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Dammarane-type saponins from the flower buds of *Panax ginseng* and their effects on human leukemia cells

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ABSTRACT

Six dammarane-type saponins, including three new compounds, floralginsenosides Ta–Tc (**1**–**3**), and three known, floralginsenoside Td (**4**), ginsenoside F_1 (**5**), and ginsenoside F_5 (**6**), were isolated from the flower buds of *Panax ginseng*. Floralginsenoside Td (**4**) was first isolated from natural plant sources. Their structures were elucidated on the basis of extensive chemical and spectroscopic methods. Compounds **1**, **5**, and **6** showed cytotoxic activities towards the HL-60 human leukemia cell line with respective IC_{50} values of 36.3, 23.2, and 62.4 μM. In addition, after the HL-60 cells were treated with these compounds, several apoptosis events, including chromatin condensation and increase in the population of sub-G1 hypodiploid cells, were observed.

Panax ginseng, an ancient and famous herbal drug in oriental traditional medicine, has been used as a tonic and for the treatment of various diseases.[1,2] Biologically active constituents of *P. ginseng* have been pursued extensively and many dammarane-type triterpene oligoglycosides have been characterized as the principal ingredients.[3–5]

In our present study with the aim of seeking new ginsenosides, the flower buds of *P. ginseng* were collected at Geumsan province, Korea, in May 2008 and were taxonomically identified by one of us (Kim, Y. H.). Voucher specimens (CNU08122) have been deposited at the College of Pharmacy, Chungnam National University, Daejeon, Korea. The air-dried material (1.0 kg) was extracted in hot MeOH (3 L × 3) and the combined extracts concentrated in vacuo to give a residue (204 g) that was suspended in H_2O and partitioned successively with *n*-hexane and CH_2Cl_2. The H_2O layer was subjected to a Diaion HP-20 column, followed by various silica gel and YMC reversed-phase columns (Supplementary data) to yield three new dammarane-type saponins, floralginsenosides Ta–Tc (**1**–**3**) and three known ones, floralginsenoside Td (**4**), ginsenosides F_1[6] (**5**), and F_5[7] (**6**) (Fig. 1).

Floralginsenoside Ta (**1**),[8] an amorphous powder, has the molecular formula $C_{36}H_{60}O_{10}$, deduced by high-resolution electrospray-ionization time-of-flight mass spectrometry (HRESITOFMS) (found at *m/z* $[M-H]^-$ 651.4116, calcd for $C_{36}H_{59}O_{10}$ 651.4108). The IR spectrum of **1** showed absorption bands at ν_{max} 3446, 1628, and 1064 cm^{-1}, due to hydroxy groups, an enone functionality, and a glycosidic linkage. Acid hydrolysis of **1** liberated D-glucose, confirmed by GC (Supplementary data). From the ^1H and ^{13}C NMR spectra (Tables 1–3), **1** was proposed to be a dammarane-type saponin, including a β-D-glucopyranosyl unit. The configuration of the anomeric position was determined to be β on the basis of the large coupling constant (J = 7.5 Hz) of the anomeric proton at δ_H 5.14 in the ^1H NMR spectrum of **1**. In addition, the ^1H NMR spectrum of **1** showed signals assignable to the aglycone portion [δ_H 0.94, 0.98, 1.04, 1.43, 1.53, 1.82, 1.96 (3H each, all s, H_3-30, 19, 18, 29, 21, 27, 28); 3.50 (1H, dd, J = 5.0, 11.5 Hz, H-3), 4.12 (1H, m, H-12), 4.38 (1H, m, H-6), 5.63 and 6.18 (1H each, both br s, H-26)]. The ^1H and ^{13}C NMR spectra of **1** were superimposable upon those of ginsenoside F_1[6] (**5**), except for the signals designated as the side-chain moiety (C-20–C-27) of the aglycone, which were similar to those of notoginsenoside B.[9] The proposed structure was further confirmed by assignment of ^1H–^1H COSY, HMQC, and HMBC spectra. As shown in Figure 2, interpretation of the ^1H–^1H COSY spectra indicated the connectivity of partial structures written in bold lines, with key HMBC correlations observed between the following protons and carbons: H-6 and C-8; H-12 and C-9, 17; H-18 and C-7, 9, 14; H-19 and C-1, 5, 9; H-21 and C-17, 22; H-22 and C-24; H-26 and C-24, 27; H-27 and C-24, 26; H-1′ and C-20. On the basis of the above evidence, floralginsenoside Ta (**1**) was characterized as 3β,6α,12β,20β-tetrahydroxydammar-25(26)-ene-24-one 20-*O*-β-D-glucopyranoside.

