Gymnasterkoreaynes A−F, Cytotoxic Polyacetylenes from *Gymnaster koraiensis*

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Six new polyacetylenes, gymnasterkoreaynes A−F (**1**−**6**), were isolated from the roots of *Gymnaster koraiensis*, together with 2,9,16-heptadecatrien-4,6-diyn-8-ol (**7**) and 1,9,16-heptadecatriene-4,6-diyn-3,8-diol (**8**), by bioassay-guided fractionation using the L1210 tumor cell line as a model for cytotoxicity. The structures of compounds **1**−**6** were established spectroscopically, which included 2D NMR experiments. Gymnasterkoreaynes A−F (**1**−**6**) are linear diacetylenes and are structurally related to falcarinol, panaxynol, panaxydiol, and panaxytriol. Of the compounds isolated, gymnasterkoreaynes B (**2**), C (**3**), F (**6**), and 1,9,16-heptadecatrien-4,6-diyn-3,8-diol (**8**) exhibited significant cytotoxicity against L1210 tumor cells with ED_{50} values of 0.12−3.3 μg/mL.

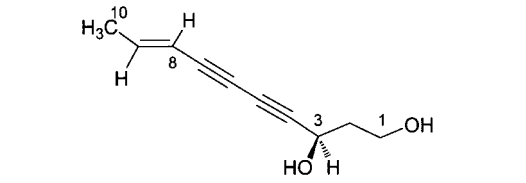
Polyacetylenes have been found in many families of higher plants, such as the Araliaceae, Campanulaceae, Compositae, Olacaceae, Pottosporaceae, Santalaceae, and Umbelliferae.[1−4] More recently, linear polyacetylenes have become a major element in the search for bioactive substances from marine sponges.[5−7] It has been reported that these compounds exhibit potent cytotoxic, antimicrobial, antiviral, RNA-cleaving, sedative, and enzyme-inhibitory activities, as well as brine-shrimp lethality.[8−14] *Gymnaster koraiensis* (Nakai) Kitamura (Compositae) is an endemic species in Korea, but its constituents and biological activities have not been investigated previously.

As a part of our continuing studies to discover novel antitumor agents from natural sources, we established that the CH_2Cl_2-soluble fraction of an 80% EtOH extract of the roots of *G. koraiensis* had an appreciable cytotoxicity against L1210 mouse leukemia cells (ED_{50}, 2.5 μg/mL). From the CH_2Cl_2 fraction of this plant, eight polyacetylenes (**1**−**8**) were isolated by bioassay-guided fractionation. In this paper, we report the isolation, structure elucidation, and cytotoxicity of these substances, including six new compounds (**1**−**6**).

Results and Discussion

The roots of *G. koraiensis* were extracted at room temperature with 80% aqueous EtOH. A CH_2Cl_2-soluble fraction of the aqueous EtOH extract was purified by silica gel column chromatography using a stepwise gradient (hexane and EtOAc), preparative TLC on silica gel, and preparative HPLC (Metachem C_{18}, MeOH and H_2O) to afford eight polyacetylenes (**1**−**8**). Two known compounds were identified as 2,9,16-heptadecatrien-4,6-diyn-8-ol (**7**) and 1,9,16-heptadecatrien-4,6-diyn-3,8-diol (**8**), respectively, by comparing their spectral data with those previously reported.[1,15]

Gymnasterkoreayne A (**1**) was obtained as a light yellow oil with a negative optical rotation ($[\alpha]_D$, −14°). The IR (2232 and 1640 cm^{-1}) and UV absorptions (240, 252, 266, and 282 nm) suggested the presence of two triple bonds and one double bond in conjugation.[16] The HREIMS revealed the molecular formula of **1** to be $C_{10}H_{12}O_2$. The ^1H NMR spectrum showed signals for two olefinic protons



