Steamed Ginseng-Leaf Components Enhance Cytotoxic Effects on Human Leukemia HL-60 Cells

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Three new dammarane-type glycosides, named ginsenosides SL_1—SL_3 (1—3), and eleven known compounds (4—14) were isolated from the heat-processed leaves of *Panax ginseng*. Their structures were elucidated on the basis of extensive chemical and spectroscopic methods. Cytotoxic-activity testing of compounds 1—14 against human leukemia HL-60 cells showed that ginsenosides Rh_3 (11) and Rk_2 (12) exhibited potent effects with IC_{50} values of 0.8 and 0.9 μM. In addition, ginsenosides SL_3 (3), 20*S*-Rg_2 (7), F_4 (10), 20*S*-Rh_2 (13) displayed strong activity with IC_{50} values of 9.0, 9.0, 7.5, and 8.2 μM, respectively. This is the first report on chemical components of the steamed ginseng leaves.

Key words *Panax ginseng*; Araliaceae; ginsenoside SL_1; ginsenoside SL_2; ginsenoside SL_3; cytotoxicity

Panax ginseng (C.A. Meyer, Araliaceae), 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 whole parts of *P. ginseng* have been pursued extensively and many dammarane-type triterpene oligoglycosides have been characterized as the principal components.[1—4]

Traditionally, the root of *P. ginseng* (ginseng), the most used and valuable part, has been processed to make white ginseng (WG, roots air-dried after peeling) and red ginseng (RG, roots steamed at 98—100 °C without peeling) to enhance its preservation and efficacy. In which, RG is more common as an herbal medicine than WG, because steaming induces changes in the chemical constituents and enhances the biological activities of ginseng.

Extracts from roots and leaves have similar multifaceted pharmacological activities (*e.g.* central nervous and cardiovascular systems). Moreover, in terms of costs and source availability, ginseng leaf has advantages over its root.[5] Nevertheless, there has been no study concerning processed leaves of this plant. In our ongoing research on *P. ginseng*,[6,7] it was found that a saponin extract of steamed leaves showed potent cytotoxic effect on HL-60 cells. Subsequently, the current study on chemical components of the steamed leaves led to the isolation of three new damarane-type saponins, ginsenosides SL_1—SL_3 (**1**—**3**), and eleven known ones (**4**—**14**). Here, this paper deals with the structure elucidation of the new ginsenosides SL_1—SL_3 (**1**—**3**) and evaluation of cytotoxic activity against HL-60 cells of all compounds.

Results and Discussion

The methanolic extract of the steamed leaves of *P. ginseng* was suspended in H_2O and partitioned with CH_2Cl_2. Then, the H_2O layer was subjected to a Diaion HP-20 column, followed by various silica gel and YMC reversed-phase columns to yield three new dammarane-type saponins, ginsenoside SL_1—SL_3 (**1**—**3**) (Fig. 1), and eleven known ones (**4**—**14**), including (20*S*)-ginsenoside Rh_1 (**4**),[8] ginsenoside F_1 (**5**),[9] ginsenoside Rh_4 (**6**),[8] (20*S*)-ginsenoside Rg_2 (**7**),[10] (20*R*)-ginsenoside Rg_2 (**8**),[10] ginsenoside Rg_6 (**9**),[11] ginsenoside F_4 (**10**),[11] ginsenoside Rh_3 (**11**),[8] ginsenoside Rk_2 (**12**),[8] (20*S*)-ginsenoside Rh_2 (**13**),[12] and (20*R*)-ginsenoside Rh_2 (**14**),[12] respectively (Fig. 2).

