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Phytochemical Constituents from the Flowers of *Gymnaster koraiensis* and Their Cytotoxic Activities *in vitro*

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Gymnaster koraiensis (Nakai) Kitamura (Compositae) is widely distributed in the northern parts of Korea. This indigenous herb is used as a folk medicine for antitussive and antibacterial activities.[1] Previous phytochemical studies on this plant showed the presence of polyacetylenes, and benzofurans.[2,3,4] We have recently reported the isolation of sesquiterpenes and flavonoids from this plant.[5] In a continuing study on this source, we have further isolated two new sesquiterpene glucopyranosides (**1**-**2**), together with ten known compounds (**3**-**12**) by repeated column chromatography of the EtOH extract. Compounds **3**-**12** were identified as gymnasterkoreayne B (**3**),[3] gymnasterkoreayne E (**4**),[3] gymnasterkoreayne F (**5**),[3] 1,9(*Z*), 16-heptadecatriene-4,6-diyne-3,8-diol (**6**),[3,6] apigenin (**7**),[7] naringenin (**8**),[8,9] apigenin-3-*O*-β-D-glucopyranoside (**9**),[10] quercetin-3-*O*-β-D-glucopyranoside (**10**),[10] isorhamnetin-3-*O*-β-D-glucopyranoside (**11**),[12] apigenin-3-*O*-β-D-glucuronide (**12**)[7] by comparing the ^{1}H-NMR, ^{13}C-NMR, and mass spectral data with the literature data. Compounds **7**-**12** were isolated from this plant for the first time. The isolated compounds were tested for their cytotoxicity against four human tumor cell lines *in vitro* using the SRB assay.

Compound **1** was obtained as colorless gum with a molecular formula of $C_{21}H_{36}O_8$ from the $[M+Na]^+$ peak at m/z 439.2306 (calcd. for $C_{21}H_{36}O_8Na$: 439.2308) in the positive-ion HRFA-BMS. The IR spectrum indicated that 1 possessed a hydroxyl (3386 cm^{-1}) group and a C=C double bond (1650 cm^{-1}). In the ^{13}C-NMR (including DEPT) spectrum, 21 carbon signals appeared, which included four methyl carbons at δ_C = 21.9, 21.9, 21.8 and 9.3, two methylene carbons at δ_C = 32.6 and 30.8, three oxygenated methine carbons at δ_C = 81.3, 79.6 and 76.6, two olefinic carbons at δ_C = 136.4 and 120.6, three methine carbons at δ_C = 52.1, 51.9 and 28.9, one quaternary carbon at δ_C = 42.4, and six signals assignable to the glucose moiety (δ_C = 104.6, 78.4, 77.3, 76.1, 72.1, and 63.4). These data indicated that compound **1** was a eudesmane type sesquiterpene glucopyranoside.[13] Moreover, the above NMR data, except for the glucose part, were similar to 1β,6β-dihydroxy-7-epi-eudesm-3-ene isolated from *Pluchea dioscoridis*.[13] The differences were the chemical shifts at C-1, C-6, and C-9 : $\delta_{C\text{-}1}$ = 79.6, $\delta_{C\text{-}6}$ = 76.6 and $\delta_{C\text{-}9}$ = 81.3 in **1**, and $\delta_{C\text{-}1}$ = 76.6, $\delta_{C\text{-}6}$ = 68.4 and $\delta_{C\text{-}9}$ = 35.3 in 1β,6β-dihydroxy-7-epi-eudesm-3-ene,[13] implying that **1** was glycosylated at C-6 and oxygenated at C-9. The coupling constant (*J* = 7.5 Hz) of the anomeric proton at δ_H = 4.36 of D-glucose was in the β-form.[14] The glycosidic position was established by HMBC, with a long-range correlation observed between H-1′ (δ_H = 4.36, d, *J* = 7.5 Hz) and C-6 (δ_C = 76.6) (Figure 1). Thus, the structure of **1** was 1,6,9-trihydroxy-*trans*-eudesm-3-ene-6-*O*-β-D-glucopyronoside. The configuration of the hydroxyl group at C-1 was β-form based on the *J* value (δ_C = 3.69, dd, *J* = 11.5, 6.3 Hz)[15,16] and NOESY spectrum (Figure 1). The configurations of hydroxyl groups at C-6 and C-9 were β- and α-forms, respectively, based on the NOESY correlations: the correlation of H-6 with H-5 (not with H-7), and the correlations of H-9 with H-7 and H-14 (Figure 1). The proposed structure of **1** was in accordance with ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY spectra. Therefore, the structure of **1** was 1β,6β,9α-trihydroxy-*trans*-eudesm-3-ene-6-*O*-β-D-glucopyranoside.

