Two New Dammarane-Type Saponins from the Leaves of *Panax ginseng*

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Two new dammarane-type saponins, named ginsenoside Ki (1a) and ginsenoside Km (1b), along with 15 known ones (2—16), were isolated from the leaves of *Panax ginseng*. Their structures were elucidated on the basis of chemical and spectroscopic methods.

Key words *Panax ginseng*; Araliaceae; ginsenoside Ki; ginsenoside Km; dammarane-type triterpene

Ginseng (the root of *Panax ginseng* C. A. Meyer, Araliaceae) is one of the most commonly used traditional medicines in Korea, China, Japan, and other Asian countries for the treatment of various conditions. Biologically active constituents of ginseng have been pursued extensively, and many dammarane-type triterpene oligoglycosides have been characterized as the principal ingredients.[1,2] Recently, the chemical components of the leaves and flower buds of *P. ginseng* have been reported including also various dammarane-type saponins.[3—5] In our studies of the chemical constituents of *P. ginseng*, two new dammarane-type saponins, ginsenoside Ki (**1a**) and ginsenoside Km (**1b**), along with 15 known ones (**2**—**16**), were isolated from the leaves of *P. ginseng*. This paper deals with the isolation and structural elucidation of the new ginsenosides **1a** and **1b**.

Results and Discussion

The methanolic extract from the leaves of *P. ginseng* was suspended in water and partitioned successively with *n*-hexane and CH_2Cl_2. The H_2O layer was subjected to Diaion HP-20 column chromatography, followed by various silica gel and YMC reversed-phase columns to afford ginsenoside Ki (**1a**), ginsenoside Km (**1b**), ginsenoside Re (**2**),[6] ginsenoside Rg_1 (**3**),[6] notoginsenoside R_1 (**4**),[7] floralginsenoside M (**5**),[4] floralginsenoside N (**6**),[4] ginsenoside F_1 (**7**),[8] ginsenoside F_5 (**8**),[9] ginsenoside F_3 (**9**),[9] vinaginsenoside R_4 (**10**),[10] ginsenoside Ia (**11**),[11] ginsenoside Rd (**12**),[12] ginsenoside Rc (**13**),[4] ginsenoside Rb_2 (**14**),[10] ginsenoside Rb_1 (**15**),[10] and ginsenoside Rh_6 (**16**)[10] (Fig. 1). To the best of our knowledge, floralginsenoside M (**5**) and floralginsenoside N (**6**) were isolated for the first time from the leaves of *P. ginseng*.

