml) was added to a solution of **1a** (2.0 mg) in CCl_4 (0.2 ml). Work-up as described above gave an (*S*)-MTPA ester (**1c**, 1.5 mg) as a colorless oil. ^{1}H-NMR ($CHCl_3$): δ 6.39 (1H, m, H-9), 5.61 (1H, t, J=6.9 Hz, H-3), 5.53 (1H, ddd, J=15.8, 1.8, 0.7 Hz, H-8), 4.34 (1H, m, H-1a), 4.20 (1H, m, H-1b), 3.57 (3H, d, J=1.2 Hz, MTPA–OC$\underline{H}_3$), 3.53 (3H, d, J=1.0 Hz, MTPA–OC$\underline{H}_3$), 2.20 (2H, m, H-2), 1.84 (3H, dd, J=6.9, 1.8 Hz, H-10).

Determination of Sugars in 1 and 2 Each sample (2 mg) was refluxed with 4 N HCl–dioxane (1 : 1, 2 ml) for 2 h. The mixture was extracted with EtOAc (5 ml×3). The residual water layer was neutralized with Amberlite MB-3 and dried to give a residue. The residue was dissolved with pyridine (1 ml), to which 0.1 M L-cysteine methyl ester hydrochloride in pyridine (2 ml) was added. The mixture was stored at 60 °C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with hexamethyldisilazane–trimethylchlorosilane (0.1 ml) at 60 °C for 1 h. The mixture was partitioned between hexane and H_2O (0.3 ml each), and the hexane extract was analyzed by GC-MS. In the acid hydrolysate of **1** and **2**, D-glucose and D-apiose were confirmed by comparison of the retention times of their derivatives with those of D-glucose, L-glucose, and D-apiose derivatives prepared in a similar way, which showed retention times of 21.30, 22.00, and 17.30 min, respectively.

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