Antiproliferative Effects of Saponins from the Roots of *Platycodon grandiflorum* on Cultured Human Tumor Cells

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Three new triterpenoid saponins, platyconic acid B lactone (**1**), deapio-platyconic acid B lactone (**2**), and deapio-platycodin D_2 (**3**), together with 17 known triterpenoid saponins, were isolated from a root extract of *Platycodon grandiflorum*. The structures of **1**−**3** were determined on the basis of spectroscopic data interpretation and chemical transformation. Saponins with a platycodigenin or polygalacic acid unit as a sapogenin demonstrated significant inhibitory effects on the proliferation of a small panel of cultured human tumor cells.

Platycodon grandiflorum A. DC. (Campanulaceae), commonly called "balloon flower" or "bell flower", is a perennial flowering plant widespread in northeast Asia. The roots of this species, either dried or fresh, are used as an ingredient in salads and traditional cuisine in Korea. The annual domestic consumption of the plant as a food material in 2001 was estimated as over 4000 tonnes in Korea. The roots of *P. grandiflorum* are also used as an expectorant and antitussive to treat coughs, colds, sore throats, tonsillitis, and chest congestion in Traditional Chinese Medicine. Several biological activities of the extract from the plant have been reported, including chemopreventive,[1−5] antioxidant,[6] antihyperglycemic,[7] anti-inflammatory,[8,9] hepatoprotective,[10−12] neuroprotective,[13−15] and antiobesity[16] effects. As the main chemical constituents of *P. grandiflorum*, 39 triterpenoidal saponins with an oleanene backbone have been reported so far and are regarded as being responsible for these various biological effects.[17−25]

Result and Discussion

In previous work, the isolation of the triterpenoid saponins deapioplatycoside E and platyconic acid A from the root extract of *P. grandiflorum* was reported.[1,25] In a continuation of the phytochemical investigation of the plant extract, three new saponins, platyconic acid B lactone (**1**), deapio-platyconic acid B lactone (**2**), and deapio-platycodin D_2 (**3**), were isolated, together with 17 related saponins. In this paper, the isolation and structure determination of **1**−**3** is reported, as well as the inhibitory effects of all compounds isolated on the proliferation of four cultured human tumor cell lines. The known compounds were identified as platyconic acid A (**4**),[25] platycodin D (**5**),[17] 2″*O*-acetylplatycodin D (**6**),[17] 3″*O*-acetylplatycodin D (**7**),[17] deapio-platycodin D,[17] platycodin D_2 (**8**),[17] platycodin D_3,[17] deapio-platycodin D_3,[17] platycoside E,[17] deapioplatycoside E,[1] polygalacin D (**9**),[17] 2″*O*-acetylpolygalcin D (**10**),[17] 3″*O*-acetylpolygalacin D (**11**),[17] polygalacin D_2 (**12**),[17] 2″*O*-acetylpolygalacin D_2 (**13**),[17] 3″*O*-acetylpolygalacin D_2 (**14**),[17] and polygalacin D_3 (platycoside G3),[21] respectively. All isolates of known structure were identified by comparison of their spectroscopic data with values in the literature.

