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Dammarane-Type Glycosides from the Steamed Flower-Buds of *Panax ginseng*

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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* (roots, leaves, flower buds, and fruits) have been pursued extensively and many dammarane-type triterpene oligoglycosides have been characterized as the principal components.[1-4]

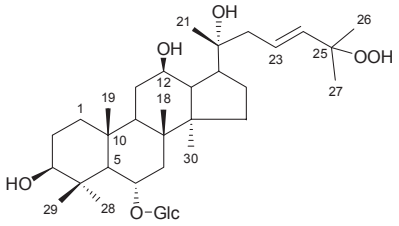

Figure 1. Ginsenoside SF (**1**).

Extracts from roots and flower buds have similar multifaceted pharmacological activities (e.g. central nervous system).[5] 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. 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. Moreover, there has been no study on chemical components from processed flower-buds of this plant to date. Subsequently, in continuation of our research on *P. ginseng*,[6,7] the present study on chemical components of the steamed flower-buds led to the isolation of one new damarane-type saponin, named ginsenoside SF (**1**) (Fig. 1), and nineteen known saponins (**2-20**) (Fig. 2), including ginsenoside Rh_4 (**2**),[8] ginsenoside Rk_3 (**3**),[8] ginsenoside F_1 (**4**),[9] (20*E*)-ginsenoside F_4 (**5**),[10] ginsenoside Rg_2 (**6**),[11]

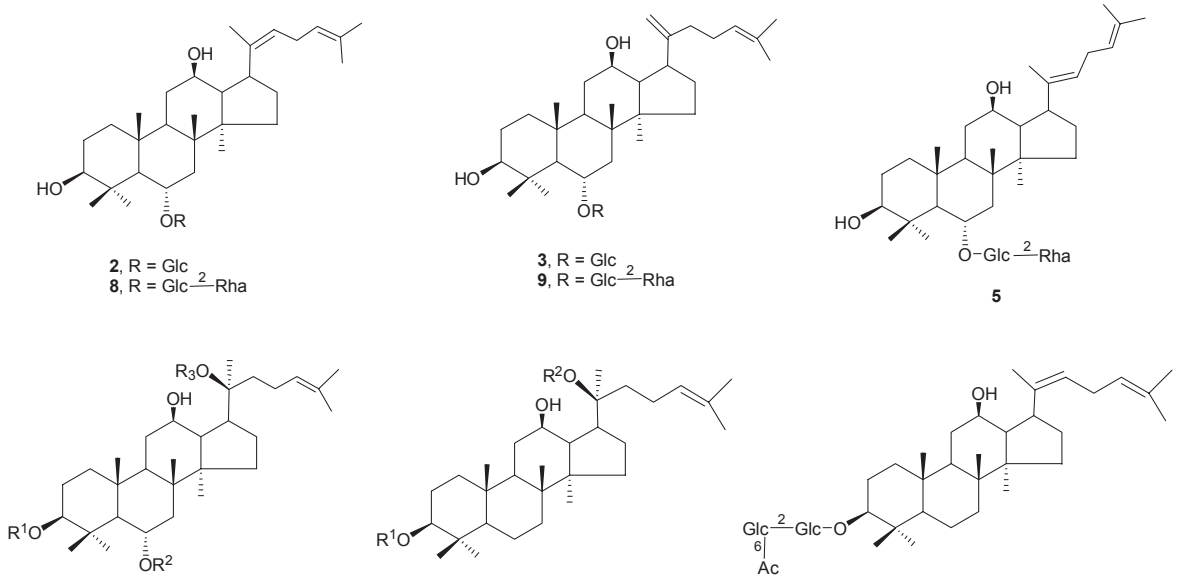

Figure 2. Structures of known saponins **2-20**.

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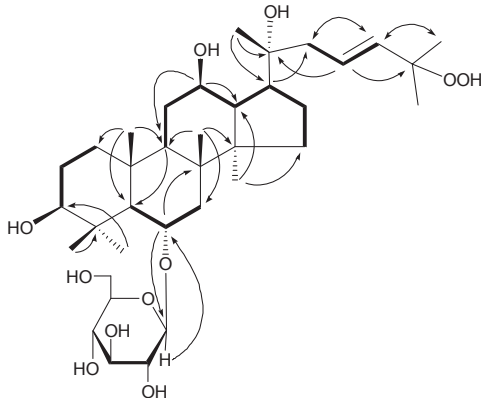

Figure 3. COSY (bold lines), selected HMBC Correlations (arrows) of ginsenoside SF (**1**).

pseudoginsenoside RC_1 (**7**),[4] ginsenoside Rg_6 (**8**),[12] ginsenoside F_4 (**9**),[12] ginsenoside Rg_1 (**10**),[13] 6'-acetyl-ginsenoside Rg_1 (**11**),[14] ginsenoside Rd (**12**),[6] ginsenoside Rc (**13**),[6] ginsenoside Rb_2 (**14**),[6] ginsenoside Re (**15**),[6] vinaginsenoside R_4 (**16**),[6] ginsenoside Mb (**17**),[15] ginsenoside Rb_1 (**18**),[6] ginsenoside Rs_4 (**19**),[16] and 6'-acetyl-ginsenoside F_1 (**20**),[14] respectively, on the basis of NMR, ESI-MS data, and comparison with those reported in the literature.