Ginsenoside SL_1 (**1**), an amorphous powder, has the molecular formula $C_{36}H_{62}O_{11}$ as deduced by a high-resolution Fourier-transform ion-cyclotron-resonance mass spectrometry (HR-FT-ICR-MS) experiment (Found at *m*/*z* $[M+Na]^+$ 693.4141, Calcd for $C_{36}H_{62}O_{11}Na$ 693.4190). Acid hydrolysis of **1** liberated D-glucose, confirmed by gas chromatography (GC) analysis. It was proposed to possess a hydroperoxyl group due to positive response to *N*,*N*-dimethyl-*p*-phenylenediammonium dichloride reagent. The ^{1}H-NMR spectrum of **1** showed signals due to the aglycone part [δ 0.88, 1.07, 1.25, 1.43, 1.65, 1.92, 2.09 (3H each, s, H_3-30, 19, 18, 21, 29, 27,

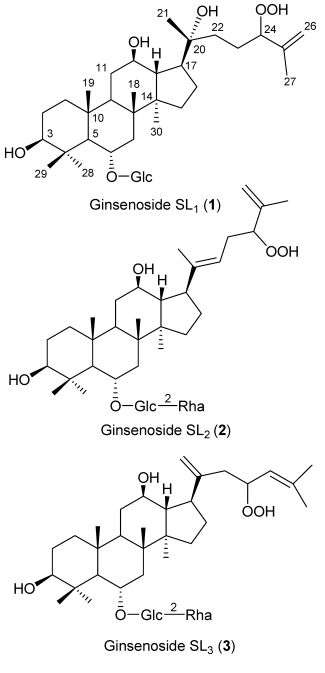

Fig. 1. Structures of Ginsenosides SL_1—SL_3 (**1**—**3**)

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28), 3.54 (1H, dd, J=11.6, 4.8 Hz, H-3), 3.98 (1H, m, H-12), 4.42 (1H, m, H-6), 5.11 and 5.27 (1H each, br s, H-26)] and an anomeric proton at δ 5.07 (d, J=7.2 Hz, H-1′), which was assignable to a β-glucopyranosyl unit. The ^{13}C-NMR spectrum of **1** exhibited 36 signals including a set of six signals (δ 106.0, 75.4, 79.6, 71.8, 78.2, 63.0) revealing a β-D-glucopyranosyl unit and 30 remaining ones of a sapogenol moiety. The signal of C-5 at δ 61.4 is a characteristic of a protopanaxatriol-type aglycone common among dammarane-type saponins in *P. ginseng* with variations in its side-chain. Furthermore, the ^{1}H- and ^{13}C-NMR data of **1** were similar to those of (20*R*)-ginsenoside Rh$_1$[13,14] except for the signals belonging to the side-chain part (C-24—C-27) of the aglycone, which was identical to that of floralginsenosides A and C.[4] 20*R*-Configuration was suggested based on the ^{13}C-NMR evidence of C-17 at δ 51.1 and C-21 at δ 22.5, which were compatible with those of related structures.[13,14] The proposed structure of **1** was further confirmed by the ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC) (Fig. 3), and rotating frame Overhauser effect spectroscopy (ROESY) spectra, respectively. As shown in Fig. 2, the ^{1}H–^{1}H COSY experiment on **1** indicated the presence of partial structures written in bold lines; and in the HMBC spectrum, the long-range correlations were 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; H-23 and C-20,25; H-24 and C-26; H-26 and C-24; H-27 and C-24; H-1′ and C-6. Consequently, the structure of ginsenoside SL$_1$ (**1**) was characterized as (20*R*)-24-hydroperoxyl-3β,6α,12β,20α-tetrahydroxy-dammar-25-ene 6-*O*-β-D-glucopyranoside.