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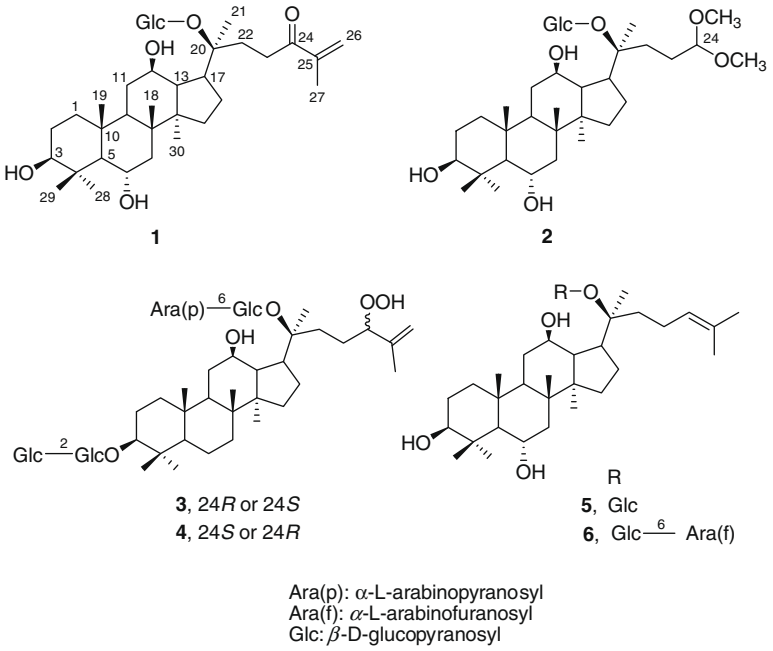

Figure 1. The structures of compounds **1**–**6**.

Table 1
^{13}C NMR data for compounds **1**–**4** in C_5D_5N

Position	**1**[a]	**2**[a]	**3**[b]	**4**[b]
1	39.3	39.3	39.5	39.5
2	28.1	28.1	27.1	27.0
3	78.5	78.5	89.3	89.2
4	40.3	40.3	39.9	39.9
5	61.7	61.7	56.7	56.6
6	67.7	67.7	18.7	18.6
7	47.4	47.5	35.4	35.3
8	41.1	41.2	40.3	40.2
9	49.9	49.9	50.5	50.4
10	39.4	39.4	37.2	37.1
11	30.7	32.0	31.0	31.0
12	70.2	70.2	70.5	70.4
13	49.1	49.2	50.0	49.9
14	52.0	51.6	51.7	51.6
15	30.9	30.9	31.0	31.0
16	26.7	26.6	26.9	26.8
17	51.4	51.3	52.1	51.6
18	17.5	17.6	16.6	16.5
19	17.4	17.4	16.2	16.2
20	83.1	83.0	83.6	83.6
21	21.9	22.4	22.8	22.6
22	32.8	30.7	32.8	33.0
23	29.8	27.9	26.5	26.8
24	202.4	105.7	90.4	90.3
25	144.4		146.2	146.4
26	124.9		113.9	113.6
27	17.8		17.8	17.9
28	31.9	32.0	28.4	28.3
29	16.5	16.5	16.9	16.8
30	17.4	17.4	17.6	17.5
OCH_3		52.8		
OCH_3		52.8		
Glc-1′	98.0	98.2	105.4	105.3
Glc-2′	75.0	75.1	83.8	83.7
Glc-3′	79.3	79.3	78.6	78.5
Glc-4′	71.6	71.7	71.9	71.8
Glc-5′	78.3	78.3	78.4	78.4
Glc-6′	62.9	63.0	63.2	63.1
Glc-1″			106.4	106.2
Glc-2″			77.5	77.3
Glc-3″			78.6	78.5
Glc-4″			71.9	72.0
Glc-5″			78.4	78.3
Glc-6″			63.0	63.1
Glc-1‴			98.4	98.2
Glc-2‴			75.1	75.0
Glc-3‴			79.4	79.3
Glc-4‴			71.9	71.8
Glc-5‴			77.1	78.1
Glc-6‴			69.3	69.4
Ara-1⁗			104.8	104.7
Ara-2⁗			72.4	72.4
Ara-3⁗			74.3	74.3
Ara-4⁗			68.8	68.8
Ara-5⁗			65.7	65.8