Compound **1** was obtained as a white, amorphous powder, and its molecular formula established as $C_{63}H_{98}O_{33}$, based on the HRESIMS. The IR spectrum exhibited absorptions at 3427 cm^{-1} (OH) and 1755 cm^{-1} (γ-lactone carbonyl). The ^1H and ^{13}C NMR spectroscopic data (Tables 1−3) of **1** indicated the presence of an unusual sapogenin and six sugar moieties. All ^1H and ^{13}C NMR data of **1** were assigned with the aid of the COSY, HMQC, and HMBC pulse sequences. The HMQC and HMBC experiments revealed that the sapogenin of **1** is platycogenic acid A lactone (3β,16α,23-trihydroxyolean-12-en-2,24-lacton-28-oic acid), produced via intramolecular esterification between the 24-carboxylic acid and the 2-hydroxy group of platycogenic acid A,[17,25] which was reported as a sapogenin of platyconic acid A lactone.[26] The lactone carbon of C-24 (δ_C 178.5) was observed to be correlated with H-2 (δ_H 5.42) on the basis of the HMBC spectrum. The chemical shifts of C-3 (δ_C 89.5) and C-28 (δ_C 176.0) implied that **1** is a bisdesmosidic glycoside, with sugar chains at C-3 and C-28, respectively. The ^1H and ^{13}C NMR spectra of **1** exhibited signals for six sugar anomeric protons at δ_H 6.42 (brs), 5.64 (brs), 5.02 (d, J = 7.2 Hz), 6.15 (d, J = 1.8 Hz), 5.18 (d, J = 7.2 Hz), and 4.99 (d, J = 8.1 Hz) and for the analogous carbons at δ_C 93.4, 101.3, 106.5, 111.1, 105.1, and 105.5. The sequence and linkage pattern of each sugar unit attached to C-3 were identified from the HMBC experiments. The H-1″ signal (δ_H 4.99, J = 8.1 Hz) of the terminal glucose was correlated with C-6 (δ_C 70.0) of the inner glucose, and H-1 (δ_H 5.18, J = 7.2 Hz) of the inner glucose with C-3 (δ_C 89.5) of the sapogenin, which indicated that a gentiobiose [6-*O*-β-D-glucopyranosyl-D-glucose] unit is attached to the C-3 hydroxy with a β-glycosidic linkage. Similarly, the sugar moiety attached to C-28 was elucidated as α-L-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranose (Figure 1). On the basis of all the foregoing evidence, **1** was determined to be a new saponin, with the structure platycogenic acid A 3-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-28-*O*-[α-L-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinoside]. This compound has been assigned the trivial name platyconic acid B lactone.

Compound **2** was obtained as a white, amorphous powder with a molecular formula of $C_{58}H_{90}O_{29}$, as based on the HRESIMS. When the ^1H and ^{13}C NMR spectra of **2** (Tables 1−3) were compared with those of **1**, the proton and carbon signals of the apiosyl group observed in the NMR spectra of **1** were absent, and the C-3 chemical shift of the xylose unit was shifted upfield from δ_C 84.8 to 78.5 (Table 3), which indicated that **2** is a congener of **1** lacking the terminal apiose linked to C-3 of xylose. Thus, **2** (deapio-platyconic acid B) was determined as platycogenic acid A 3-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinoside]. Moreover, compound **1** was found to slowly convert to **2** by mild acid hydrolysis in 0.1 N HCl.

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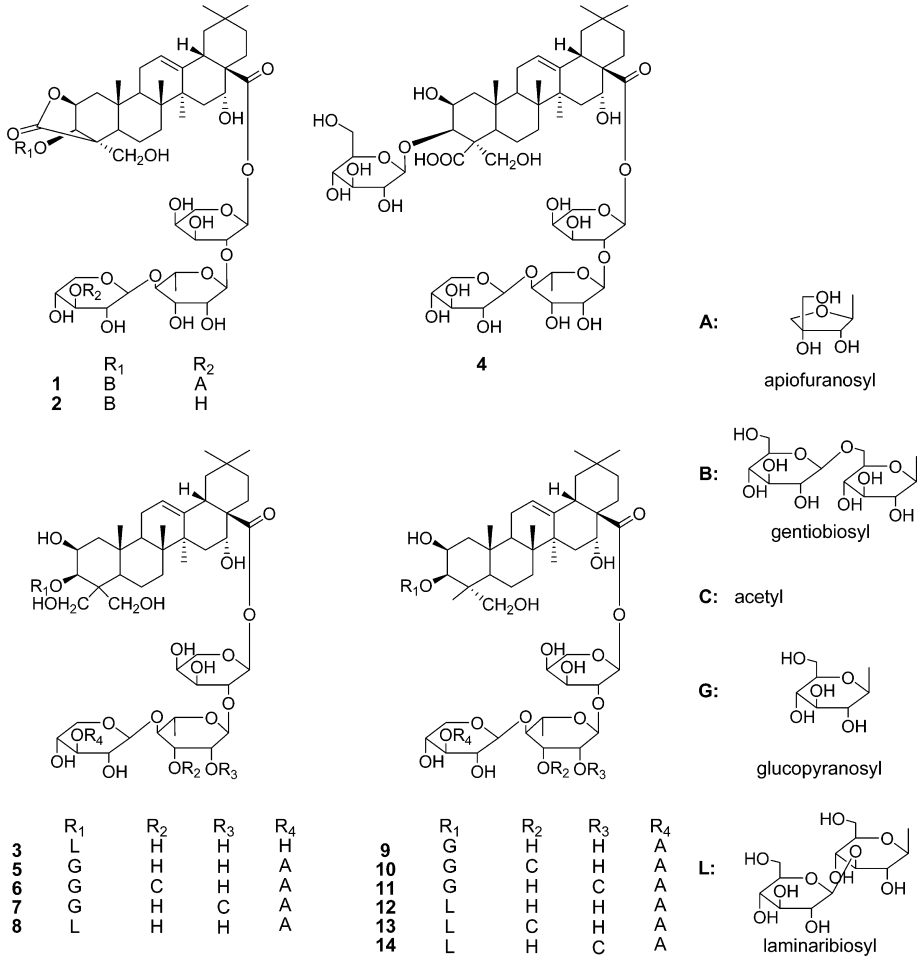
Chart 1