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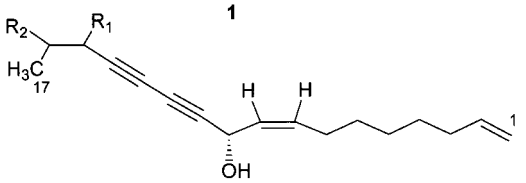
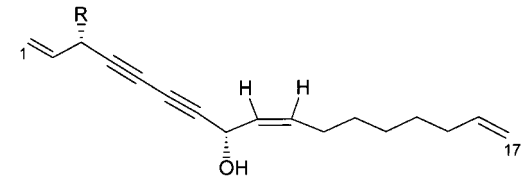
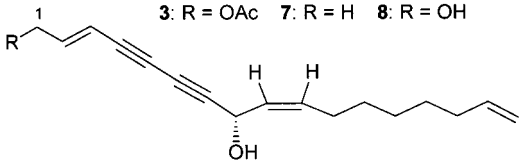


	R_1	R_2
2:	-O-	
5:	OH	OH



3: R = OAc **7**: R = H **8**: R = OH



4: R = OAc **6**: R = OH

at δ 5.50 (dd, J = 15.8 and 1.7 Hz) and 6.30 (dd, J = 15.8 and 6.9 Hz), an oxymethine at δ 4.68 (t, J = 6.3 Hz), and an oxymethylene at δ 3.79 (t, J = 5.6 Hz) (Table 1). The ^{13}C NMR spectrum, aided by the DEPT and HETCOR pulse sequence, showed signals for four nonprotonated acetylene carbons (δ 81.7, 69.8, 71.5, and 77.8), two oxygen-bearing carbons at δ 59.7 and 61.4, two olefinic carbons at δ 109.5 and 144.1, a methyl at δ 18.8, and a methylene at δ 38.8 (Table 2). The $^1H-^1H$ COSY NMR spectrum showed two terminal spin systems that began with methyl (δ 1.77) and oxymethylene (δ 3.79) protons. The methyl protons coupled to an olefinic proton (δ 6.30) that was further coupled to a second olefinic proton (δ 5.50). The oxygen-bearing methylene protons (δ 3.79) correlated with two methylene protons (δ 1.94) and an oxymethylene proton (δ 4.68),

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**−**6** ($CDCl_3$)

position	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[a]	**6**[b]
1c	3.79 t (5.6)	4.95 ddt (10.2, 2.0, 1.6)	5.28 d (10.1)	4.60 dd (5.7, 1.6)	4.94 ddt (10.2, 2.0, 1.6)	4.24 dd (4.8, 2.0)
1t		5.01 ddt (17.1, 2.0, 1.6)	5.47 d (16.9)		4.99 ddt (17.0, 2.0, 1.6)	
2	1.94 m	5.81 ddt (17.1, 10.2, 6.8)	5.79 ddd (16.9, 10.1, 6.3)	6.28 dt (16.3, 5.7)	5.81 ddt (17.0, 10.2, 6.8)	6.41 dt (15.9, 4.8)
3	4.68 t (6.3)	2.05 dt (7.3, 6.8)	5.84 d (6.3)	5.76 d (16.3)	2.05 dt (7.1, 6.9)	5.82 dd (15.9, 1.9)
4		1.37 m			1.35 m	
5		1.37 m			1.35 m	
6		1.37 m			1.35 m	
7		2.11 dtd (9.0, 7.3, 1.3)			2.11 dt (6.9, 6.3)	
8	5.50 d (15.8, 1.7)	5.61dtd (10.4, 7.3, 1.3)	5.13 d (8.3)	5.21 d (8.2)	5.62 dtd (10.9, 6.9, 0.8)	5.23 d (8.3)
9	6.30 dq (15.8, 6.9)	5.51 ddt (10.4, 8.3, 1.0)	5.44 ddt (10.4, 8.3, 1.0)	5.50 dd (10.1, 8.2)	5.51 dd (10.9, 8.1)	5.52 ddt (10.4, 8.3, 1.3)
10	1.77 dd (6.9, 1.7)	5.19 d (8.3)	5.54 dtd (10.4, 7.6, 2.0)	5.57 dt (10.1, 7.3)	5.20 br d (8.1)	5.60 dtd (10.4, 7.6, 1.0)
11			2.04 dtd (9.0, 7.6, 2.0)	2.10 dt (7.3, 7.2)		2.12 dtd (7.6, 7.3, 1.3)
12			1.28 m	1.34 m		1.36 m
13			1.28 m	1.34 m		1.36 m
14			1.28 m	1.34 m		1.36 m
15		3.13 d (2.1)	1.98 dt (7.0, 6.9)	2.07 dt (7.0, 6.9)	4.61 dd (4.1, 0.6)	2.05 dt (7.1, 6.9)
16		3.19 qd (5.2, 2.1)	5.74 ddt (17.1, 10.2, 6.9)	5.78 ddt (17.1, 10.2, 7.0)	4.04 dq (6.3, 4.1)	5.81 ddt (17.1, 10.1, 7.1)
17c		1.35 d (5.2)	4.87 ddt (10.2, 1.3, 1.0)	4.92 dd (10.2, 1.5)	1.36 d (6.3)	4.94 dd (10.1, 1.6)
17t			4.93 ddt (17.1, 1.6, 1.3)	4.98 dd (17.1, 1.5)		5.00 dd (17.1, 1.6)
CH_3CO			2.03 s	2.07 s		