[a] Recorded at 500 MHz.
[b] Recorded at 400 MHz.

Floralginsenoside Tb (**2**),[8] an amorphous powder, has the molecular formula $C_{35}H_{62}O_{11}$, based on HRESITOFMS (found at *m/z* $[M-H]^-$ 657.4221, calcd for $C_{35}H_{61}O_{11}$ 657.4214). Acid hydrolysis liberated D-glucose, which was identified by GC. The ^{1}H NMR (C_5D_5N) spectrum of **2** (Tables 2 and 3) showed six methyl singlets [δ_H 0.94, 0.98, 1.06, 1.42, 1.57, and 1.96 (3H each, all s, H_3-30, 19, 18, 29, 21, 28), two methoxy protons [δ_H 3.26 and 3.28 (each 3H, both s, 24-OCH_3), four oxymethine protons [δ_H 3.50 (dd, J = 5.0, 11.5 Hz, H-3), 4.12 (m, H-12), 4.38 (m, H-6), and 4.51 (t, J = 5.5 Hz, H-24)], and an anomeric proton at δ_H 5.16 (d, J = 7.5 Hz, H-1′). The ^{13}C NMR signals of **2** (Table 1) were similar to those of ginsenoside F_1[6] (**5**), except for the signals belonging to the side-chain moiety (C-22 part) of the aglycone, composed notably of two methylene [δ_C 30.7 (C-22) and 27.9 (C-23)], one methine (δ_C 105.7, C-24), and two methoxy (both δ_C 52.8, 24-OCH_3) carbons. The structure of **2**, especially for its side-chain, was confirmed by analysis of the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra. As shown in Figure 2, the ^{1}H–^{1}H COSY of **2** indicated the presence of partial structures written in bold lines, and in the HMBC experiment, long-range correlations were observed between the following protons and carbons: H-12 and C-9, 17; H-18 and C-7, 9, 14; H-19 and C-1, 5, 9; H-21 and C-17, 22; H-22 and C-24; H-23 and C-20; H-24 and C-22; 24-OCH_3 and C-24; H-

Table 2
^{1}H NMR data for the aglycone moieties of compounds **1**–**4** in C_5D_5N