Compound **3** was also obtained as a white, amorphous powder, with a molecular formula of $C_{58}H_{94}O_{29}$ based on the HRESIMS. In the IR spectrum, absorptions at 3400 cm^{-1} (OH), 1742 cm^{-1} (ester carbonyl), and 1644 cm^{-1} (double bond) were observed. The ^1H NMR spectrum coupled with the ^{13}C NMR spectrum (Tables 1−3) indicated that the sapogenin of **3** is a platycodigenin (2β,3β,16α,23,24-pentahydroxyolean-12-en-28-oic acid) unit.[26] The chemical shifts of C-3 (δ_C 86.4) and C-28 (δ_C 176.1) suggested that **3** is a bisdesmosidic glycoside with two sugar chains linked to C-3 and C-28, respectively. The sequence and linkage pattern of each sugar unit attached to C-3 and C-28 were identified by the aid of 2D NMR spectra obtained by DEPT, COSY, HMQC, and HMBC experiments, as described in Figure 1. Thus, **3** (deapio-platycodin D_2) was determined as platycodigenin 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]. The ^1H and ^{13}C NMR spectra of **3** were closely comparable with those of platycodin D_2 (**8**) except for signals assignable to an apiose moiety. Accordingly, compound **8** was converted to **3** by the treatment with 0.1 N HCl. A compound described as deapio-platycodin D_2 was reported earlier by Yoshimura and co-workers, with detailed spectroscopic data.[17] However, these literature data are quite different from those obtained in the present investigation for **3**.

All isolated saponins were evaluated for their inhibitory activity on the proliferation of cultured human tumor cell lines using the sulforhodamine B method. The compounds that showed a significant inhibitory activity (IC_{50} < 10 μM) for the proliferation of the test cancer cell lines used are summarized in Table 4. Certain saponins with platycodigenin (**3**, **5**−**8**) and polygalacic acid (**9**−**14**) units showed cytotoxic activity.

Table 1. ^1H NMR Spectroscopic Data of the Sapogenins of **1**−**3** (900 MHz, C_5D_5N)[a]