[a] 300 MHz. [b] 600 MHz. δ values in ppm and coupling constants (in parentheses) in Hz.

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**6** (75 MHz, in $CDCl_3$)

carbon	**1**	**2**	**3**	**4**	**5**	**6**
1	59.7 t	114.3 t	119.7 t	63.5 t	114.3 t	62.6 t
2	38.8 t	138.9 d	131.9 d	140.0 d	138.9 d	145.8 d
3	61.4 d	33.7 t	64.4 d	111.5 d	33.6 t	108.6 d
4	81.7 s	28.6 t	74.8 s	76.3 s	28.6 t	77.3 s
5	69.8 s	28.7 t	70.8 s	74.6 s	28.7 t	73.8 s
6	71.5 s	29.1 t	68.5 s	69.0 s	29.0 t	69.5 s
7	77.8 s	20.9 t	80.0 s	82.5 s	27.6 t	81.9 s
8	109.5 d	134.6 d	58.5 d	58.5 d	134.6 d	58.7 d
9	144.1 d	127.7 d	127.7 d	127.9 d	127.5 d	128.0 d
10	18.8 q	58.6 d	134.5 d	134.0 d	58.5 d	134.2 d
11		78.7 s	27.6 t	27.5 t	81.0 s	27.6 t
12		67.5 s	29.1 t	29.0 t	68.3 s	29.1 t
13		68.8 s	28.7 t	28.6 t	72.0 s	28.7 t
14		76.6 s	28.6 t	28.5 t	74.2 s	28.6 t
15		46.1 d	33.6 t	33.6 t	54.9 d	33.6 t
16		56.9 d	138.9 d	138.9 d	70.6 d	139.0 d
17		17.3 q	114.3 t	114.2 t	18.8 q	114.3 t
CH_3CO			20.8 q	20.7 q		
CH_3CO			169.4 s	170.5 s		

indicating the end of the spin system (Figure 1). In addition, partial structures were linked using the HMBC NMR technique. Long-range correlations between δ_H 4.68 (H-3) and δ_C 69.8 (C-5)/81.7 (C-4), and δ_H 5.50 (H-8) and δ_C 77.8 (C-7)/71.5 (C-6), confirmed that the conjugated diynes were connected to C-3 and C-8, respectively. This finding was further supported by the presence of the prominent fragment ion peaks at *m/z* 89 $[C_7H_5]^+$ and 119 $[C_8H_7O]^+$ in the EIMS. The C-8,9 alkene bond was assigned as *E*, as evidenced by a vicinal coupling constant of J = 15.8 Hz.[17] To determine the absolute stereochemistry at C-3, compound **1** was subjected to derivatization by the modified Mosher method.[18,19] Compound **1** was treated with (+)- and (−)-α-methoxyl-α-trifluoromethylphenylacetyl chloride (MTPA-Cl) in the presence of 4-(dimethylamino)-pyridine (DMAP) and gave the (*R*)- and (*S*)-MTPA esters