Ginsenoside SL$_2$ (**2**), also an amorphous powder, has the molecular formula $C_{42}H_{70}O_{14}$ on the basis of a HR-FT-ICR-MS experiment. Like compound **1**, the molecule of **2** was proposed to have a hydroperoxyl group due to positive response to *N*,*N*-dimethyl-*p*-phenylenediammonium dichloride reagent. On the acid hydrolysis, it yielded D-glucose and L-rhamnose as identified by the GC procedure. The ^{1}H- and ^{13}C-NMR (Table 1) spectra of **2** due to the dammarane-type triterpene part and 6-*O*-β-D-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] moiety were superimposable on those of (20*E*)-ginsenoside F$_4$[11] except for the signals of the side-chain part (C-24—C-27), which were similar to that of **1**. Moreover, comprehensive analyses of the ^{1}H–^{1}H COSY, heteronuclear multiple quantum coherence (HMQC), HMBC (Fig. 3), and ROESY spectra of **2** (Fig. 4) permitted complete assignments of its NMR data as well as partial structures. *E*-Geometry of the double bond at C-20(22) of **2** was concluded on the basis of the methyl carbon signal C-21 at δ 13.2; whereas in case of *Z*-form, the chemical shift of C-21 is expected at *ca.* δ 30.0,[15] respectively. Hence, the structure of ginsenoside SL$_2$ (**2**) was identified as (20*E*)-24-hydroperoxyl-3β,6α,12β-trihydroxydammar-20(22),25-diene 6-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Ginsenoside SL$_3$ (**3**), an amorphous powder, has the molecular formula $C_{42}H_{70}O_{14}$ from a HR-FT-ICR-MS experiment. The molecule of **3** was also proposed to have a hydroperoxyl group according to positive response to *N*,*N*-dimethyl-*p*-phenylenediammonium dichloride reagent. Like compound **2**, the acid hydrolysis of **3** gave D-glucose and L-rhamnose. The ^{1}H- and ^{13}C-NMR (Table 1) spectra of **3** resembled those of ginsenoside Rg$_6$ (**9**)[11] except for the signals of the side-chain part (C-22—C-27). Furthermore, the structure of **3**, especially the side chain, was assigned by ^{1}H–^{1}H COSY, HMQC, HMBC (Fig. 3), and ROESY spectra (Fig. 4). Accordingly, the location of hydroperoxyl group at C-23 and double bond at C-24 were assured from following HMBC correlations: H-21 with C-22, H-22 with C-24, H-24 with C-22,26, respectively. Thus, the structure of ginsenoside SL$_3$ (**3**) was identified as 23-hydroperoxyl-3β,6α,12β-trihydroxydammar-20(21),24-diene 6-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

On the basis of traditionally-oriental medical theories, the herbs need processing for different purposes. Like roots of *P. ginseng*, chemical compositions of the steamed leaves were significantly different from those in the raw materials (Fig. 5). Because steaming was carried out under high temperature, the new monodesmosides should be formed by chemical degradation of the C-20 glycosyl moiety of the dammarane skeleton during the processing.

To evaluate the potential of the steamed-leaf components for leukemia treatment, their cytotoxic activity was tested against the HL-60 cell line, a type of human leukemia, using the 3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[16] As the results, ginsenosides Rh$_3$ (**11**) and Rk$_2$ (**12**) exhibited potent activity with IC$_{50}$ values of 0.8 and 0.9 μM. In addition, new ginsenoside SL$_3$ (**3**), ginsenosides 20*S*-Rg$_2$ (**7**), F$_4$ (**10**), and 20*S*-Rh$_2$ (**13**) displayed strong activity with IC$_{50}$ values of 9.0, 9.0, 7.5, and 8.2 μM, respectively. Besides, the activity of ginsenosides SL$_2$ (**2**) and Rg$_6$ (**9**) was relatively weak with IC$_{50}$ values of 78.6 and 35.7 μM as compared with mitoxantrone (MX) used as the positive control with the IC$_{50}$ value of 7.9 μM (Table 2). It is noteworthy that these components are unique in steamed leaves and not found in non-processed samples as reported previously.[6]

Structurally, it is suggested that variations in structures of ginsenosides, especially the side-chain, influenced the cytotoxic activity against HL-60 cells. In particular, *S* stereospecificity at C-20 in cytotoxic action against HL-60 cells