Position	**1**[a]	**2**[a]	**3**[b]	**4**[b]
1	1.02 m	1.03 m	0.74 m	0.74 m
	1.71 m	1.73 m	1.51 m	1.50 m
2	1.87 m	1.88 m	1.81 m	1.81 m
	1.97 m	1.97 m	2.17 m	2.17 m
3	3.50 dd	3.50 dd	3.26 dd	3.26 dd
	(5.0, 11.5)	(5.0, 11.5)	(4.4, 11.6)	(4.4, 11.6)
4				
5	1.20 d (10.0)	1.22 d (10.0)	0.67 m	0.66 m
6	4.38 m	4.38 m	1.36 m	1.36 m
			1.50 m	1.50 m
7	1.88 m	1.88 m	1.22 m	1.22 m
	1.98 m	1.98 m	1.45 m	1.45 m
8				
9	1.58 m	1.58 m	1.37 m	1.36 m
10				
11	1.36 m	1.36 m	1.41 m	1.41 m
	2.12 m	2.12 m	1.92 m	1.92 m
12	4.12 m	4.12 m	4.14 m	4.14 m
13	2.04 m	2.03 m	1.97 m	1.98 m
14				
15	1.09 m	1.09 m	1.02 m	1.02 m
	1.59 m	1.60 m	1.55 m	1.55 m
16	1.36 m	1.36 m	1.58 m	1.58 m
	1.82 m	1.82 m	2.00 m	2.00 m
17	2.54 m	2.58 m	2.52 m	2.51 m
18	1.04 s	1.06 s	0.98 s	0.95 s
19	0.98 s	0.98 s	0.83 s	0.81 s
20				
21	1.53 s	1.57 s	1.64 s	1.61 s
22	2.13 m	2.07 m	2.18 m	2.17 m
	2.53 m	2.49 m	2.51 m	2.48 m
23	2.67 m	1.92 m	1.82 m	1.81 m
	3.05 m	2.24 m	2.20 m	2.20 m
24		4.51 t (5.5)	4.79 m	4.75 m
25				
26	5.63 s		5.10 br s	5.06 br s
	6.18 s		5.27 br s	5.22 br s
27	1.82 s		1.96 s	1.96 s
28	1.96 s	1.96 s	1.29 s	1.28 s
29	1.43 s	1.42 s	1.11 s	1.10 s
30	0.94 s	0.94 s	0.93 s	0.94 s
24-OC*H*$_3$		3.26[c] s		
24-OC*H*$_3$		3.28[c] s		

[a] Recorded at 500 MHz.
[b] Recorded at 400 MHz.
[c] Interchangeable within one column.

Table 3
^{1}H NMR data for the sugar moieties of compounds **1**–**4** in C_5D_5N

Position	**1**[a]	**2**[a]	**3**[b]	**4**[b]
Glc-1′	5.14 d (7.5)	5.16 d (7.5)	4.93 d (7.6)	4.92 d (7.6)
Glc-2′	3.98 t (8.0)	3.98 t (8.0)	4.24 m	4.23 m
Glc-3′	4.28 m	4.27 m	4.25 m	4.25 m
Glc-4′	4.21 m	4.20 m	4.14 m	4.13 m
Glc-5′	3.92 m	3.91 m	3.91 m	3.92 m
Glc-6′	4.38 dd	4.38 dd	4.32 m	4.33 m
	(5.0, 10.0)	(5.0, 12.0)		
	4.48 br d	4.48 br d	4.56 br d	4.57 br d
	(10.0)	(12.0)	(12.0)	(12.0)
Glc-1″			5.37 d (7.6)	5.35 d (7.6)
Glc-2″			4.12 m	4.11 m
Glc-3″			4.30 m	4.29 m
Glc-4″			4.33 m	4.33 m
Glc-5″			3.94 m	3.94 m
Glc-6″			4.32 m	4.31 m
			4.50 m	4.48 m
Glc-1‴			5.11 d (7.6)	5.10 d (7.6)
Glc-2‴			4.01 m	4.01 m
Glc-3‴			4.26 m	4.26 m
Glc-4‴			4.20 m	4.20 m
Glc-5‴			3.97 m	3.97 m
Glc-6‴			4.25 m	4.25 m
			4.70 br d (11.2)	4.70 br d (11.2)
Ara-1⁗			5.02 d (5.6)	4.99 d (5.6)
Ara-2⁗			4.13 m	4.12 m
Ara-3⁗			4.28 m	4.28 m
Ara-4⁗			4.40 m	4.40 m
Ara-5⁗			3.90 m	3.89 m
			4.35 m	4.34 m

[a] Recorded at 500 MHz.
[b] Recorded at 400 MHz.

1′ and C-20. Thus, floralginsenoside Tb (**2**) was established as 3β,6α,12β,20β-tetrahydroxy-24,24-dimethoxy-25,26,27-trinordammar 20-*O*-β-D-glucopyranoside.