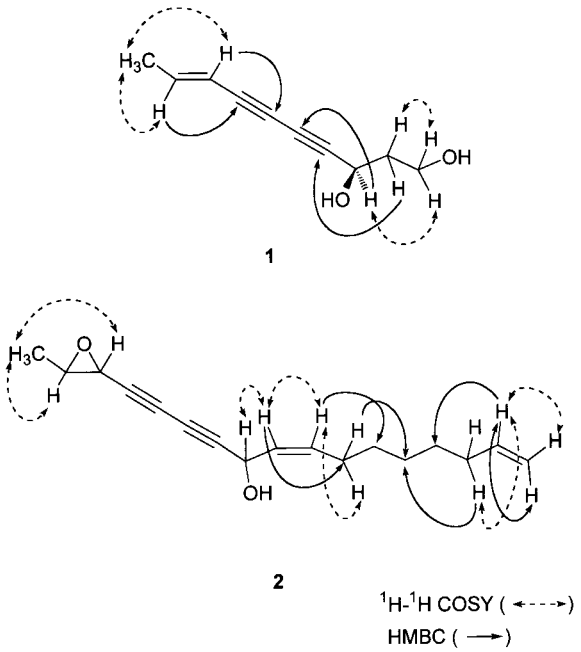
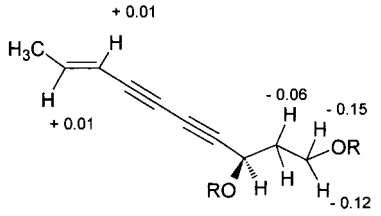


Figure 1. ^{1}H−^{1}H COSY and HMBC correlations for **1** and **2**.

(**1a** and **1b**). In the ^{1}H NMR spectrum of the (*S*)-MTPA ester (**1b**), proton signals assigned to H_2-1 and H_2-2 were observed at a higher field than those of the (*R*)-MTPA ester (**1a**), while signals due to H-8 and H-9 in the former ester were shifted to a lower field than those in the latter ester (Figure 2). Therefore, the absolute configuration at C-3 was concluded to be 3*R*. Consequently, the structure of gymnasterkoreayne A (**1**) was determined as (3*R*)-8-decen-4,6-diyn-1,3-diol.

Gymnasterkoreayne B (**2**) was isolated as an optically active oil, $[\alpha]_D$ +267°, and was attributed a molecular



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

Figure 2. Chemical shift difference for the (*S*)-MTPA ester (**1b**) and (*R*)-MTPA ester (**1a**) in ppm.

