

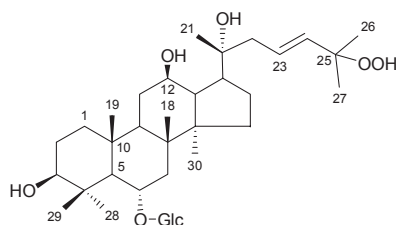
Dammarane-Type Glycosides from the Steamed Flower-Buds of *Panax ginseng*Nguyen Huu Tung,^a Kyoungwon Cho,^a Jeong Ah Kim, Gyu Yong Song, and Young Ho Kim*

College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea. *E-mail: yhk@cnu.ac.kr

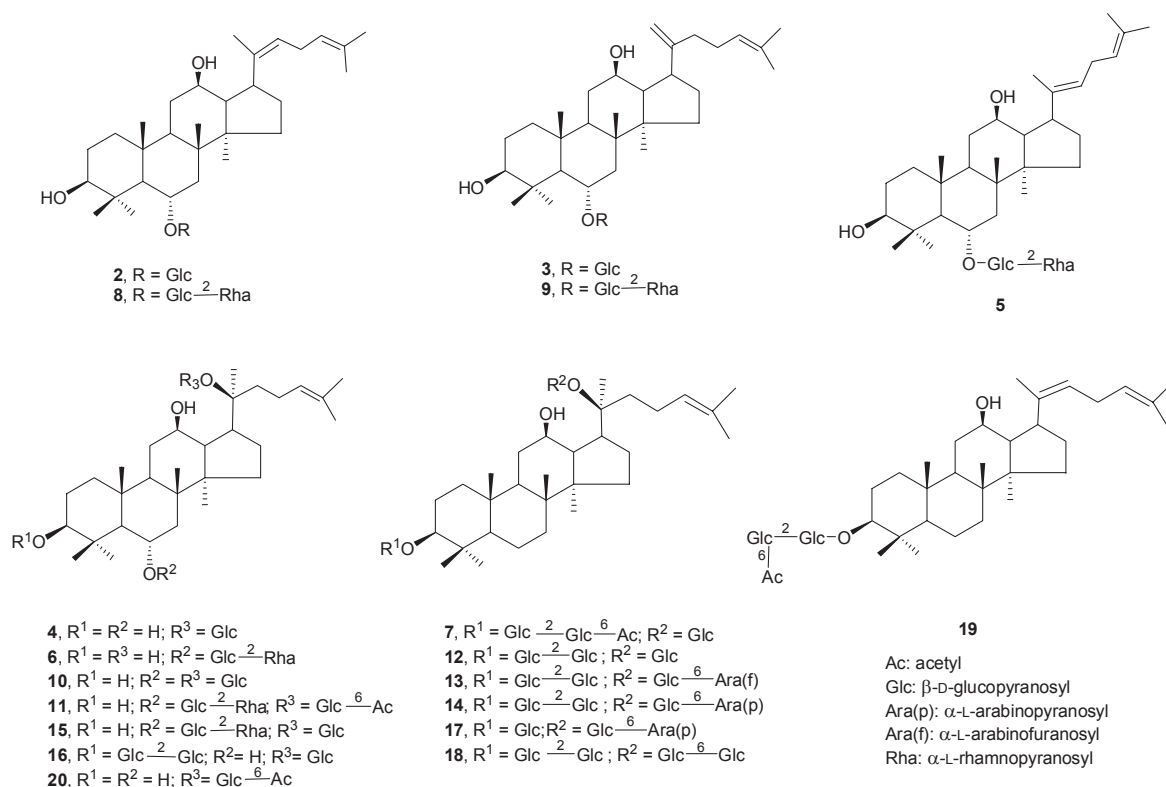
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Panax ginseng C.A. Meyer (Araliaceae), an ancient and famous herbal drug in oriental traditional medicine, has been used as a tonic and for the treatment of various diseases.^{1,2} Biologically active constituents of whole parts of *P. ginseng* (roots, 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 dammarane-type saponin, named ginsenoside SF (1) (Fig. 1), and nineteen known saponins (2-20) (Fig. 2), including ginsenoside Rh₄ (2),⁸ ginsenoside Rk₃ (3),⁸ ginsenoside F₁ (4),⁹ (20*E*)-ginsenoside F₄ (5),¹⁰ ginsenoside Rg₂ (6),¹¹

**Figure 2.** Structures of known saponins 2-20.^aThese authors contributed equally to this work.

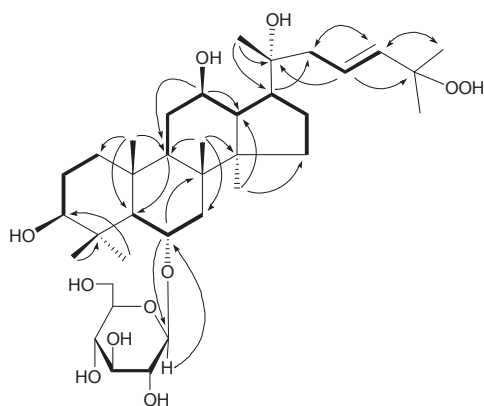


Figure 3. COSY (bold lines), selected HMBC Correlations (arrows) of ginsenoside SF (**1**).