Floralginsenosides Tc (**3**) and Td (**4**),[8] obtained as amorphous powders, were shown to possess a hydroperoxy group from their positive responses with *N,N*-dimethyl-*p*-phenylenediammonium dichloride.[4,10] The molecular formulas of **3** and **4** were identical to each other, $C_{53}H_{90}O_{24}$, which were deduced from HRESITOFMS. Acid hydrolysis of **3** and **4** liberated D-glucose and L-arabinose, which were determined by GC. The ^{1}H NMR (C_5D_5N) spectra of **3** and **4** disclosed seven methyl singlets [**3**: δ_H 0.83, 0.93, 0.98, 1.11, 1.29, 1.64, 1.96 (3H each, all s, H_3-18, 30, 19, 29, 28, 21, 27); **4**: δ_H 0.81, 0.94, 0.95, 1.10, 1.28, 1.61, 1.96 (3H each, all s, H_3-18, 30, 19, 29, 28, 21, 27)], three oxymethine protons [**3**: δ_H 3.26 (dd, J = 4.4, 11.6 Hz, H-3), 4.14 (m, H-12), 4.79 (m, H-24); **4**: δ_H 3.26 (dd, J = 4.4, 11.6 Hz, H-3), 4.14 (m, H-12), 4.75 (m, H-24)], and two geminal olefinic protons [**3**: δ_H 5.10, 5.27 (1H each, both br s, H_2-26); **4**: δ_H 5.06, 5.22 (1H each, both br s, H_2-26)], which were attributed to the aglycone moiety, together with four anomeric protons [**3**: δ_H 4.93 (d, J = 7.6 Hz, H-1′), 5.02 (d, J = 5.6 Hz, H-1⁗), 5.11 (d, J = 7.6 Hz, H-1‴), 5.37 (d, J = 7.6 Hz, H-1″); **4**: δ_H 4.92 (d, J = 7.6 Hz, H-1′), 4.99 (d, J = 5.6 Hz, H-1⁗), 5.10 (d, J = 7.6 Hz, H-1‴), 5.35 (d, J = 7.6 Hz, H-1″)], assignable to three β-D-glucopyranosyl and one α-L-arabinopyranosyl units. Furthermore, the ^{1}H and ^{13}C NMR spectra of **3** and **4** were superimposable on those of either ginsenoside I or ginsenoside II,[11] except for the signals due to 20-O-glycoside moiety, which were similar to those of ginsenoside Rb_2 and ginsenoside F_3, with a 20-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl] moiety.[4] As shown in Figure 2, the structures of **3** and **4** were further confirmed by ^{1}H–^{1}H COSY, HMQC, and HMBC correlations. Based on these evidence, **3** and **4** were found to be stereoisomeric at the 24-position with each other. Like ginsenosides I and II,[11] comparison of ^{13}C NMR data of **4** with those of **3** indicated that they were in very good agreement, regarding the parts of the tetracarbocyclic and sugar moieties, except for slight differences in the C-22–C-27 signals. Further comparison with those of metabolized product VII from ginsenoside Rb_2 showed that **4** and oxygenated product VII seem to be identical.[12] As in the previous study, the structure of **4** was proposed based on the oxidation mechanism and ^{1}D NMR without ^{2}D NMR and HRMS,[12] thus not all NMR assignments were presented. In this study, **4** was a naturally occurring product, and its structure was completely characterized by chemical and spectroscopic methods (^{1}D, ^{2}D NMR, and HRMS). Hence, floralginsenoside Td (**4**) was identified as 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]-3β,12β,20β-trihydroxy-24ξ-hydroperoxydammar-25-ene, with an uncertain C-24 configuration, and floralginsenoside Tc (**3**) was the another isomer, one more new ginsenoside from *P. ginseng*.

To evaluate the potential of the isolates for leukemia treatment, their cytotoxic activity was first tested against the HL-60 cell line, a type of human leukemia, using the 3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[13] Among them, floralginsenoside Ta (**1**), ginsenoside F_1 (**5**), and ginsenoside F_5 (**6**) showed moderate cytotoxic activity with IC_{50} values of 36.3, 23.2, and 62.4 μM, respectively. The other compounds lacked