formula of $C_{17}H_{22}O_2$ by HRFABMS and ^{13}C NMR. Its UV spectrum was similar to that of **1**, indicating the presence of a conjugated diyne. This was supported by the ^{13}C, DEPT, and HETCOR NMR spectra, which showed signals for four nonprotonated acetylenic carbons (δ 78.7, 67.5, 68.8, and 76.6) and four olefinic carbons (δ 114.3, 138.9, 134.6, and 127.7). The ^1H NMR spectrum exhibited the presence of five olefinic protons including terminal vinyl group protons (δ 5.01, 4.95, and 5.81) and three oxymethine protons (δ 5.19, 3.19, and 3.13). The chemical shifts of two oxymethine protons (δ 3.19 and 3.13) indicated the presence of an epoxide including the ^{13}C NMR signals at δ 46.1 and 56.9 when compared to those of chloropanaxydiol.[20] This was further supported by a prominent fragment ion peak at *m*/*z* 55 $[C_3H_5O]^+$ in the CIMS. Analysis of ^1H and $^1H-^1H$ COSY NMR spectra indicated that one of the terminal spin systems began with a methyl proton (δ 1.35, d, J = 5.2 Hz). The terminal methyl was coupled to an oxymethine proton (δ 3.19, qd, J = 5.2, 2.1 Hz) that was further linked to another oxymethine proton (δ 3.13, d, J = 2.1 Hz). The COSY spectrum also showed a separate spin system that began at an allylic oxymethylene proton (δ 5.19). This proton was coupled to an olefinic proton (δ 5.51), which, in turn, was coupled to its vicinal partner (δ 5.61). A correlation was observed between this latter proton and an allylic methylene (δ 2.11). The other olefinic proton (δ 5.81) was *cis*-coupled to one proton (δ 4.95), *trans*-coupled to another (δ 5.81), and vicinally coupled to an allylic methylene (δ 2.05). This latter signal also correlated with the overlapped methylenes (H_2-4 and H_2-5). The five continuous methylenes were confirmed by HMBC (Figure 1) and the CIMS spectrum, which showed a prominent fragment ion peak at *m*/*z* 161 $[M - C_7H_{14} + H]^+$. In addition, two conjugated diynes were connected to the C-10 and C-15 oxymethines, and this was confirmed by the HMBC spectrum correlation peaks, similar to those of **1** (Figure 1).

The configuration of the epoxide was assigned as *trans* on the basis of the coupling constant (J = 2.1 Hz).[21] The C-8 double bond was determined to be in the *Z* configuration based on the vicinal coupling constant (J = 10.4 Hz). The absolute stereochemistry of the asymmetric carbon at C-10 was assigned as *S* based on ^{13}C NMR chemical shifts of C-9−C-12 (δ 127.7, 58.6, 78.7, and 67.5) and its optical rotation, which resembled that of dendroarboreol A, isolated from *Dendropanax arboreus*.[10] From these data, the structure of gymnasterkoreayne B (**2**) was established as (10*S*)-15,16-epoxy-1,8-heptadecadien-11,13-diyn-10-ol.

Gymnasterkoreayne C (**3**) was isolated as a light yellow oil, and its molecular formula of $C_{19}H_{24}O_3$ was established using CIMS and NMR data. Compound **3** showed spectral data that matched those of **2**. Its IR spectrum displayed an intense carbonyl absorption (1750 cm^{-1}), and this was supported by a fragment ion at *m*/*z* 241 $[M - AcOH + H]^+$ in the CIMS. In the ^1H NMR spectrum (Table 1), a new methyl signal at δ 2.03 and three olefinic protons at δ 5.28, 5.47, and 5.79 appeared in place of the methyl and epoxide protons of **2** (Table 1). Corresponding changes were also observed in the ^{13}C NMR spectrum in which two new sp^2 olefinic carbons (δ 119.7 and 131.9) and acetyl group carbons (δ 20.8 and 169.4) appeared. A combination of $^1H-^1H$ COSY, HETCOR, and HMBC NMR experiments was used to show that the two olefinic carbons correlated with an oxymethine (δ_H 5.84, δ_C 64.4), which constituted a terminal group. The connectivity of the acetyl group at C-3 was confirmed by the HMBC correlations of δ_H 5.84 (H-3) and δ_C 20.3 (C-19). The absolute configurations at C-3 and C-8 were assigned as 3*S*,8*S* based on ^{13}C NMR chemical shifts of C-1−C-9 and from the optical rotation ($[\alpha]_D$, +263°), which resembled analogous data of dehydrofalcarindiol isolated from *Dendropanax arboreus*.[10] Consequently, the structure of gymnasterkoreayne C (**3**) was established as (3*S*,8*S*)-1,9,16-heptadecatrien-4,6-diyn-3,8-diol-3-acetate.