Ginsenoside SF (**1**), an amorphous powder, has the molecular formula $C_{36}H_{62}O_{11}$ as deduced by a high-resolusion electrospray-ionization time-of-flight mass spectrometry (HRESITOFMS) 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 as 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.[4,5] The ^{1}H-NMR spectrum of **1** showed signals due to the aglycone part [δ 0.88, 1.07, 1.25, 1.43, 1.56, 1.57, 1.65, 2.09 (3H each, all s, H_3-30, 19, 18, 21, 26, 27, 29, 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), 6.08 (1H, d, J = 16.0 Hz, H-24), and 6.38 (1H, m, H-23)] and an anomeric proton at δ 5.07 (d, J = 7.2 Hz, H-1'), which was assignable to a β-glucopranosyl 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, and 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 feature of a protopanaxatriol-type aglycone, which is 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[17,18] except for the signals of the side-chain part (C-22 ~ C-27) resembling those of floralquinquenosides A and C.[19] 20*R*-Configuration was suggested based on the ^{13}C-NMR evidence of C-17 at δ 51.1 and C-21 at δ 22.7, which were compatible with those of related structures.[17,18] Accordingly, compound **1** was found to be the 20*R*-epimer of floralquinquenoside A. The proposed structure of **1** was further confirmed by the ^{1}H-^{1}H correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) spectra, respectively. As shown in Fig. 3, 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-22,26; H-26 and C-24; H-1' and C-6. Consequently, the structure of ginsenoside SF (**1**) was characterized as (20*R*)-25-hydroperoxyl-3β,6α,12β,20α-tetrahydroxy-dammar-23-ene 6-*O*-β-D-glucopyranoside.

On the basis of oriental medicine, the herbs need processing for different purposes. Like roots of *P. ginseng*,[20-22] chemical compositions of the steamed flower-buds were significantly different from those of the raw materials. Because steaming was carried out under high temperature, new monodesmosides should be formed by chemical degradation of the C-20 glycosyl moiety of the dammarane skeleton during the processing.[10,12] It is apparent that rich dammarane-type monodesmosides presented are not only chemically characteristic of the steamed flower buds but also give special biological activities to this processed herb. To the best of our knowledge, this is the first report on chemical components of the steamed flower-buds of *P. ginseng*. The pharmaceutical evaluation of the steamed flowers is now in progress.

Experimental

General procedures. Optical rotations were obtained using a DIP-360 digital polarimeter (Jasco, Easton, MD). IR spectra were measured using a Perkin-Elmer 577 spectrometer (Perkin Elmer, Waltham, MA). NMR spectra were recorded on Bruker DRX 400 and 500 NMR spectrometers (Bruker, Billerica, MA). ESI-MS spectra were recorded on a Model 1100 LC-MSD Trap spectrometer (Agilent, Santa Clara, CA). HRESITOFMS measurements 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 was performed on silica gel (70 - 230 and 230 - 400 mesh, Merck), 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 flower buds of *P. ginseng* were collected in Geumsan province, which is well-known for ginseng cultivation in Korea, in August 2008, and were taxonomically identified by one of us (Young Ho Kim). Voucher specimens (CNU 08202) have been deposited at the College of Pharmacy, Chungnam National University. The air-dried sample (2.7 kg) was then steamed at 120 °C for 4 h under 0.15 MPa pressure, without mixing with water, to give the steamed sample, which was used for extraction and isolation in this study.

Extraction and isolation. The steamed-flowers sample was extracted in MeOH (5.0 L × 3, 50 °C) and the combined extracts were concentrated in vacuo to dryness. The MeOH residue (850 g) was suspended in H_2O (2.5 L), then partitioned with CH_2Cl_2 (2.5 L × 3), and the water layer was subjected to a

Table 1. ^{1}H- and ^{13}C-NMR Data[a] for Ginsenosides SF (**1**) in Pyridine-d_5