Ginsenoside Ki (**1a**), an amorphous powder, has the molecular formula $C_{36}H_{62}O_{10}$ as deduced by a high-resolusion electrospray-ionization time-of-flight mass spectrometry (HR-ESI-TOF-MS) experiment (Found at *m/z* $[M+H]^+$, 655.4410; Calcd for $C_{36}H_{63}O_{10}$, 655.4421). The IR spectrum of **1a** showed absorption bands at ν_{max} 3456, 1062, and 1648 cm^{-1} due to hydroxy groups, a glycosidic linkage, and a double bond. The acid hydrolysis of **1a** liberated D-glucose as confirmed in a gas chromatography (GC) experiment. From the ^{1}H- and ^{13}C-NMR spectra (Table 1), **1a** was proposed to be a β-D-glucopyranosyl and an aglycone with five oxygenated carbons and one double bond. The configuration of the anomeric position was determined to be β on the basis of the large coupling constant (J=8.0 Hz) of the anomeric proton at δ 5.19 in the ^{1}H-NMR spectrum of **1a**. In addition, the ^{1}H-NMR spectrum of **1a** showed signals assignable to the aglycone part [δ 0.97, 1.03, 1.10, 1.47, 1.59, 1.95, 2.01 (3H each, all s, H_3-30, 19, 18, 29, 21, 28, 27), 3.52 (1H, dd, J=11.6, 4.8 Hz, H-3), 4.02 (1H, ddd, J=12.4, 10.0, 4.4 Hz, H-12), 4.38 (2H, overlapped, H-26), 4.40 (1H, ddd, J=12.0, 10.0, 3.2 Hz, H-6), 5.42 (1H, br t, J=6.4 Hz, H-24)]. The ^{13}C-NMR spectrum of **1a** showed 36 carbon signals including a set of six signals (δ 98.5, 75.4, 79.6, 72.0, 78.6, 63.2) contributing to a β-D-glucopyranosyl unit and 30 remaining ones of a sapogenol moiety. The signal of C-5 at δ 62.0 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 **1a** were similar to those of ginsenoside F_1[9] except for the signals belonging to the side-chain part (C-22—C-27) of the aglycone. The structure of **1a**, especially the side-chain, was based on the ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC), and rotating frame Overhauser effect spectroscopy (ROESY) spectra, respectively. As shown in Fig. 2, the ^{1}H–^{1}H COSY experiment on **1a** indicated the presence of partial structures shown in bold lines; and in the HMBC experiment, long-range correlations were observed between the following protons and carbons: H-6 and C-8; H-12 and C-9, 13, 17; H-17 and C-20; H-18 and C-7, 9, 14; H-19 and C-1, 5, 9, 10; H-21 and C-17, 22; H-23 and C-20, 25; H-24 and C-22, 27; H-26 and C-24, 25, 27; H-27 and C-24, 26; H-1′ and C-20. Consequently, the configuration of the double bond at C-24 was identified to be (*Z*) since the NOE correlation of H_3-27/H-24, was seen in the ROESY spectrum of **1a** (Fig. 3). On the basis of the above evidence, the structure of ginsenoside Ki (**1a**) was characterized as (20*S*,24*Z*)-3β,6α,12β,20β,26-pentahydroxydammar-24-ene 20-*O*-β-D-glucopyranoside.

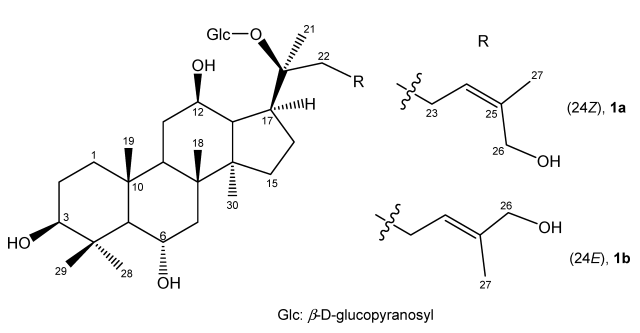

Fig. 1. Structures of the New Ginsenosides **1a** and **1b**

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Table 1. ^{1}H- and ^{13}C-NMR Data for **1a** and **1b** in Pyridine-d_5