The molecular formula of gymnasterkoreayne D (**4**) was assigned as $C_{19}H_{24}O_3$ by HRFABMS and ^{13}C NMR, which was identical to that of **3**. In addition, the NMR spectral data of **4** were almost identical to those of **3**. However, careful examination of the ^1H and ^{13}C NMR spectra of both compounds showed considerable differences in the olefinic group signals of C-1−C-3. Therefore, the terminal structure of **4** was structurally modified when compared with **3**. This was confirmed using a combination of 2D NMR spectra. The $^1H-^1H$ COSY NMR spectrum showed that the hydroxy-bearing proton (δ 4.60) was spin-coupled to an olefinic proton (δ 6.28) and further coupled to the other olefinic proton (δ 5.76), thus forming the same enol functionality as **3**. Significant HMBC correlations of the olefinic protons at δ 5.76 (H-3) and 63.5 (C-1)/δ 76.3 (C-4)/δ 74.6 (C-5), respectively, showed that the enol and diyne groups were connected to each other. Similarly, the long-range HMBC correlation of the oxygen-bearing proton at δ 4.60 (H-1) and the carbonyl carbon at δ 170.5 confirmed the location of the acetyl group at C-1. This was further supported by the prominent fragment ion peak at *m*/*z* 99 $[C_5H_7O_2]^+$ in the CIMS. The geometry of the C-2 double bond was assigned as *E* on the basis of the vicinal coupling constant (J = 16.3 Hz). Consequently, the structure of gymnasterkoreayne D (**4**) was determined as (8*S*)-2,9,16-heptadecatrien-4,6-diyn-1,8-diol-1-acetate.

Gymnasterkoreayne E (**5**) was assigned a molecular formula of $C_{17}H_{24}O_3$, from its HRFABMS and ^{13}C NMR spectra. The NMR spectrum of **5** was very similar to that of **2**. Careful examination of the spectral data, however, revealed several significant differences. The most notable changes were among chemical shifts of H-15 (δ 4.61), H-16 (δ 4.04), and H-17 (δ 1.36), which were lower by 1.48, 0.85, and 0.1 ppm, respectively, compared to the corresponding ^1H NMR signals of **2**. Furthermore, the ^{13}C NMR signals of C-15 (δ 54.9), C-16 (δ 70.6), and C-17 (δ 18.8) were shifted to lower fields by 8.8, 13.7, and 1.5 ppm, respectively, versus analogous data of **2**. These results indicated the presence of a diol group in **5**, instead of the epoxide group in **2**. This was supported by a fragment ion peak at *m*/*z* 97 $[C_5H_8O_2]^+$ in the CIMS. The diol group was assigned with the *erythro* configuration on the basis of the J coupling constant (4.1 Hz).[22] The structure of gymnasterkoreayne E (**5**) was determined as *erythro*-(10*S*)-1,8-heptadecadien-11,13-diyn-10,15,16-triol.

Gymnasterkoreayne F (**6**) was also isolated as a yellowish oil. Its molecular formula was deduced as $C_{17}H_{22}O_2$ by

Table 3. Cytotoxicity of the Compounds **1**−**8** against L1210 Tumor Cells

compound	ED_{50} (μg/mL)
1	>10
2	3.3
3	2.1
4	7.7
5	9.6
6	3.1
7	10.4
8	0.12
cisplatin[a]	0.02

[a] Positive control.

HRFABMS and ^{13}C NMR. The ^{1}H NMR spectrum of **6** was similar to that of **4**, except for the absence of a methyl signal at δ 2.03. This suggested the presence of a hydroxyl group instead of an acetyoxy group. Corresponding changes were also observed in the ^{13}C NMR spectrum in which the acetyl carbon signals at δ 20.7 and 170.5 of **4** were absent. Thus, the structure of gymnasterkoreayne F (**6**) was assigned as (8*S*)-2,9,16-heptadecatrien-4,6-diyn-1,8-diol.