position	1	2	3
1	1.65 m, 2.07 m	1.66 m, 2.07 m	1.55 m, 1.95 m
2	5.42 d (4.5)	5.42 d (4.5)	4.06 m
3	4.73 s	4.72 s	4.65 s
4			
5	2.19 d (12.6)	2.18 m	1.83 m
6	1.64 m, 2.21 m	1.23 m, 2.18 m	1.53 m, 1.81 m
7	1.42 d (12.6), 1.75 m	1.39 d (11.7), 1.75 m	1.41 m, 1.66 m
8			
9	2.03 m	2.03 m	1.89 m
10			
11	1.93 m, 2.03 m	1.92 m, 2.03 m	1.97 m, 2.03 m
12	5.52 brs	5.51 brs	5.62 brs
13			
14			
15	1.25 d (11.7), 2.22 m	1.23 m, 2.20 m	1.78 m, 2.25 m
16	5.17 brs	5.16 brs	5.21 m
17			
18	3.51 dd (4.0 14.0)	3.50 dd (3.6 13.5)	3.53 dd (4.0 14.4)
19	1.30 m, 2.68 t (13.5)	1.28 m, 2.67 t (13.5)	1.32 d (9.0), 2.71 t (13.5)
20			
21	1.70 m, 2.35 m	1.68 m, 2.35 m	1.25 m, 2.35 m
22	2.14 m, 2.25 m	2.15 m, 2.25 m	2.13 m, 2.29 m
23	4.30 m, 4.78 d (10.8)	4.30 m, 4.78 m	4.00 m, 4.50 m
24			4.13 m, 4.55 m
25	1.30 s	1.28 s	1.43 s
26	1.03 s	1.00 s	1.07 s
27	1.68 s	1.67 s	1.69 s
28			
29	0.95 s	0.94 s	0.95 s
30	1.10 s	1.10 s	1.09 s

[a] Assignments are based on HMQC and HMBC experiments, and chemical shifts are given in ppm.

Table 2. ^{1}H NMR Spectroscopic Data for the Sugar Moieties of **1**−**3** (900 MHz, C_5D_5N)[a]

position	1	2	3
glucose (inner) 1′	5.18 d (7.2)	5.20 d (8.1)	4.99 brs
2′	3.92 m	3.92 m	4.00 m
3′	4.13 m	4.10 m	4.07 m
4′	4.07 m	4.10 m	4.05 m
5′	3.98 m	3.98 m	3.85 m
6′	4.23 m, 4.75 d (10.8)	4.23 m, 4.75 d (10.8)	4.20 m, 4.41 d (10.8)
glucose (terminal) 1″	4.99 d (8.1)	5.00 d (8.1)	5.19 d (8.1)
2″	3.98 m	3.98 m	4.05 m
3″	4.17 m	4.13 m	4.20 m
4″	4.15 m	4.15 t (9.0)	4.11 m
5″	3.86 m	3.87 m	4.00 m
6″	4.29 m, 4.45 m	4.29 m, 4.45 d (9.9)	4.25 m, 4.53 m
arabinose 1	6.42 brs	6.42 brs	6.43 brs
2	4.45 m	4.49 brs	4.52 m
3	4.49 m	4.49 m	4.50 m
4	4.37 m	4.37 m	4.38 m
5	3.91 m, 4.50 m	3.91 m, 4.49 m	3.93 m, 4.50 m
rhamnose 1	5.64 brs	5.71 brs	5.73 brs
2	4.49 m	4.52 brs	4.53 m
3	4.49 m	4.53 m	4.55 m
4	4.33 m	4.35 m	4.37 m
5	4.30 m	4.33 m	4.36 m
6	1.65 d (5.4)	1.68 d (5.4)	1.70 d (5.4)
xylose 1	5.02 d (7.2)	5.12 d (8.1)	5.14 d (8.1)
2	3.98 m	3.99 m	4.00 m
3	3.99 m	4.05 t (9.0)	4.07 m
4	4.00 m	4.49 m	4.15 m
5	3.35 m, 4.15 m	3.45 t (10.8), 4.18 m	3.45 t (10.8), 4.19 m
apiose 1	6.15 d (1.8)		
2	4.77 d (2.7)		
3			
4	4.30 m, 4.71 d (9.0)		
5	4.13 m		

[a] The coupling constants (*J*) are in parentheses and reported in Hz, and chemical shifts are given in ppm. The assignments are based upon DEPT, HMQC, and HMBC experiments. Overlapped signals are labeled as multiplets (m).