Position	**1a**		**1b**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	39.6	1.03 m	39.8	1.03 m
		1.74 m		1.75 m
2	28.4	1.88 m	28.5	1.88 m
		1.97 m		1.96 m
3	78.7	3.52 dd (11.6, 4.8)	78.8	3.54 dd (12.0, 4.8)
4	40.6		40.7	
5	62.0	1.22 d (10.0)	62.1	1.23 d (9.6)
6	68.0	4.40 ddd (12.0, 10.0, 3.2)	68.1	4.42 ddd (12.8, 9.6, 3.2)
7	47.7	1.88 t (10.0)	47.9	1.89 t (9.6)
		1.98 dd (12.0, 3.2)		1.98 dd (12.8, 3.2)
8	41.4		41.6	
9	50.2	1.58 dd (12.0, 2.8)	50.3	1.58 dd (12.8, 3.2)
10	39.6		39.8	
11	31.2	1.36 m	31.3	1.34 m
		2.12 m		2.09 m
12	70.4	4.02 ddd (12.4, 10.0, 4.4)	70.5	4.04 ddd (12.8, 10.4, 4.8)
13	49.4	2.03 t (10.0)	49.6	2.02 t (10.4)
14	51.6		51.7	
15	31.0	1.09 m	31.2	1.10 t (10.4)
		1.60 m		1.64 m
16	26.8	1.36 m	27.0	1.40 m
		1.82 m		1.84 ddd (12.8, 10.4, 3.2)
17	51.9	2.58 dd (10.0, 7.2)	51.9	2.60 dd (10.4, 7.2)
18	17.9	1.10 s	18.0	1.11 s
19	17.7	1.03 s	17.9	1.03 s
20	83.5		83.7	
21	22.5	1.59 s	22.7	1.63 s
22	36.6	1.87 m	36.4	1.86 m
		2.48 m		2.48 m
23	23.0	2.63 m	23.2	2.66 m
24	128.0	5.42 br t (6.4)	125.8	5.78 br t (6.4)
25	136.4		136.7	
26	61.1	4.38 overlapped	68.5	4.24 s
27	22.1	2.01 s	14.4	1.82 s
28	32.2	1.95 s	32.4	2.00 s
29	16.8	1.47 s	16.9	1.47 s
30	17.6	0.97 s	17.8	0.96 s
Glc-1′	98.5	5.19 d (8.0)	98.6	5.22 d (8.0)
Glc-2′	75.4	4.01 t (8.0)	75.5	4.03 t (8.0)
Glc-3′	79.6	4.27 m	79.7	4.25dd (8.8, 8.0)
Glc-4′	72.0	4.20 m	72.0	4.18 m
Glc-5′	78.6	3.96 m	78.8	3.96 m
Glc-6′	63.2	4.30 dd (12.0, 6.0)	63.3	4.33 dd (12.0, 6.4)
		4.50 br d (12.0)		4.51 dd (12.0, 2.4)

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

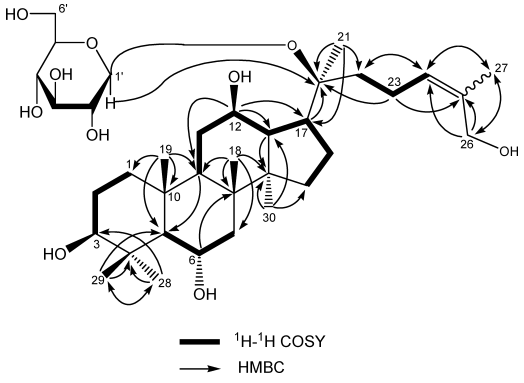

Fig. 2. H–H COSY, Selected HMBC Correlations of **1a** and **1b**

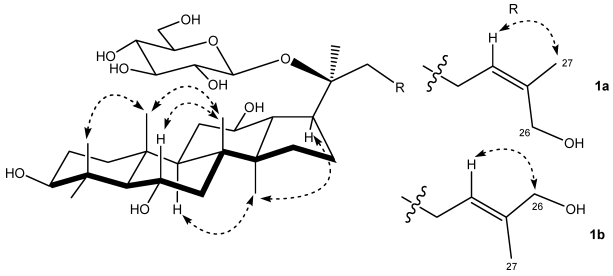

Fig. 3. Selected ROESY Correlations of **1a** and **1b**

Ginsenoside Km (**1b**), also an amorphous powder, has the molecular formula $C_{36}H_{62}O_{10}$ based on a HR-ESI-TOF-MS experiment. On acid hydrolysis, it yielded D-glucose as identified using the GC procedure. The ^{1}H-NMR (pyridine-d_5)

and ^{13}C-NMR (Table 1) spectra of **1b** due to the dammarane-type triterpene moiety and 20-*O*-β-D-glucopyranosyl unit were superimposable on those of ginsenoside F_1,[9] except for the signals of the side-chain part (C-22—C-27) which consisted of two olefinic, one hydroxylated-methylene, two methylene, and one methyl carbons like **1a**. Further analyses of the COSY and HMBC spectra of **1b** suggested the location of the double bond at C-24 and the hydroxy group at C-26, respectively, and therefore the planar structure of the side chain of **1b** was similar to that of **1a** (Fig. 2). The configuration of the double bond at C-24 of **1b** was inferred to be (*E*) from the differences in the ^{13}C-NMR data between **1a** and **1b** [**1a**: δ 128.0 (C-24), 61.1 (C-26), 22.1 (C-27); **1b**: δ 125.8 (C-24), 68.5 (C-26), 14.4 (C-27)] and the NOE correlation between H-26 at δ 4.24 (2H, s) and H-24 at δ 5.78 (1H, br t, J=6.4 Hz) in the ROESY spectrum of **1b** (Fig. 3). Consequently, the structure of ginsenoside Km (**1b**) was identified as (20*S*,24*E*)-3β,6α,12β,20β,26-pentahydroxydammar-24-ene 20-*O*-β-D-glucopyranoside.