The compounds (**1**−**8**) were tested for their cytotoxicity against L1210 (mouse leukemia) tumor cell lines (Table 3). Compounds **3** and **8** exhibited significant cytotoxicity against the cell lines tested, with ED_{50} values of 2.1 and 0.12 μg/mL, respectively, while compounds **2** and **4**−**7** were less potently cytotoxic (ED_{50}, 3.1−10.4 μg/mL). However, gymnasterkoreayne A (**1**) was inactive to L1210 cells. These results suggested that the two terminal double bonds in the compounds tested are important for the enhancement of cytotoxicity against L1210 tumor cells.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in MeOH. UV spectra were recorded on a Shimadzu-160A spectrometer. FT-IR spectra were obtained on a JASCO-40 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 300 or 600 spectrometer, the chemical shifts being represented as ppm with tetramethylsilane as an internal standard. EIMS and CIMS were measured on an Autospec mass spectrometer (Micromass). Preparative HPLC was performed on a Shimadzu LC-10AD pump and SPD-10AV UV-detector.

Plant Material. Roots of *G. koraiensis* 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 Purification. The air-dried roots (4.8 kg) of *G. koraiensis* were 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- (140 g) and BuOH-soluble fractions (123 g), respectively. The CH_2Cl_2-soluble fraction was chromatographed on a silica gel column. The column was eluted using a stepwise gradient of hexane and EtOAc to give nine fractions. Repeated column chromatography of each fraction on silica gel (hexane−acetone) and preparative TLC (hexane−acetone), followed by HPLC on RP-C_{18} (50−70% aqueous MeOH), afforded **1** (40 mg), **2** (50 mg), **3** (50 mg), **4** (40 mg), **5** (50 mg), **6** (40 mg), **7** (40 mg), and **8** (50 mg).

Gymnasterkoreayne A (1): yellow oil; $[\alpha]_D^{20}$ −14° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 240 (0.68), 252 (1.18), 266 (1.59), 282 (1.25) nm; IR (CCl_4) ν_{max} 3324, 2928, 2856, 2232, 2640, 1640, 1010, 870 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 164 $[M]^+$ (5), 146 (25), 119 (100), 89 (42); HREIMS *m*/*z* 164.0827 (calcd for $C_{10}H_{12}O_2$, 164.0837).

(*R*)-MTPA Ester of 1 (1a): (+)-MTPA chloride (1.5 mg) and DMAP (10 mg) in pyridine (0.2 mL) were added to a solution of **1** (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 by preparative thin-layer chromatography (TLC) [hexane−acetone (1:1)] to give the (*R*)-MTPA ester (**1a**, 1.5 mg) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 4.35 (1H, m, H_a-1), 4.46 (1H, m, H_b-1), 2.26 (2H, m, H-2), 5.58 (1H, t, J = 6.9 Hz, H-3), 5.52 (1H, ddd, J = 15.8, 1.8, 0.7 Hz, H-8), 6.38 (1H, m, H-9), 1.84 (3H, dd, J = 6.9, 1.8 Hz, H-10), 3.51 (3H, d, J = 1.0 Hz, MTPA-OCH_3), 3.52 (3H, d, J = 1.2 Hz, MTPA-OCH_3).

(*S*)-MTPA Ester of 1(1b): (−)-MTPA chloride (1.5 mg) and DMAP (10 mg) in pyridine (0.2 mL) were added to a mixture of **1** (2 mg) in CCl_4 (0.2 mL). Workup as described above gave a (*S*)-MTPA ester (**1b**, 1.5 mg) as colorless oil: ^{1}H NMR ($CDCl_3$) δ 4.20 (1H, m, H_a-1), 4.34 (1H, m, H_b-1), 2.20 (2H, m, H-2), 5.61 (1H, t, J = 6.9 Hz, H-3), 5.53 (1H, ddd, J = 15.8, 1.8, 0.7 Hz, H-8), 6.39 (1H, m, H-9), 1.84 (3H, dd, J = 6.7, 1.8 Hz, H-10), 3.53 (3H, d, J = 1.2 Hz, MTPA-OCH_3), 3.57 (3H, d, J = 1.2 Hz, MTPA-OCH_3).