Table 3. ^{13}C NMR Spectroscopic Data of **1**−**3** (225 MHz, C_5D_5N)[a]

position	1	2	3	position	1	2	3
1	41.1	41.2	45.1	glucose (inner) 1	105.1	105.2	105.7
2	83.4	83.5	69.7	2	74.9	74.9	74.2
3	89.5	89.5	86.4	3	78.1	78.1	88.5
4	54.0	54.1	48.1	4	71.2	71.3	69.8
5	51.8	51.9	46.7	5	77.1	77.2	78.1
6	19.4	19.5	19.4	6	70.0	70.1	62.2
7	33.4	33.6	33.7	glucose (terminal) 1′	105.5	105.5	105.6
8	40.4	40.5	40.5	2′	75.2	75.1	75.5
9	48.0	48.1	47.8	3′	78.3	78.3	78.2
10	37.6	37.7	37.5	4′	71.6	71.6	71.6
11	24.6	24.7	24.3	5′	78.4	78.5	78.8
12	122.3	122.4	123.2	6′	62.6	62.7	62.6
13	145.0	145.1	144.5	arabinose 1	93.4	93.6	93.6
14	42.3	42.4	42.4	2	75.5	75.4	75.3
15	36.0	36.0	36.1	3	70.0	70.1	70.1
16	73.8	73.9	74.1	4	65.8	66.1	66.1
17	49.6	49.6	49.7	5	62.8	63.1	63.2
18	41.3	41.4	41.6	rhamnose 1	101.3	101.3	101.3
19	47.0	47.1	47.1	2	71.8	71.9	71.9
20	31.0	31.0	31.0	3	72.5	72.6	72.6
21	36.0	36.0	36.2	4	83.5	83.7	83.6
22	32.1	32.2	32.2	5	68.6	68.7	68.7
23	57.0	57.1	63.6	6	18.3	18.5	18.5
24	178.5	178.5	63.5	xylose 1	106.5	106.9	106.8
25	17.5	17.5	18.3	2	75.0	76.0	76.0
26	18.2	18.2	17.7	3	84.8	78.5	78.5
27	27.3	27.4	27.2	4	69.3	71.0	71.0
28	176.0	176.1	176.1	5	66.9	67.5	67.5
29	33.3	33.4	33.4	apiose 1	111.1		
30	24.8	24.9	24.8	2	77.7		
				3	80.4		
				4	75.1		
				5	65.2		

[a] Assignments are based on HMQC and HMBC experiments, and chemical shifts are given in ppm.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Rudolph Autopol IV polarimeter. IR spectra were measured on a Bruker Equinox 55 FT-IR spectrometer. NMR spectra were obtained by Bruker AM 300 and 500 and Bruker AVANCE II 900 spectrometers, using TMS as an internal standard. HRESIMS was recorded on an Applied Biosystems Mariner time-of-flight mass spectrometer with an electrospray interface. For column chromatography, Diaion HP-20 and ODS (Cosmosil 140 C_{18}) were used as stationary phases. Preparative HPLC was performed on a Futecs P-4000 system with a Shim-pack prep-ODS (H) column (5 μm, 20 mm × 25 cm). Isolation and purification were also carried out using a medium-pressure liquid chromatographic (MPLC) system [Buchi pump module C-601, silica gel 60 (230−400 mesh, Merck), ODS (Cosmosil 140 C_{18})].

Plant Material. The roots of *P. grandiflorum* were cultivated for three years on a mountainside in Kyungnam Province, Korea. The plant was harvested in September 2003 and authenticated by one of the authors (K.R.L.). A voucher specimen (herbarium no. JS-03024) has been preserved at the Herbarium of JangSaeng Doraji Co., Ltd., Jinju, Korea.