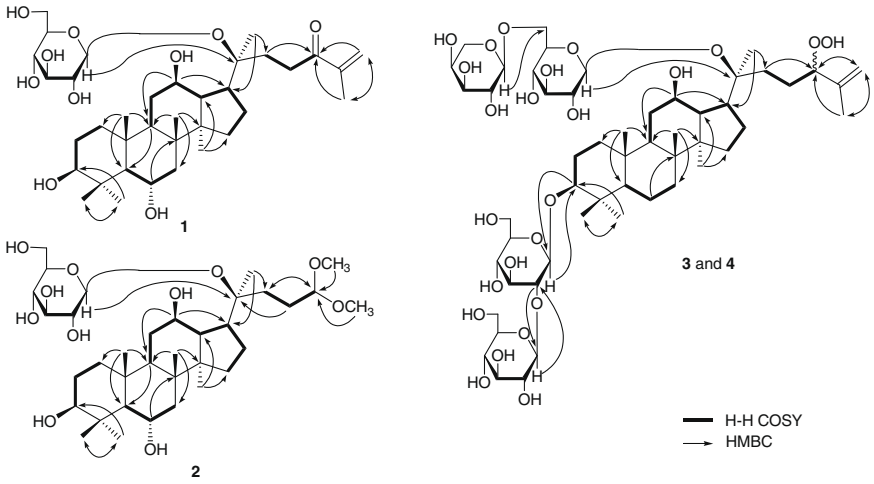

Figure 2. H–H COSY and selected HMBC correlations of **1**–**4**.

Table 4
Effects of compounds **1**–**6** on the growth of HL-60 human leukemia cells

Compound	IC_{50} (μM)[a]
1	36.3 ± 2.1
2	>100
3	>100
4	>100
5	23.2 ± 1.5
6	62.4 ± 2.0
Mitoxantrone[b]	6.8 ± 0.9

[a] Results are the means ± SD of three independent experiment in triplicate, and values <100 μM are considered to be active.
[b] Positive control.

cytotoxic activity up to 100.0 μM (Table 4). Generally, the cytotoxic compounds show effects on diverse cellular pathways associated with cell survival and apoptosis, such as extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) pathway, as well as the phosphoinositide 3 (PI3) kinase/Akt pathway.[14–17]

In order to elucidate the cytotoxic mechanism, we investigated whether the inhibitory effects of compounds **1**, **5**, and **6** on the growth of HL-60 cells might arise from the induction of apoptosis.[18] The apoptotic characteristics were examined after the HL-60 cells were treated with the IC_{50} of **1**, **5**, and **6** for 24 h. The percentage of the sub-G1 hypodiploid cells by the treatment of **1**, **5**, and **6** increased to 29.8%, 19.2%, and 21.4%, respectively (Fig. 3).