Compound **2** was obtained as colorless gum with a molecular formula of $C_{21}H_{36}O_9$ from the $[M+Na]^+$ peak at m/z 455.2259 (calcd. for $C_{21}H_{36}O_9Na$: 455.2257) in the positive-ion HRFA-BMS. The IR spectrum indicated that **2** possessed a hydroxy (3382 cm^{-1}) and a C=C double bond (1658 cm^{-1}). The NMR spectra of **2** were similar to those of compound **1**, except for an additional oxygenated carbon signal in the ^{13}C-NMR spectrum of **2**; four oxygenated carbon signals (δ_C 81.1, 80.5, 79.5 and 72.8) exist in **2**, with only three oxygenated carbon signals (δ_C 81.3, 79.6 and 76.6) in **1**. The coupling pattern of methyl protons at C-12 and C-13 in the ^{1}H-NMR spectrum was different [δ_H = 1.00 (d), 0.95 (d), *J* = 6.3 Hz in **1**; δ_H = 1.36 (s), 1.25 (s) in **2**]. The position of the hydroxylated carbon at δ_C = 72.8 was

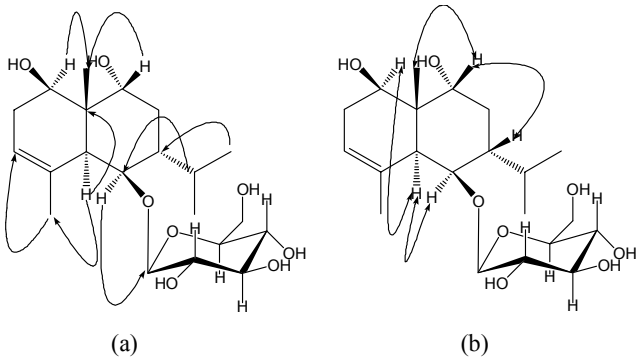
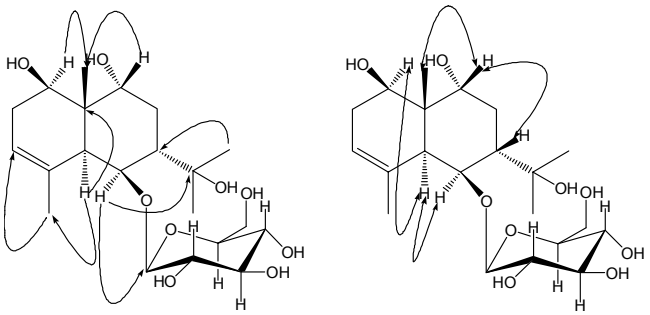


Figure 1. Key HMBC (⌒) (a) and NOESY (⌒) (b) correlations of **1**.



(a) (b)

Figure 2. Key HMBC ( ) (a) and NOESY ( ) (b) correlations of **2**.

established by HMBC (Figure 2). The relative stereochemistry was the same as **1** based on the NMR data (chemical shifts and *J* values) and reconfirmed by the NOESY spectrum (Figure 2). Thus, the structure of compound **2** was 1β,6β,9α,11-tetrahydroxy-*trans*-eudesm-3-ene-6-*O*-β-D-glucopyranoside.