Extraction and Isolation. Dried roots of *P. grandiflorum* (5.0 kg) were extracted with MeOH by percolation for seven days at room temperature to give 1.4 kg of a dark, syrupy extract, which was suspended in 5 L of water and extracted with EtOAc (3 × 5 L). The aqueous layer was further extracted with *n*-BuOH (3 × 5 L). The *n*-BuOH layer (130 g) was suspended in 2 L of H_2O and poured into a Diaion HP-20 column (Φ = 5.0 × 100 cm), which was stabilized with H_2O. The column was washed with 5 L of H_2O and then eluted with 5 L of MeOH. The eluate was concentrated under reduced pressure to give 75 g of a crude saponin mixture. A part of this mixture (50 g) was subjected to MPLC (ODS, Φ = 5.0 × 70 cm), performed by stepwise gradient elution with water−MeOH mixtures, changing the ratio of water and MeOH from 9:1, 7:3, 5:5, 2:8, and 1:10, to give five fractions (Fr.1−Fr.5). Fr.5 (4.8 g) was divided into three further fractions (Fr.51−Fr.53) by MPLC (silica gel, 230−400 mesh, Φ = 5.0 × 5 cm) eluted with CH_2Cl_2−MeOH (30% to 100% MeOH

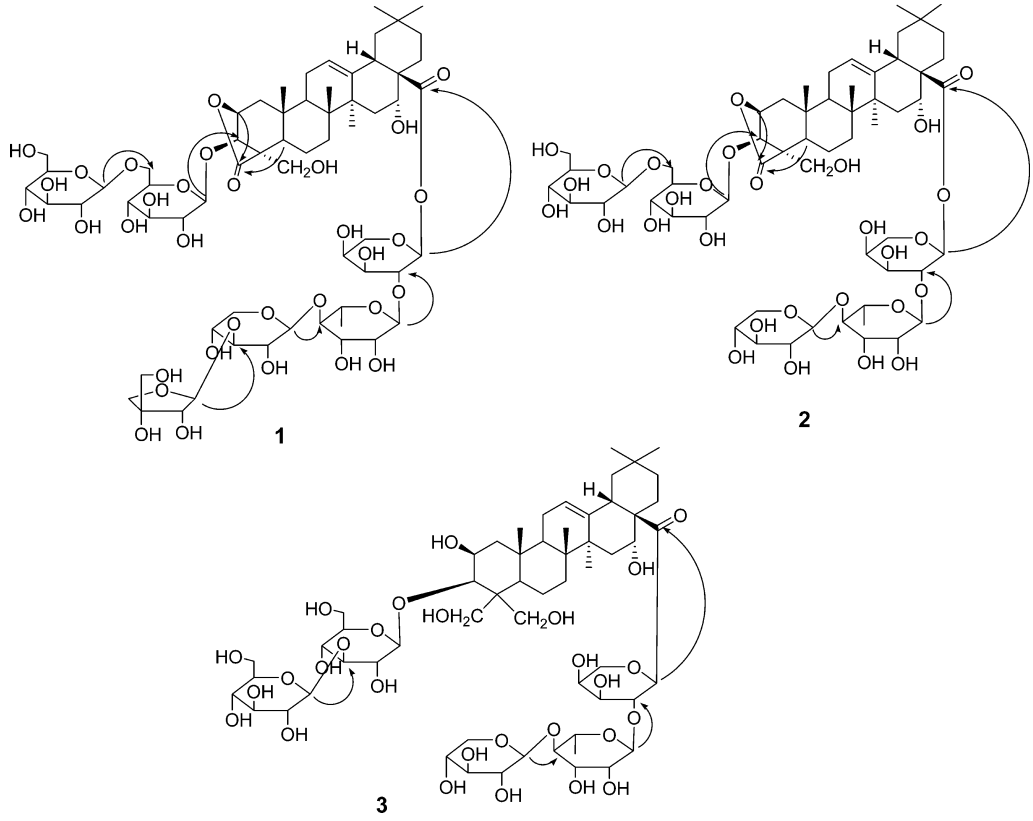


Figure 1. Key HMBC correlations of **1**−**3**.