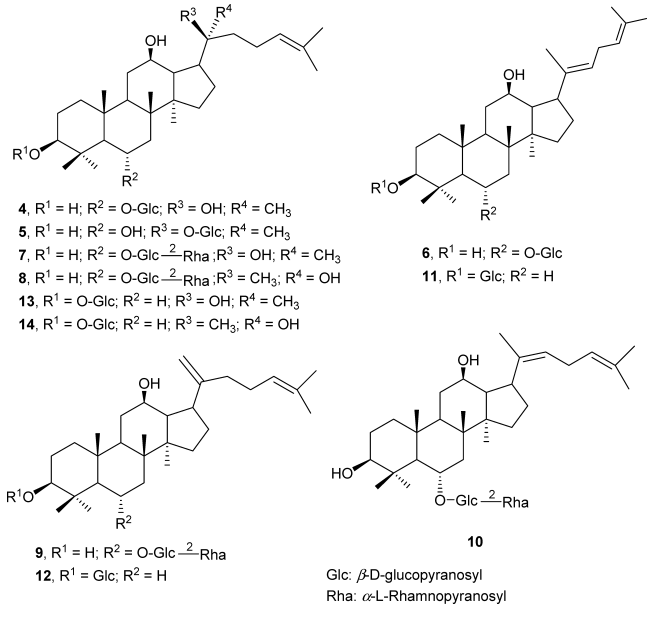

Fig. 2. Structures of Known Ginsenosides **4**—**14**

Table 1. ^{1}H- and ^{13}C-NMR Data for Ginsenosides SL_1—SL_3 (**1**—**3**) in Pyridine-d_5

Position	**1**		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	39.3	1.03 m	39.6	1.00 m	39.6	1.01 m
		1.73 m		1.72 m		1.73 m
2	27.9	1.87 m	27.8	1.44 m	27.7	1.44 m
		1.95 m		1.83 m		1.84 m
3	78.5	3.54 dd (11.6, 4.8)	78.4	3.50 dd (11.6, 5.2)	78.4	3.51 dd (11.6, 5.2)
4	40.4		40.0		40.0	
5	61.4	1.42 d (9.2)	60.8	1.41 d (10.0)	60.8	1.42 d (10.0)
6	80.1	4.42 m	74.5	4.70 m	74.3	4.70 m
7	45.1	1.97 m	46.0	2.00 m	46.2	2.02 m
		2.54 m		2.32 m		2.32 m
8	41.0		41.4		41.3	
9	50.2	1.61 m	49.7	1.60 m	50.0	1.62 m
10	39.6		39.5		39.4	
11	31.2	1.46 m	32.2	1.44 m	32.1	1.44 m
		2.13 m		2.04 m		2.04 m
12	70.9	3.98 m	72.1	3.92 m	72.3	3.91 m
13	49.6	2.02 m	50.6	1.98 m	52.0	2.00 m
14	51.6		50.9		51.2	
15	31.7	1.10 m	32.4	0.98 m	32.6	0.98 m
		1.61 m		1.58 m		1.60 m
16	26.3	1.29 m	27.6	1.74 m	25.2	1.76 m
		1.85 m		1.85 m		1.88 m
17	51.1	2.38 m	50.5	2.81 m	50.2	3.03 m
18	17.7	1.25 s	17.7	1.23 s	17.7	1.23 s
19	17.4	1.07 s	17.8	0.97 s	17.6	0.98 s
20	73.7		142.6		154.4	
21	22.5	1.43 s	13.2	1.80 s	114.0	4.82 br s
						5.12 br s
22	40.4	2.16 m	121.9	5.80 m	37.1	2.50 m
		2.54 m				2.78 m
23	25.3	1.83 m	30.7	2.25 m	89.0	4.71 m
		2.23 m		2.80 m		
24	90.2	4.73 m	90.1	4.71 m	128.8	5.27 d (8.0)
25	146.1		145.7		137.2	
26	113.5	5.11 br s	113.9	5.08 br s	25.1	1.38 s
		5.27 br s		5.25 br s		
27	17.7	1.92 s	18.1	1.88 s	17.6	1.56 s
28	31.7	2.09 s	32.2	2.13 s	32.1	2.15 s
29	16.8	1.65 s	16.8	1.34 s	16.8	1.38 s
30	17.3	0.88 s	17.3	0.97 s	17.1	0.98 s
Glc-1′	106.0	5.07 d (7.2)	101.9	5.28 d (7.2)	101.8	5.29 d (7.2)
2′	75.4	4.13 t (8.0)	79.5	4.37 m	79.3	4.38 m
3′	79.6	4.28 t (8.4)	78.4	4.35 m	78.4	4.34 m
4′	71.8	4.22 m	72.4	4.20 m	72.3	4.22 m
5′	78.2	3.97 m	78.2	3.98 m	78.3	3.98 m
6′	63.0	4.40 m	63.0	4.38 m	62.9	4.38 m
		4.57 br d (11.2)		4.53 br d (11.2)		4.54 br d (11.2)
Rha-1″			102.0	6.52 br s	101.9	6.53 br s
2″			71.9	4.81 br s	72.1	4.82 br s
3″			72.2	4.70 m	72.4	4.70 m
4″			74.0	4.37 m	74.0	4.37 m
5″			69.4	4.98 m	69.4	4.98 m
6″			18.8	1.84 d (6.4)	18.7	1.82 d (6.4)