Cytotoxic activities of the isolated compounds (**1**-**12**) were evaluated against A549, SK-OV-3, SK-MEL-2, and HCT15 human tumor cell lines *in vitro* using the SRB assay. Compounds **7**, **9** and **12** showed moderate cytotoxicity against A549, SK-OV-3, SK-MEL-2 and HCT15 cells, with ED_{50} values of **7**: 9.11, 9.26, 5.94, 8.32 ; **9**: 12.07, 11.36, 7.53, 13.51 ; **12**: 17.92, 15.04, 10.83, 17.40 μM, respectively, but other compounds did not (ED_{50} > 30 μM).

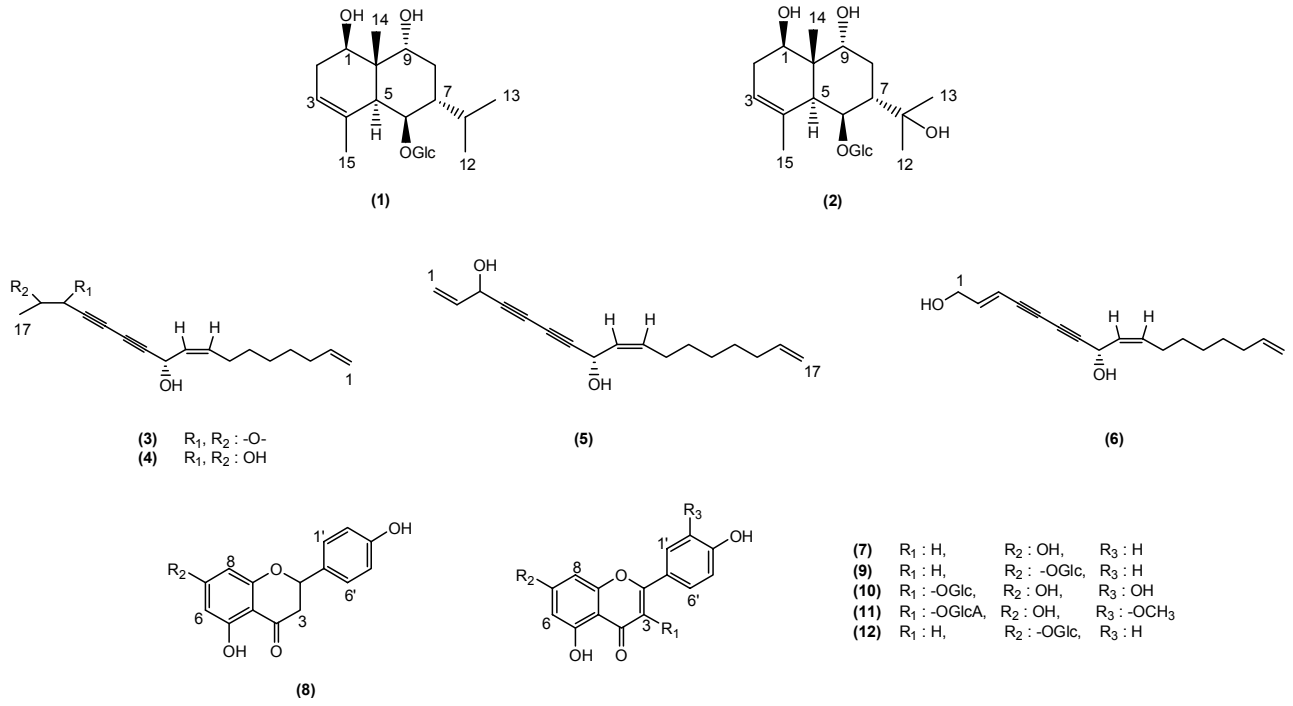
Experimental Section

General Procedures. All melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 Polarimeter. UV spectra were obtained using a Shimadzu UV-1601 UV/Visible spectrophotometer (Shimadzu Co.). NMR spectra were recorded on a Varian UNITY INOVA 500 NMR and Bruker Avance 500 NMR spectrometer. FAB-MS data were obtained on a JEOL JMS700 mass spectrometer. Preparative HPLC was performed using a Gilson 306 pump, Shodex refractive index detector, and either an Apollo silica 5μ column (250 × 22 mm) or an Econosil® RP-18 10μ column (250 × 22 mm). Silica gel 60 (Merck, 70 - 230 mesh and 230 - 400 mesh) was used for column chromatography. TLC was performed with Merck precoated silica gel F_{254} plates and RP-18 F_{254s} plates. The packing material in the molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). Low pressure liquid chromatography was performed over Merck LiChroprep Lobar®-A Si 60 (240 × 10 mm) or LiChroprep Lobar®-A RP-18 (240 × 10 mm) columns with an FMI QSY-0 pump (ISCO).

Plant Materials. The flower parts of *Gymnaster koraiensis* (Nakai) Kitamura (Compositae) (5 kg) were collected at Pyeongchang in Gangwon province, Korea, in August, 2006. A voucher specimen of the plant (SKK-07-006) was deposited at the College of Pharmacy in Sungkyunkwan University.