Position	δ_C	δ_H (J in Hz)
1	39.3	1.03 m 1.73 m
2	27.9	1.87 m 1.95 m
3	78.5	3.54 dd (11.6, 4.8)
4	40.4	
5	61.4	1.42 d (8.0)
6	80.1	4.42 m
7	45.1	1.97 m 2.54 m
8	41.0	
9	50.2	1.61 m
10	39.6	
11	31.2	1.46 m 2.13 m
12	70.9	3.98 m
13	49.6	2.02 m
14	51.6	
15	31.7	1.10 m 1.61 m
16	26.3	1.29 m 1.85 m
17	51.1	2.38 m
18	17.7	1.25 s
19	17.4	1.07 s
20	73.7	
21	22.7	1.43 s
22	40.6	2.16 m 2.56 m
23	126.8	6.38 m
24	137.9	6.08 d (16.0)
25	81.3	
26	25.3[b]	1.56 s
27	25.2[b]	1.57 s
28	31.7	2.09 s
29	16.4	1.65 s
30	17.3	0.88 s
Glc-1'	106.0	5.07 d (7.2)
2'	75.4	4.13 t (8.0)
3'	79.6	4.28 t (8.4)
4'	71.8	4.22 m
5'	78.2	3.97 m
6'	63.0	4.40 m 4.57 br d (11.2)

[a]Assignments were confirmed by COSY, HMQC, and HMBC spectra. [b]Reversible.

Diaion HP-20 column eluted with a gradient of MeOH in H_2O (25, 50, 75, and 100% MeOH; v/v) to give six fractions (fr. 1.1 ~ fr. 1.6). Next, fr. 1.3 (46 g) was chromatographed on a silica gel column using CH_2Cl_2-MeOH (15:1 - 1:1) to afford nine subfractions (fr. 2.1 ~ fr. 2.9). Fr. 2.3 (5.6 g) was further chromatographed on a silica gel column with $CHCl_3$-MeOH-H_2O (7:1:0.1), followed by a reversed-phase (RP) column with MeOH-H_2O (2:1) to obtain ginsenoside Rh_4 (**2**, 100 mg), ginsenoside Rk_3 (**3**, 110 mg), ginsenoside F_1 (**4**, 54 mg) and 6'-acetyl-ginsenoside F_1 (**20**, 13 mg). Fr. 2.7 (9.0 g) was rechromatographed on a silica gel column with $CHCl_3$-MeOH-H_2O (3:1:0.2) to afford eleven subfractions (fr. 3.1 ~ fr. 3.11). Next, fr. 3.2 (1.4 g) was subjected to a RP column with MeOH-H_2O (5:2) to furnish (20*E*)-ginsenoside F_4 (**5**, 18 mg), ginsenoside Rg_2 (**6**, 35 mg), pseudoginsenoside RC_1 (**7**, 30 mg), ginsenoside Rg_6 (**8**, 40 mg), and ginsenoside F_4 (**9**, 36 mg). Similarly, fr. 3.5 (1.1 g) was repeatedly chromatographed on a RP column with MeOH-H_2O (3:1) to give ginsenoside Rg_1 (**10**, 114 mg), 6'-acetyl-ginsenoside Rg_1 (**11**, 118 mg), and ginsenoside Rd (**12**, 134 mg). Fr. 3.8 (1.2 g) was chromatographed on a RP column with MeOH-H_2O (2:1) to afford ginsenoside Rc (**13**, 35 mg), ginsenoside Rb_2 (**14**, 92 mg), and vinaginsenoside R_4 (**16**, 12 mg). Again, fr. 3.10 (1.4 g) was chromatographed on a RP column with MeOH-H_2O (3:1) to give ginsenoside Re (**15**, 100 mg), ginsenoside Mb (**17**, 14 mg), and ginsenoside Rb_1 (**18**, 119 mg), respectively.

Fr. 1.6 (15 g) was subjected to a silica gel column with CH_2Cl_2-MeOH (15:1-1:1) to furnish seven subfractions (fr. 4.1 ~ fr. 4.7). Then, fr. 4.3 (2.6 g) was repeatedly chromatographed on a silica gel column with $CHCl_3$-MeOH-H_2O (5:1:0.1), followed by a RP column with MeOH-H_2O (4:1) to afford ginsenoside SF (**1**, 28 mg) and ginsenoside Rs_4 (**19**, 13 mg).

Ginsenoside SF (1): white amorphous powder; $[\alpha]_D^{20}$ +18° (*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; HRESITOFMS *m*/*z* 693.4141 $[M+Na]^+$ (Calcd for $C_{36}H_{62}O_{11}Na$: 693.4190).

Acid hydrolysis and sugar determination of ginsenosides SF (1). A solution of the compound (2.0 mg) in 1.0 M HCl (4.0 mL) was heated under reflux for 4 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 peak of the hydrolysate of **1** was detected at t_R 14.12 min for D-glucose. The retention times for the authentic samples (Sigma), after being treated in the similar manner, were 14.12 min (D-glucose) and 14.25 min (L-glucose), respectively. Co-injection of the hydrolysates of the ginsenoside with standard D-glucose gave single peaks.

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