Assignments were confirmed by COSY, HMQC, HMBC, and ROESY spectra.

was observed since of two isomeric pairs at C-20 of ginsenoside Rg_2 (**7**, **8**) and ginsenoside Rh_2 (**13**, **14**), only the 20*S*-isomers (**7**, **13**) were strongly active.

After oral administration of ginseng, ginsenosides are well known to be metabolized by intestinal microflora, and thus intestinal bacterial metabolites of saponin extracts of steamed ginseng-leaves as well as their activities should be investigated in further study. On the other hand, it is noticeable that oral administration of ginsenosides such as Rg_1, Rg_3 and Rh_2 has been reported to show anti-cancer activity.[17—19]

Taken together, it is apparent that rich dammarane-type monodesmosides presented are not only chemically characteristic of the steamed leaves but also give special biological activities to this processed herb. These results warrant further studies concerning potential of saponin extracts of steamed ginseng-leaves for leukemia treatments.

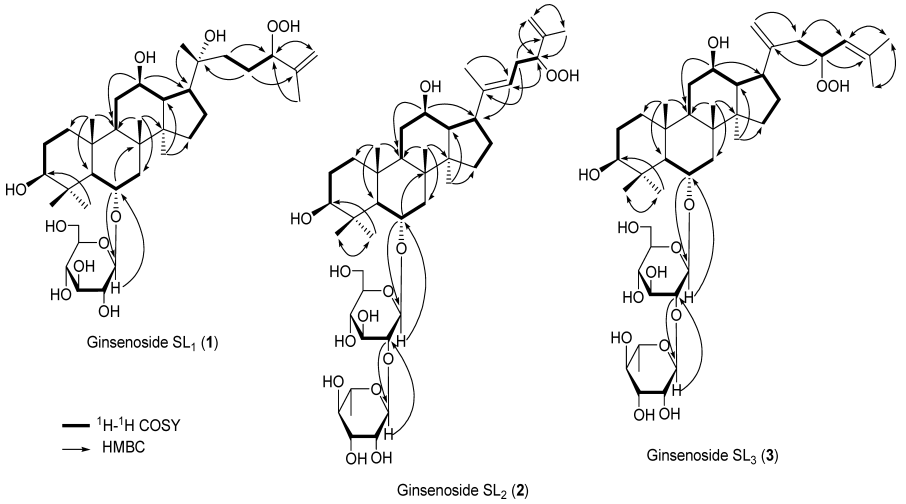

Fig. 3. COSY, Selected HMBC Correlations of Ginsenosides SL_1—SL_3 (**1**—**3**)