Table 4. Inhibition of Tumor Cell Proliferation (IC_{50} value, μM) by Saponins Isolated from *P. grandiflorum*[a]

	IC_{50} (μM)			
compound	MES-SA	MES-SA/DX5	HCT	HCT15/CL02
5	3.9 ± 0.45	>10.0	5.7 ± 0.64	7.9 ± 0.77
6	2.3 ± 0.63	2.2 ± 0.82	3.4 ± 0.76	4.2 ± 0.64
7	2.3 ± 0.83	2.2 ± 0.94	3.4 ± 0.52	4.1 ± 0.53
8	3.5 ± 0.43	5.2 ± 0.32	9.6 ± 0.35	5.7 ± 0.63
9	5.0 ± 0.74	7.7 ± 0.63	8.5 ± 0.45	>10.0
10	3.6 ± 0.55	4.2 ± 0.76	6.7 ± 0.54	>10.0
11	6.3 ± 0.36	6.2 ± 0.37	>10.0	>10.0
12	2.6 ± 0.73	3.4 ± 0.85	3.8 ± 0.56	5.8 ± 0.44
13	3.4 ± 0.46	3.4 ± 0.93	3.6 ± 0.86	5.9 ± 0.35
14	3.3 ± 0.54	3.4 ± 0.64	3.6 ± 0.36	5.8 ± 0.46
etoposide[b]	0.3 ± 0.63	>10.0	1.6 ± 0.54	>10.0

[a] Data are expressed as the mean ± SD of three distinct experiments. [b] Positive control substance.

gradient). Each fraction was subjected to preparative HPLC (Shimpack ODS column) with 50% MeOH to give compounds **1** (3 mg), **2** (4 mg), platycoside E (350 mg), and deapio-platycoside E (32 mg), respectively. Fr.3 (28.0 g) was chromatographed on MPLC (silica gel, 230−400 mesh, Φ = 5.0 × 15 cm), eluted with EtOAc−EtOH (10% to 90% EtOH gradient), to give four fractions (Fr.31−Fr.34). Each of these fractions was subjected to preparative HPLC (Shim-pack ODS column) with 55% MeOH to give compounds **3** (17 mg), **5** (800 mg), deapio-palatycodin D (15 mg), **8** (450 mg), **9** (150 mg), **10** (130 mg), **11** (40 mg), **12** (230 mg), **13** (100 mg), and **14** (32 mg). Fr.2 (1.2 g) was chromatographed by MPLC (silica gel, 230−400 mesh, Φ = 2.0 × 10 cm), eluted with EtOAc−EtOH (10% to 90% EtOH gradient), to give compounds **6** (35 mg) and **7** (12 mg), respectively. Fr.4 (10.0 g) was purified by MPLC (silica gel, 230−400 mesh, Φ = 5.0 × 10 cm) eluted with CH_2Cl_2−MeOH (30% to 100% MeOH gradient) to give three fractions (Fr.41−Fr.43). Each fraction was purified by preparative HPLC (Shim-pack ODS column), eluted with 53% MeOH, to give compound **4** (790 mg), platycodin D_3 (420 mg), deapio-platycodin D_3 (28 mg), and polygalacin D_3 (64 mg).

Platyconic Acid B Lactone (1): white, amorphous powder; $[\alpha]_D^{25}$ −23.4 (*c* 1, MeOH); IR (KBr) ν_{max} 3423, 1754, 1074 cm^{-1}; ^1H NMR (C_5D_5N, 900 MHz, see Tables 1 and 2); ^{13}C NMR (C_5D_5N, 225 MHz, see Table 3); HRESIMS *m*/*z* 1405.5887 $[M + Na]^+$ (calcd for $C_{63}H_{98}O_{33}Na$, 1405.5883).

Deapio-platyconic Acid B Lactone (2): white, amorphous powder; $[\alpha]_D^{25}$ −29.2 (*c* 1, MeOH); IR (KBr) ν_{max} 3427, 1755, 1074 cm^{-1}; ^1H NMR (C_5D_5N, 900 MHz, see Tables 1 and 2); ^{13}C NMR (C_5D_5N, 225 MHz, see Table 3); HRESIMS *m*/*z* 1273.5466 $[M + Na]^+$ (calcd for $C_{58}H_{90}O_{29}Na$, 1273.5460).

Deapio-platycodin D_2 (3): white, amorphous powder; $[\alpha]_D^{25}$ −14.4 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3419, 1735, 1077 cm^{-1}; ^1H NMR (C_5D_5N, 900 MHz, see Tables 1 and 2); ^{13}C NMR (C_5D_5N, 225 MHz, see Table 3); HRESIMS *m*/*z* 1277.5774 $[M + Na]^+$ (calcd for $C_{58}H_{94}O_{29}Na$, 1277.5773).