Test for Cytotoxicity *in vitro*. A sulforhodamine B bioassay (SRB) was used to determine compound cytotoxicity against four human cancer cell lines[17] *in vitro* at the Korea Research Institute of Chemical Technology. The tumor cell lines were A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin was used as the positive control. The cytotoxicity in ED_{50} of doxorubicin against A549, SK-OV-3, SK-MEL-2, and HCT15 were 0.001, 0.011, 0.001 and 0.027 μM, respectively.

Extraction and Isolation. The half-dried flower parts of *G.*



(1) **(2)**

(3) R_1, R_2 : -O-
(4) R_1, R_2 : OH

(5) **(6)**

(7) R_1 : H, R_2 : OH, R_3 : H
(9) R_1 : H, R_2 : -OGlc, R_3 : H
(10) R_1 : -OGlc, R_2 : OH, R_3 : OH
(11) R_1 : -OGlcA, R_2 : OH, R_3 : $-OCH_3$
(12) R_1 : H, R_2 : -OGlc, R_3 : H

(8)

Figure 3. Structures of isolated compounds (**1**-**12**).

Table 1. NMR data for compounds **1** and **2**

Position	1		2	
	δ_H[a]	δ_c[b]	δ_H[a]	δ_c[b]
1	3.69 (dd, 11.5, 6.3)	79.6	3.69 (dd, 9.7, 6.3)	79.5
2α	2.07 (m)	32.6	2.09 (m)	32.3
2β	2.04 (m)		2.07 (m)	
3	5.28 (br. s)	120.6	5.23 (br. s)	120.9
4		136.4		136.5
5	1.81 (m)	52.1	1.81 (m)	52.5
6	4.38 (br. s)	76.6	4.45 (br. s)	80.5
7	1.71 (m)	51.9	1.47 (m)	51.9
8α	1.81 (m)	30.8	2.12 (m)	27.9
8β	1.81 (m)		1.79 (m)	
9	3.71 (dd, 10.4, 5.7)	81.3	3.77 (dd, 12.0, 4.6)	81.1
10		42.4		42.4
11	1.99 (m)	28.9		72.8
12	1.00 (d, 6.3)	21.9	1.36 (s)	29.3
13	0.95 (d, 6.3)	21.9	1.25 (s)	29.4
14	1.10 (s)	9.3	1.13 (s)	9.6
15	1.80 (s)	21.8	1.73 (s)	22.2
1′	4.36 (d, 7.5)	104.6	4.36 (d, 7.5)	105.4
2′	3.14 (br. t, 8.5)	76.1	3.16 (m)	75.8
3′	3.33 (m)	78.4	3.37 (m)	78.2
4′	3.20 (m)	72.1	3.27 (m)	72.3
5′	3.29 (m)	77.3	3.23 (m)	77.2
6a′	3.66 (dd, 12.0, 7.5)	63.4	3.65 (dd, 12.0, 7.5)	63.8
6b′	3.86 (dd, 12.0, 3.0)		3.81 (dd, 12.0, 3.0)	