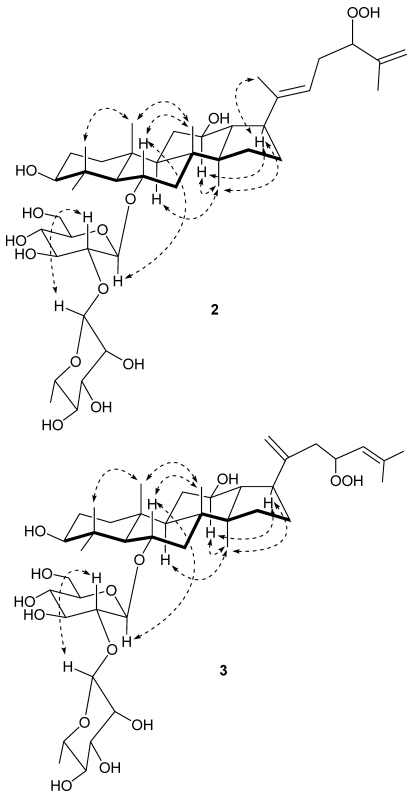

Fig. 4. Selected ROESY Correlations of **2** and **3**

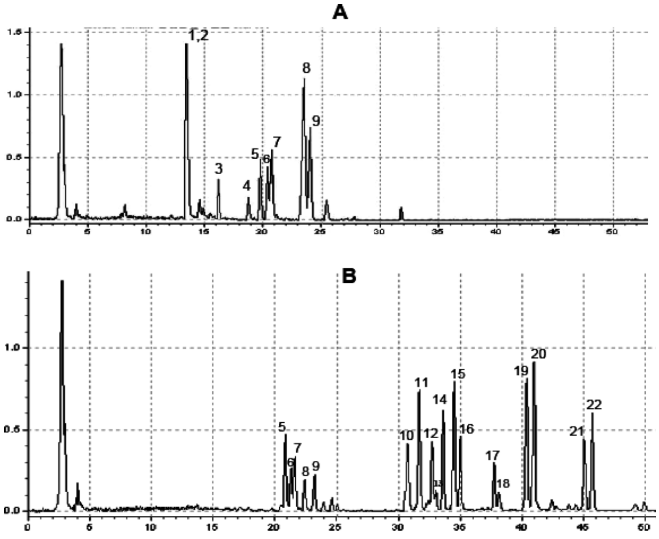

Fig. 5. HPLC/ELSD Profiles of Raw Leaves (A) and Steamed Leaves (B) of *P. ginseng*

Key to peak identity: 1, Rg_1; 2, Re; 3, F_5; 4, F_3; 5, Rb_1; 6, Rc; 7, Rb_2; 8, Rd; 9, F_1; 10, Rh_1; 11, Rh_2(20*S*); 12, Rh_2(20*R*); 13, SL_1; 14, Rh_4; 15, Rg_6; 16, F_4; 17, SL_2; 18, SL_3; 19, Rg_2(20*S*); 20, Rg_2(20*R*); 21, Rk_2; 22, Rh_3.

Table 2. Cytotoxic Effects of Ginsenosides **1**—**14** on HL-60 Human Leukemia Cells

Ginsenoside	IC_{50} (μM)[a]
1	>100
2	78.6±1.6
3	9.0±0.4
4	>100
5	>100
6	>100
7	9.0±0.5
8	>100
9	35.7±1.1
10	7.5±1.5
11	0.8±0.1
12	0.9±0.1
13	8.2±0.1
14	>100
Mitoxantrone[b]	7.9±0.5

a) Results are the means±S.D. of three independent experiment in triplicate, and values <100 μM are considered to be active. *b*) Positive control.

Experimental

General Procedures Optical rotations were obtained using a DIP-360 digital polarimeter (Jasco, Easton, MD, U.S.A.). IR spectra were measured using a Perkin-Elmer 577 spectrometer (Perkin Elmer, Waltham, MA, U.S.A.). NMR spectra were recorded on Bruker DRX 400 and 500 NMR spectrometers (Bruker, Billerica, MA, U.S.A.). HR-FT-ICR-MS measurements utilized a Variant 910 FT-ICR mass spectrometer (Varian, CA, U.S.A.). GC (Shimadzu-2010, Kyoto, Japan) using a DB-05 capillary column (0.5 mm i.d.×30 m) [column temperature: 210 °C; detector temperature: 300 °C; injector temperature: 270 °C; He gas flow rate: 30 ml/min (splitting ratio: 1/20)] was used for sugar determination. Column chromatography was performed on silica gel (70—230 and 230—400 mesh, Merck, Darmstadt, Germany), YMC RP-18 resins (30—50 μm, Fuji Silysia Chemical Ltd., Aichi, Japan), and HP-20 Diaion (Mitsubishi Chemical, Tokyo, Japan). TLC was performed on Kieselgel 60 F_{254} (1.05715; Merck, Darmstadt, Germany) or RP-18 F_{254s} (Merck) plates. Spots were visualized by

spraying with 10% aqueous H_2SO_4 solution, followed by heating.