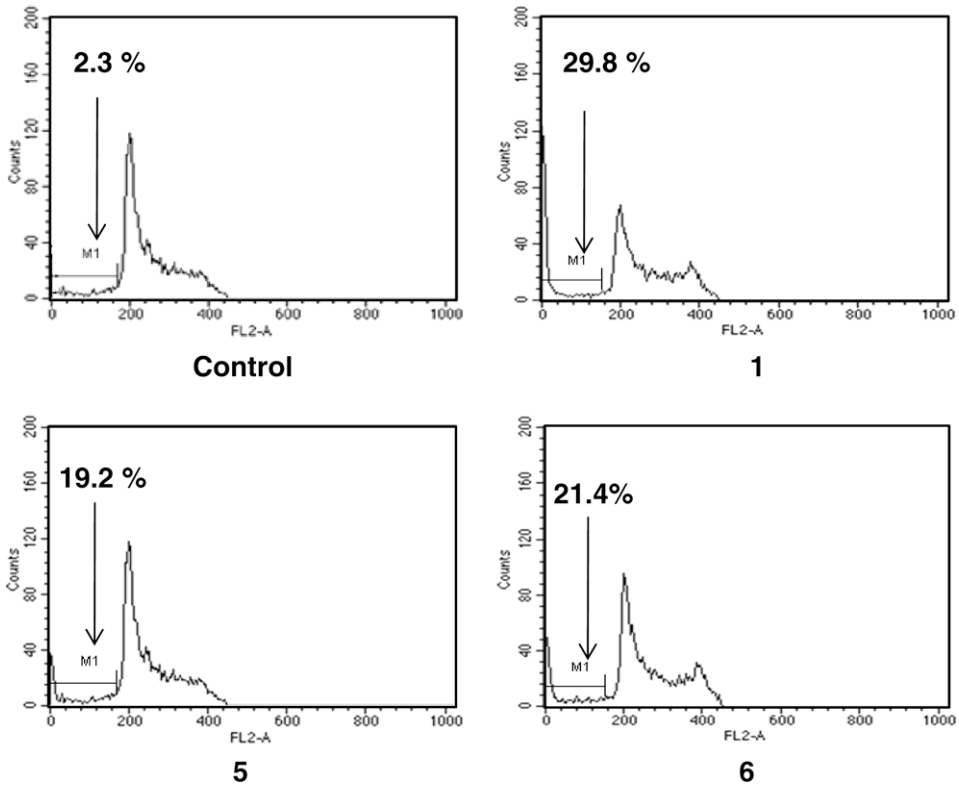

Figure 3. The degree of apoptosis represented as the DNA content measured by flow cytometric analysis in HL-60 cells.

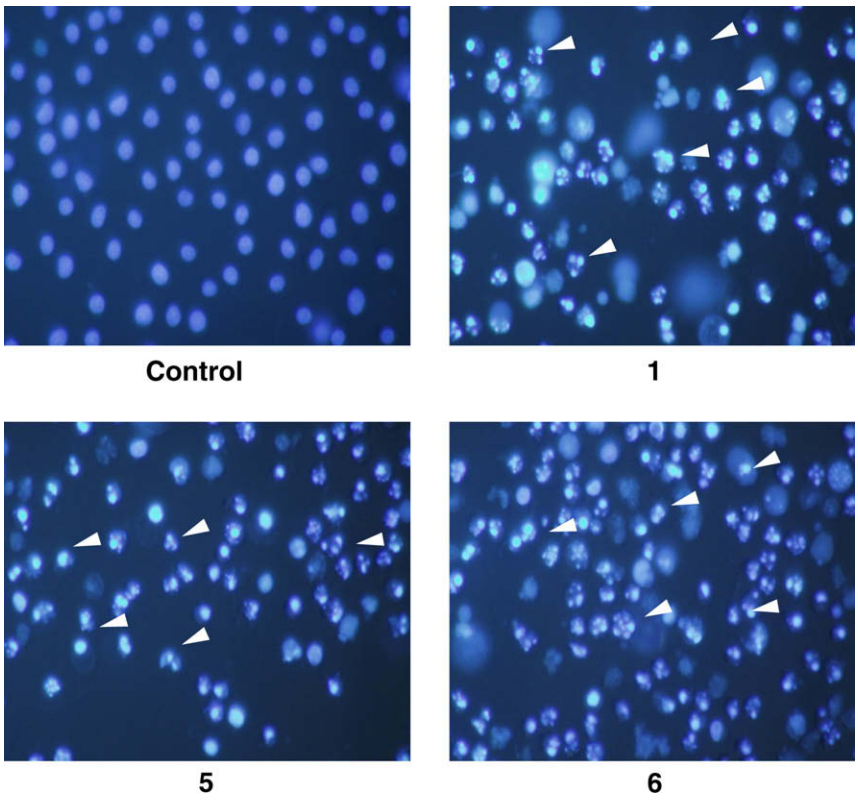

Figure 4. The degree of apoptosis represented as the fluorescent image of nuclei in HL-60 cells by fluorescent microscope.

These data showed that the three compounds induced apoptosis in the HL-60 cells and were supported by the increase in the number of apoptotic bodies easily found by H33342 staining in the compound-treated cells after 24 h-incubation (Fig. 4). This study described that compounds **1**, **5**, and **6** remarkably inhibited the growth of HL-60 cells associated with the apoptosis pathway.