[a,b]Assignments were performed with DEPT, COSY, HMQC, HMBC and NOESY. Measured in CD_3OD.

koraiensis (5.0 kg) were extracted with 100% EtOH at room temperature and evaporated under reduced pressure to give residue (250 g), which was dissolved in water (800 mL × 3) and solvent partitioned to give hexane (27 g) and BuOH fractions (85 g). The hexane fraction (27 g) was separated over a silica gel column using a gradient solvent system of hexane : EtOAc (5 : 1 - 1 : 1) as the eluent to yield seven fractions (H1 - H7). Fraction H5 (1.8 g) was also subjected to silica gel column chromatography (hexane : EtOAc = 5 : 1 - 1 : 1) and was purified with a silica gel prep HPLC with hexane : EtOAc (2.5 : 1) to yield compound **3** (75 mg). Fraction H3 (3.0 g) was also subjected to silica gel column chromatography (hexane : EtOAc = 7 : 1 - 2 : 1) and was purified with a silica gel prep HPLC with hexane : $CHCl_3$: EtOAc (9 : 9 : 1) to yield compounds **4** (14 mg), **5** (10 mg) and **6** (5 mg). The BuOH fraction (85 g) was separated over a silica gel column with a solvent system of $CHCl_3$: MeOH : Water (35 : 10 : 1 - 10 : 5 : 1) to give nine fractions (B1 - B9). Fraction B1 (6.0 g) was also subjected to silica gel column chromatography ($CHCl_3$: MeOH : Water = 35 : 10 : 1) and was purified with a silica gel prep HPLC with $CHCl_3$: MeOH (6 : 1) to yield compounds **1** (60 mg) and **2** (70 mg). Fraction B2 (6.8 g) was also subjected to silica gel column chromatography ($CHCl_3$: MeOH : Water = 35 : 10 : 1) and was purified with a silica gel prep HPLC with $CHCl_3$: MeOH (12 : 1) to yield compounds **7** (400 mg) and **8** (4 mg). Fraction B3 (600 mg) was also subjected to RP C-18 column chromatography (20% MeCN) and was purified with a silica gel prep HPLC with 50% MeOH to yield compound **9** (6 mg). Fraction B5 (1.2 g) was also subjected to RP C-18 column chromatography (30% MeOH) and was purified with a silica gel prep HPLC with 50% MeOH to yield compound **10** (12 mg). Fraction B6 (1.6 g) was subjected to LH-20 column chromatography (CH_2Cl_2 : MeOH = 1 : 1) and was purified with a silica gel prep HPLC with 30% MeOH to yield compounds **11** (12 mg) and **12** (18 mg).

1β,6β,9α-Trihydroxy-*trans*-eudesm-3-ene-6-*O*-β-D-glucopyranoside (1): Colorless gum; $[\alpha]_D^{25}$: − 19.6° (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 3386, 2956, 1650, 1362, 1079 cm^{-1}; ^{1}H-, ^{13}C-NMR : see Table 1.; HR FAB-MS (positive-ion mode) m/z: 493.2306 $[M+Na]^+$.

1β,6β,9α,11-Tetrahydroxy-*trans*-eudesm-3-ene-6-*O*-β-D-glucopyranoside (2): Colorless gum; $[\alpha]_D^{25}$: + 2.66° (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 3382, 2925, 1658, 1361, 1077 cm^{-1}; ^{1}H-, ^{13}C-NMR : see Table 1. HR FAB-MS (positive-ion mode) m/z: 455.2259 $[M+Na]^+$.

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