Gymnasterkoreayne B (2): yellow oil; $[\alpha]_D^{20}$ +267° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 237 (1.15), 250 (1.17), 264 (0.82) nm; IR (KBr) ν_{max} 3603, 2930, 2857, 2154, 1641, 1549, 990 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; CIMS *m*/*z* 259 $[M + H]^+$ (14), 241 (60), 197 (100), 161 (15), 155 (50), 135 (56), 95 (68), 55 (53); HRFABMS *m*/*z* 281.1517 (calcd for $C_{17}H_{22}O_2Na$, 281.1519).

Gymnasterkoreayne C (3): yellow oil; $[\alpha]_D^{20}$ +263° (*c* 0.5, MeOH); UV λ_{max} (log ϵ) 233 (1.34), 246 (1.19), 260 (0.71) nm; IR (KBr) ν_{max} 3466, 2929, 2857, 2159, 1750, 1640, 1222, 990, 910, 868 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; CIMS *m*/*z* 301 $[M + H]^+$ (14), 283 (84), 241 (100), 171 (76), 131 (51), 93 (36); HRFABMS *m*/*z* 323.1622 (calcd for $C_{19}H_{24}O_3Na$, 323.1624).

Gymnasterkoreayne D (4): yellow oil; $[\alpha]_D^{20}$ +281° (*c* 0.5, MeOH); UV λ_{max} (log ϵ) 242 (0.81), 255 (1.39), 269 (1.82), 285 (1.50) nm; IR (KBr) ν_{max} 3465, 2929, 2857, 2360, 1748, 1640, 1382, 1225, 1000, 910 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; CIMS *m*/*z* 301 $[M + H]^+$ (16), 283 (43), 241 (100), 171 (76), 131 (51), 99 (10), 93 (36); HRFABMS *m*/*z* 323.1632 (calcd for $C_{19}H_{24}O_4Na$, 323.1624).

Gymnasterkoreayne E (5): yellow oil; $[\alpha]_D^{20}$ +260° (*c* 0.5, MeOH); UV λ_{max} (log ϵ) 236 (0.66), 250 (0.69), 263 (0.37) nm; IR (KBr) ν_{max} 3436, 2927, 2856, 2347, 1639, 1550, 1383, 1000 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; CIMS *m*/*z* 277 $[M + H]^+$ (70), 259 (39), 243 (50), 223 (12), 197 (91), 135 (100), 97 (47), 95 (82); HRFABMS *m*/*z* 299.1622 (calcd for $C_{17}H_{24}O_3Na$, 299.1624).

Gymnasterkoreayne F (6): yellow oil; $[\alpha]_D^{20}$ +296° (*c* 0.5, MeOH); UV λ_{max} (log ϵ) 241 (0.73), 254 (1.26), 268 (1.70), 284 (1.37) nm; IR (KBr) ν_{max} 3324, 2928, 2856, 2232, 1721, 1640, 1549, 1371, 1010, 990, 910, 860 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; CIMS *m*/*z* 259 $[M + 1]^+$ (14), 241 (100), 223 (54), 171 (75), 131 (71), 91 (85); HRFABMS *m*/*z* 281.1513 (calcd for $C_{17}H_{22}O_2Na$, 281.1519).

Cytotoxicity Assay. The L1210 murine leukemia cell line was used with RPMI 1640 containing 5% fetal calf serum (FCS). A cell suspension (30 000−40 000 cells/mL) was prepared in the culture medium, which was then inoculated into each well of a 96-well microtiter plate. One day after plating, a time-zero control plate was made. The cells were directly treated with the compounds and incubated for 48 h in a CO_2 incubator. Cell viability was measured by a Cell Counting Kit (Dojindo, Kumamoto, Japan) containing WST-1 reagent. The reagent mixture of A and B was added, and its optical density (OD) was determined with a microplate reader at 405 nm. Growth inhibition was calculated as follows: the OD of the treated well was subtracted from the OD of the time-zero (T_z) plate and divided by the calculated value of the untreated control. A growth inhibition of 50% (ED_{50}) was calculated by the Probit method.[23]

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