Plant Material The leaves of *P. ginseng* were collected in Geumsan province, which is well-known for *P. ginseng* cultivation in Korea, in August 2008, and were taxonomically identified by one of the authors (Y. H. Kim). Voucher specimens (CNU 08201) have been deposited at the College of Pharmacy, Chungnam National University. The air-dried sample (1.0 kg) was crushed finely and then steamed at 120 °C for 4 h under 0.15 MPa pressure, without mixing with water, to give the steamed-leaf sample, which was used for extraction and isolation in this study.

Extraction and Isolation The steamed-leaf sample of *P. ginseng* was extracted in MeOH (4.0 l×3, 50 °C) and the combined extracts were concentrated *in vacuo* to dryness. The MeOH residue (160 g) was suspended in H_2O (2.0 l), then partitioned with CH_2Cl_2 (2.0 l×3), and the water layer was subjected to a Diaion HP-20 column eluted with a gradient of MeOH in H_2O (25, 50, 75, 100% MeOH; v/v) to give eight fractions (fr. 1.1—fr. 1.8). Next, fr. 1.6 (4.5 g) was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (10 : 3 : 0.4, v/v/v) to afford eleven subfractions (fr. 2.1—fr. 2.11). Fr. 2.1 (300 mg) was further chromatographed on a reversed-phase column with MeOH–H_2O (4 : 1) to obtain ginsenoside Rh_4 (**6**, 80 mg). Similarly, fr. 2.3 (350 mg) was subjected to a reversed-phase column with MeOH–H_2O (5 : 3) to afford ginsenoside Rh_1 (**4**, 23 mg) and ginsenoside F_1 (**5**, 45 mg). Fr. 2.4 (330 mg) was repeatedly chromatographed on a reversed-phase column with MeOH–H_2O (5 : 2) to furnish ginsenoside SL_1 (**1**, 13 mg), ginsenoside Rg_6 (**9**, 15 mg), and ginsenoside F_4 (**10**, 12 mg). Again, fr. 2.7 (550 mg) was chromatographed on a reversed-phase column with MeOH–H_2O (5 : 2) to give ginsenoside SL_2 (**2**, 15 mg), ginsenoside SL_3 (**3**, 12 mg), (20*S*)-ginsenoside Rg_2 (**7**, 14 mg), and (20*R*)-ginsenoside Rg_2 (**8**, 20 mg).

Fr. 1.8 (10.0 g) was subjected to a silica gel column with $CHCl_3$–MeOH–H_2O (7 : 1 : 0.1) to furnish five subfractions (fr. 3.1—fr. 3.5). Then, fr. 3.1 (130 mg) was repeatedly chromatographed on a reversed-phase column with MeOH–H_2O (8 : 1) to give ginsenoside Rh_3 (**11**, 18 mg) and ginsenoside Rk_2 (**12**, 20 mg). Finally, fr. 3.4 (350 mg) was chromatographed on a reversed-phase column with MeOH–H_2O (6 : 1) to obtain (20*S*)-ginsenoside Rh_2 (**13**, 25 mg) and (20*R*)-ginsenoside Rh_2 (**14**, 15 mg).

Ginsenoside SL_1 (**1**): White amorphous powder; $[\alpha]_D^{20}$ +12° (c=0.22, MeOH); IR (KBr) ν_{max}: 3448, 2922, 1637, 1262, 1054 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 400 MHz) and ^{13}C-NMR (pyridine-d_5, 100 MHz): see Table 1; HR-FT-ICR-MS *m/z*: 693.4141 $[M+Na]^+$ (Calcd for $C_{36}H_{62}O_{11}Na$: 693.4190).

Ginsenoside SL_2 (**2**): White amorphous powder; $[\alpha]_D^{20}$ −9° (c=0.25, MeOH); IR (KBr) ν_{max}: 3436, 2931, 1634, 1260, 1068 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 400 MHz) and ^{13}C-NMR (pyridine-d_5, 100 MHz): see Table 1; HR-FT-ICR-MS *m/z*: 821.4596 $[M+Na]^+$ (Calcd for $C_{42}H_{70}O_{14}Na$: 821.4663).