Determination of Absolute Configurations of Sugars. Compounds **1**−**3** (1 mg) were separately hydrolyzed with 2 N aqueous CF_3COOH (3 mL) for 3 h at 95 °C. After extraction with CH_2Cl_2 (3 × 5 mL), the aqueous layer was evaporated to dryness until neutral and then analyzed by TLC over silica gel (CH_2Cl_2−MeOH−H_2O, 8:5:1) by comparison with authentic samples. Furthermore, each aqueous layer was dissolved in anhydrous pyridine (100 μL), and L-cysteine methyl ester hydrochloride (0.06 mol/L) was added. Each mixture was stirred at 60 °C for 1 h; then 150 μL of HMDS−TMCS (hexamethyldisilazane−trimethylchlorosilane, 3:1) was added, and the mixture was stirred at 60 °C for another 30 min. The precipitate was centrifuged off, and the supernatant concentrated. The residue was partitioned between *n*-hexane and H_2O, and the organic layer (1 μL) was analyzed by GC.[27] Identification of D-glucose, L-arabinose, L-rhamnose, D-xylose, and D-apiose was carried out for **1**, giving peaks at 18.30, 11.74, 13.10, 13.35, and 11.54 min, respectively. D-Glucose, L-arabinose, L-rhamnose, and D-xylose were detected from **2** and **3**, giving peaks at 18.30, 11.73, 13.09, and 13.34 min for **2** and 18.34, 11.74, 13.12, and 13.39 min for **3**, respectively.

Preparation of Deapio-Congeners 2 and 3 by Mild Acid Hydrolysis of 1 and 8. Compounds **1** (1 mg) and **3** (1 mg) were separately dissolved in 0.2 mL of 0.1 N HCl at room temperature, and an aliquot of the reaction mixture was injected into a HPLC every 8 h. It was observed that **1** (t_R 28.68 min) was slowly converted to **2** (t_R 26.16 min), and **8** (t_R 60.99 min) was converted to **3** (t_R 52.98 min), in a

time-dependent manner in both cases. More than 90% of **1** and **8** were converted to **2** and **3** after 48 h (Figure S1, Supporting Information).

Tumor Cell Lines. All cell cultures were maintained using RPMI1640 cell growth medium (Gibco, Carlsbad, CA), supplemented with 5% fetal bovine serum (FBS) (Gibco), and grown at 37 °C in a humidified atmosphere containing 5% CO_2. The human uterine sarcoma cell lines, MES-SA and MES-SA/DX5, were purchased from the American Type Culture Collection, and the colorectal adenocarcinoma cell line, HCT15, was provided by the National Cancer Institute (NCI). The HCT15/CL02 cell line was established from HCT15 cells by continuous and stepwise exposure of the cells to doxorubicin at the Korea Research Institute of Chemical Technology, as described previously.[28]

Cytotoxicity Assessment. The cytotoxicity of the compounds against cultured human tumor cell lines was evaluated by the sulforhodamine B (SRB) method.[29] Each tumor cell line was inoculated over standard 96-well flat-bottom microplates and then incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. The attached cells were then incubated with the serially diluted saponin samples. After continuous exposure to the compounds for 48 h, the culture medium was removed from each well and the cells were fixed with 10% cold trichloroacetic acid at 4 °C for 1 h. After washing with tap water, the cells were stained with 0.4% SRB dye and incubated for 30 min at room temperature. The cells were washed again and then solubilized with 10 mM unbuffered Tris base solution (pH 10.5). The absorbance was measured spectrophotometrically at 520 nm with a microtiter plate reader. Each experiment was conducted in triplicate. The IC_{50} values of compounds were calculated by the nonlinear regression analysis and expressed as the mean ± SD of three distinct experiments.

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Supporting Information Available: HPLC analysis and HPLC profile of the crude saponin mixture obtained from the root extract of *P. grandiflorum*. ^{1}H and ^{13}C NMR spectra of compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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