Experimental

General Procedures Optical rotations were obtained using a DIP-360 digital polarimeter (Jasco, Easton, U.S.A.). IR spectra were measured using a Perkin-Elmer 577 spectrometer (Perkin Elmer, Waltham, U.S.A.). NMR spectra were recorded on Bruker DRX 400 and 800 NMR spectrometers (Bruker, Billerica, U.S.A.). HR-ESI-TOF-MS experiments utilized a JEOL AccuTOF^{TM} LC mass spectrometer (Jeol, Tokyo, Japan). GC (Shimadzu-2010, Tokyo, 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 (CC) was performed on silica gel (70—230 and 230—400 mesh, Darmstadt, Germany), YMC RP-18 resins (30—50 μm, Fuji Silysia Chemical Ltd., Aichi, Japan), and HP-20 diaion (Mitshubishi Chemical, Tokyo, Japan). TLC was performed on Kieselgel 60 F_{254} (1.05715; Merck) 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). A voucher specimen (CNU 08201) was deposited at the College of Pharmacy, Chungnam National University, Korea.

Extraction and Isolation The air-dried leaves of *P. ginseng* (2.3 kg) were extracted in MeOH (6 l×3, 50 °C), and the combined extracts were concentrated *in vacuo* to dryness. The MeOH residue (630 g) was suspended in H_2O (4.0 l), then, successively partitioned with *n*-hexane and CH_2Cl_2 (each 4.0 l×3) to obtain *n*-hexane and CH_2Cl_2-soluble fractions in the weights of 45 g and 20 g, respectively and the water layer, which was subjected to a Diaion HP-20 column eluted with a gradient of MeOH in H_2O (25, 50, 75, and 100% MeOH, v/v) to give four fractions (1a—d). Next, fr. 1a (1.0 g) was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (10 : 3 : 0.4, v/v/v), followed by reversed-phase columns with MeOH–H_2O (6 : 5) and MeOH–H_2O (1 : 1) to afford ginsenoside Rg_1 (**3**, 23 mg), ginsenoside Re (**2**, 90 mg), floralginsenoside M (**5**, 10 mg), and floralginsenoside N (**6**, 10 mg), respectively.

Fr. 1c (48.0 g) was fractionated on a silica gel column with a gradient of CH_2Cl_2–MeOH (20 : 1—1 : 1) to furnish five fractions (2a—e). Fr. 2b (3.0 g) was repeatedly chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (6 : 1 : 0.1) to afford four fractions (3a—d). Then, fr. 3b (450 mg) was purified on a reversed-phase column with MeOH–H_2O (2 : 1) to obtain ginsenoside F_1 (**7**, 150 mg); and fr. 3d (600 mg) was purified on a reversed-phase column with MeOH–H_2O (4 : 3) to give ginsenoside Ki (**1a**, 6.5 mg), notoginsenoside R_1 (**4**, 5.0 mg), ginsenoside Km (**1b**, 7.2 mg), ginsenoside F_5 (**8**, 100 mg), and ginsenoside Rh_6 (**16**, 6.3 mg). Fr. 2d (12.0 g) was subjected to silica gel column chromatography with a gradient of CH_2Cl_2–MeOH–H_2O (10 : 1—1 : 1), followed by reversed-phase column chromatography with MeOH–H_2O (2 : 1) and silica gel column chromatography with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.4) to obtain ginsenoside F_3 (**9**, 150 mg), vinaginsenoside R_4 (**10**, 20 mg), ginsenoside Rd (**12**, 50 mg), ginsenoside Rc (**13**, 47 mg), ginsenoside Ia (**11**, 7.2 mg), ginsenoside Rb_2 (**14**, 86 mg), and ginsenoside Rb_1 (**15**, 120 mg).