Epigallocatechin gallate (EGCG), a green tea catechin, has been reported to induce apoptosis in HL-60, acute promyelocytic leukemia cells, and cause caspase-independent necrosis-like cell death in chronic myelogenous leukemia such as K562 and C2F8.[19,20] Diallyl disulfide (DADS), an important oil-soluble organosulfur component of garlic (*Allum sativum*), has been reported to inhibit the growth of human cancer cells such as leukemia, colon, lung, skin, and breast.[21] In particular, DADS induced apoptosis in HL-60 cells via the inhibition of ERK and activation of p38.[22]

Dammarane-type saponins, generally known as ginsenosides, are the main constituents of *P. ginseng* and are believed to play a pharmacologically important role, including anticancer activity. Additionally, ginsenoside F_1 (**5**) was found to protect human HaCaT keratinocytes from ultraviolet-B-induced apoptosis by maintaining constant levels of Bcl-2.[23] Ginsenoside Rb_1 suppressed ultraviolet radiation-induced apoptosis by inducing DNA repair.[24] Ginsenoside Rg_1 attenuated beta-amyloid-induced apoptosis in mutant PS1 M146L cells.[25] Ginsenoside Rg_3 also exerted potent anti-tumor promoting effects through down-regulation of NF-κB and AP-1 transcription factors in both 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-stimulated mouse skin and HL-60 cells.[26]

In summary, this study contributed more anticancer evidence of ginsenosides from *P. ginseng*. Furthermore, one new dammarane-type saponin, floralginsenoside Ta (**1**), showing anticancer properties against HL-60 cells, and two other new ones, floralginsenosides Tb (**2**) and Tc (**3**), were investigated from the flower buds of *P. ginseng*.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.110.

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8. *Floralginsenoside Ta* (**1**): white amorphous powder; $[\alpha]_D^{20}$ +13 (*c* 0.20, MeOH); IR (KBr) ν_{max}: 3446, 2928, 1628, 1248, and 1064 cm^{-1}; HRESITOFMS *m/z*: 651.4116 $[M-H]^-$ (calcd for $C_{36}H_{59}O_{10}$, 651.4108); ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz): see Tables 1–3.
Floralginsenoside Tb (**2**): white amorphous powder; $[\alpha]_D^{20}$ +18 (*c* 0.14, MeOH); IR (KBr) ν_{max}: 3426, 2924, 1250, and 1074 cm^{-1}; HRESITOFMS *m/z*: 657.4221 $[M-H]^-$ (calcd for $C_{35}H_{61}O_{11}$, 657.4214); ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz): see Tables 1–3.
Floralginsenoside Tc (**3**): white amorphous powder; $[\alpha]_D^{20}$ +8 (*c* 0.08, MeOH); IR (KBr) ν_{max}: 3432, 2915, 1662, 1250, and 1081 cm^{-1}; HRESITOFMS *m/z*: 1109.5690 $[M-H]^-$ (calcd for $C_{53}H_{89}O_{24}$, 1109.5744); ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C

NMR (C_5D_5N, 100 MHz): see Tables 1–3.
Floralginsenoside Td (**4**): white amorphous powder; $[\alpha]_D^{20}$ +14 (*c* 0.08, MeOH); IR (KBr) ν_{max}: 3418, 2919, 1656, 1252, and 1078 cm^{-1}; HRESITOFMS *m/z*: 1109.5795 $[M-H]^-$ (calcd for $C_{53}H_{89}O_{24}$, 1109.5744); ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz): see Tables 1–3.

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18. *Flow cytometric analysis:* HL-60 cells (3×10^5 cells/mL) were treated with IC_{50} of compounds for 24 h. For the flow cytometric analysis to determine cell cycle phase distribution, the treated cells were washed twice with PBS and fixed in 70% ethanol for 30 min at 4 °C. The cells were then rinsed with PBS and incubated in 50 μg/mL propidium iodide solution (PI; Sigma) and 50 μg/mL RNase A in the dark for 30 min at 37 °C. Flow cytometry analysis was performed using an flow cytometer (Becton Dickinson FACS Caliber, BD Biosciences, USA). The DNA histograms obtained were analyzed to measure the proportion of sub-G1 hypodiploid cells.
Morphology analysis: HL-60 cells (3×10^5 cells/mL) were treated with IC_{50} of compounds for 24 h. Cells were washed twice with PBS before being stained with 1 mg/mL Hoechst 33342 for 30 min at 37 °C. Apoptotic bodies, with condensed and fragmented nuclei, were observed with a fluorescence microscope (400×, BX-50, Olympus, Japan).
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