Ginsenoside SL_3 (**3**): White amorphous powder; $[\alpha]_D^{20}$ −11° (c=0.18, MeOH); IR (KBr) ν_{max}: 3427, 2942, 1637, 1256, 1072 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 400 MHz) and ^{13}C-NMR (pyridine-d_5, 100 MHz): see Table 1; HR-FT-ICR-MS *m/z*: 821.4660 $[M+Na]^+$ (Calcd for $C_{42}H_{70}O_{14}Na$: 821.4663).

Acid Hydrolysis and Sugar Determination of Ginsenosides SL_1—SL_3 (1—3) A solution of each (2.0 mg) in 1.0 M HCl (3.0 ml) was heated under reflux for 2 h. Then, the reaction mixture was concentrated *in vacuo* to dryness. The residue was extracted with EtOAc and H_2O (5 ml each, 3 times). Next, the sugar residue, obtained by concentration of the water layer, was dissolved in dry pyridine (0.1 ml). Then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 ml) was added to the solution. After heating the reaction mixture at 60 °C for 2 h, 0.1 ml of trimethylsilylimidazole was added. Heating at 60 °C was continued for a further 2 h, and the mixture was evaporated *in vacuo* to give a dried product, which was partitioned between hexane and H_2O.[6] The hexane layer was analyzed by the GC procedure (General Procedures). The peaks of the hydrolysate of the ginsenoside were detected at t_R 14.12 min for D-glucose in **1** and at t_R 14.12 and 4.50 min for D-glucose and L-rhamnose in **2** and **3**, respectively. The retention times for the authentic samples (Sigma), after being treated in the similar manner, were 14.12 min (D-glucose), 14.25 min (L-glucose), and 4.50 min (L-rhamnose). Co-injection of the hydrolysates of the ginsenoside with standard D-glucose/L-rhamnose gave single peaks.

HPLC Analysis of Ginsenosides The standard ginsenosides were isolated from leaves and steamed leaves as identified in this study and previous report.[6] The saponin fractions (20 mg) from the leaves and steamed-leaves were dissolved in MeOH (1 ml) and filtered by 0.45 μm syringe filter. HPLC analysis was run on Shimadzu LC-6AD (Kyoto, Japan) equipped with a Varian column (Polaris XR_s5 C_{18}, 250×4.6 mm) and an ELSD-LT evaporative light scattering detector (Shimadzu, Kyoto, Japan). The separation was effected by gradient elution, using eluents (A) CH_3CN : H_2O : 5% acetic acid (15 : 80 : 5) and (B) CH_3CN : H_2O (80 : 20) according to the following profile: 0—10 min, 30% B (70% A); 10—25 min, 50% B; 25—40 min, 80% B; 40—60 min, 100% B. The solvent flow rate was held constant at 1.0 ml/min at ambient temperature throughout the analysis.

Cytotoxic Assay Cell growth inhibition by different samples was analyzed using colorimetric MTT assay in HL-60 cell lilne. HL-60 cells were obtained from the Korea Cell Ling Bank (KCLB, Seoul, Korea) and cultured in RPMI 1640 supplemented with 10% fetal bovine serum (GIBCO Inc., Grand Island, NY, U.S.A.) and 100 U/ml penicillin and 100 mg/ml streptomycin (GIBCO Inc., Grand Island, NY, U.S.A.) at 37 °C in a humidified 5% CO_2. Briefly, HL-60 cells were seeded into 96-well plates at a density of 3×10^5 cells/well. The cells were then treated with the samples at concentrations ranging from 0.1 to 100 μM. Mitoxantrone (MX) (Sigma-Aldrich, MO, U.S.A.) was used as the positive control. After 3 d, the cells were treated with 50 μl MTT (2 mg/ml, Sigma Chemical Co., MO, U.S.A.). Plates were incubated at 37 °C for 4 h, the media was carefully aspirated. 150 μl Dimethylsulfoxide (DMSO, Amresco, OH, U.S.A.) was then added to each well to dissolve the formazan crystals. The plates were read immediately at 540 nm on a microplate reader (Amersham Pharmacia Biotech., NY, U.S.A.). All the experiments were performed at least three times in triplicate and the mean absorbance values were calculated.

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