Ginsenoside Ki (**1a**): White amorphous powder; $[\alpha]_D^{20}$ +6.4° (c=0.12, MeOH); IR (KBr): ν_{max} 3456, 2915, 1648, 1252, 1062 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 400 MHz) and ^{13}C-NMR (pyridine-d_5, 100 MHz): see Table 1; HR-ESI-TOF-MS: m/z 655.4410 $[M+H]^+$ (Calcd for $C_{36}H_{63}O_{10}$, 655.4421).

Ginsenoside Km (**1b**): White amorphous powder; $[\alpha]_D^{20}$ +14° (c=0.22, MeOH); IR (KBr): ν_{max} 3453, 2913, 1654, 1248, 1060 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 800 MHz) and ^{13}C-NMR (pyridine-d_5, 200 MHz): see Table 1; HR-ESI-TOF-MS: m/z 655.4410 $[M+H]^+$ (Calcd for $C_{36}H_{63}O_{10}$, 655.4421).

Acid Hydrolysis of Ginsenosides Ki (1a) and Km (1b) A solution of each (2.0 mg) in HCl 1.0 M (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.[13] The hexane layer was analyzed using the GC procedure (General Procedures). The peak of the hydrolysate of the ginsenoside was detected at t_R 14.12 min for D-glucose. The retention times for the authentic samples (Sigma), after being treated in a similar manner, were 14.12 min (D-glucose) and 14.25 min (L-glucose). Coinjection of the hydrolysates of the ginsenoside with standard D-glucose gave single peaks.

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References

1) Park J. D., Rhee D. K., Lee Y. H., *Phytochem. Rev.*, **4**, 159—175 (2005).
2) Shibata S., *J. Korean Med. Sci.*, **16**, 28—37 (2001).
3) Dou D. Q., Chen Y. J., Liang L. H., Pang F. G., Shimizu N., Takeda T., *Chem. Pharm. Bull.*, **49**, 442—446 (2001).
4) Yoshikawa M., Sugimoto S., Nakamura S., Sakumae H., Matsuda H., *Chem. Pharm. Bull.*, **55**, 1034—1038 (2007).
5) Wu L., Wang L., Gao H., Wu B., Song X., Tang Z., *Fitoterapia*, **78**, 556—560 (2007).
6) Fujioka N., Kohda H., Yamasaki K., Kasai R., Shoyama Y., Nishioka I., *Phytochemistry*, **28**, 1855—1858 (1989).
7) Zhou J., Wu M. Z., Taniyasu S., Besso H., Tanaka O., Saruwatari Y., Fuwa T., *Chem. Pharm. Bull.*, **29**, 2844—2850 (1981).
8) Ko S. R., Choi K. J., Suzuki K., Suzuki Y., *Chem. Pharm. Bull.*, **51**, 404—408 (2003).
9) Dou D., Chen Y., Ma Z., Wen Y., Weng M., Pei Y., Wang Z., Kawai H., Fukushima H., Murakami Y., *J. Chin. Pharm. Sci.*, **5**, 48—52 (1996).
10) Duc N. M., Kasai R., Ohtani K., Ito A., Nham N. T., Yamasaki K., Tanaka O., *Chem. Pharm. Bull.*, **42**, 115—122 (1994).
11) Dou D., Wen Y., Pei Y., Yao X., Chen Y., Kawai H., Fukushima H., *Planta Med.*, **62**, 179—181 (1996).
12) Yoshikawa M., Sugimoto S., Nakamura S., Matsuda H., *Chem. Pharm. Bull.*, **55**, 571—576 (2007).
13) Min B. S., Nakamura N., Miyashiro H., Kim Y. H., Hattori M., *Chem. Pharm. Bull.*, **48**, 194—200 (2000).