pseudoginsenoside RC₁ (**7**),⁴ ginsenoside Rg₆ (**8**),¹² ginsenoside F₄ (**9**),¹² ginsenoside Rg₁ (**10**),¹³ 6'-acetyl-ginsenoside Rg₁ (**11**),¹⁴ ginsenoside Rd (**12**),⁶ ginsenoside Rc (**13**),⁶ ginsenoside Rb₂ (**14**),⁶ ginsenoside Re (**15**),⁶ vinaginsenoside R₄ (**16**),⁶ ginsenoside Mb (**17**),¹⁵ ginsenoside Rb₁ (**18**),⁶ ginsenoside Rs₄ (**19**),¹⁶ and 6'-acetyl-ginsenoside F₁ (**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 C₃₆H₆₂O₁₁ as deduced by a high-resolution electrospray-ionization time-of-flight mass spectrometry (HRESIT-OFMS) experiment (found at m/z [M+Na]⁺ 693.4141, calcd. for C₃₆H₆₂O₁₁Na 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 ¹H-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, H₃-30, 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 -glucopyranosyl unit. The ¹³C-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 ¹H- and ¹³C-NMR data of **1** were similar to those of (20*R*)-ginsenoside Rh₁^{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 ¹³C-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 ¹H-¹H correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) spectra, respectively. As shown in Fig. 3, the ¹H-¹H 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-3 β ,6 α ,12 β ,20 α -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 AccuTOFTM LC mass spectrometer (Jeol, Tokyo, Japan). GC (Shimadzu-2010, Tokyo, Japan) using a DB-05 capillary column (0.5 mm i.d. \times 30 m) [column temperature: 210 $^{\circ}$ C; detector temperature: 300 $^{\circ}$ C; injector temperature: 270 $^{\circ}$ 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 μ m, Fuji Silysia Chemical Ltd., Aichi, Japan), and HP-20 Diaion (Mitsubishi Chemical, Tokyo, Japan). TLC was performed on Kieselgel 60 F₂₅₄ (1.05715; Merck, Darmstadt, Germany) or RP-18 F_{254s} (Merck) plates. Spots were visualized by spraying with 10% aqueous H₂SO₄ 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 $^{\circ}$ 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 \times 3, 50 $^{\circ}$ C) and the combined extracts were concentrated in vacuo to dryness. The MeOH residue (850 g) was suspended in H₂O (2.5 L), then partitioned with CH₂Cl₂ (2.5 L \times 3), and the water layer was subjected to a

Table 1. ^1H - 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)

^aAssignments were confirmed by COSY, HMQC, and HMBC spectra.^bReversible.

Diaion HP-20 column eluted with a gradient of MeOH in H₂O (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 CH₂Cl₂-MeOH (15:1 - 1:1) to afford nine sub-fractions (fr. 2.1 ~ fr. 2.9). Fr. 2.3 (5.6 g) was further chro-

matographed on a silica gel column with CHCl₃-MeOH-H₂O (7:1:0.1), followed by a reversed-phase (RP) column with MeOH-H₂O (2:1) to obtain ginsenoside Rh₄ (**2**, 100 mg), ginsenoside Rk₃ (**3**, 110 mg), ginsenoside F₁ (**4**, 54 mg) and 6'-acetyl-ginsenoside F₁ (**20**, 13 mg). Fr. 2.7 (9.0 g) was rechromatographed on a silica gel column with CHCl₃-MeOH-H₂O (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-H₂O (5:2) to furnish (20*E*)-ginsenoside F₄ (**5**, 18 mg), ginsenoside Rg₂ (**6**, 35 mg), pseudoginsenoside RC₁ (**7**, 30 mg), ginsenoside Rg₆ (**8**, 40 mg), and ginsenoside F₄ (**9**, 36 mg). Similarly, fr. 3.5 (1.1 g) was repeatedly chromatographed on a RP column with MeOH-H₂O (3:1) to give ginsenoside Rg₁ (**10**, 114 mg), 6'-acetyl-ginsenoside Rg₁ (**11**, 118 mg), and ginsenoside Rd (**12**, 134 mg). Fr. 3.8 (1.2 g) was chromatographed on a RP column with MeOH-H₂O (2:1) to afford ginsenoside Rc (**13**, 35 mg), ginsenoside Rb₂ (**14**, 92 mg), and vinaginsenoside R₄ (**16**, 12 mg). Again, fr. 3.10 (1.4 g) was chromatographed on a RP column with MeOH-H₂O (3:1) to give ginsenoside Re (**15**, 100 mg), ginsenoside Mb (**17**, 14 mg), and ginsenoside Rb₁ (**18**, 119 mg), respectively.

Fr. 1.6 (15 g) was subjected to a silica gel column with CH₂Cl₂-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 CHCl₃-MeOH-H₂O (5:1:0.1), followed by a RP column with MeOH-H₂O (4:1) to afford ginsenoside SF (**1**, 28 mg) and ginsenoside Rs₄ (**19**, 13 mg).

Ginsenoside SF(1): white amorphous powder; $[\alpha]_{\text{D}}^{20} +18^{\circ}$ (c 0.22, MeOH); IR (KBr) ν_{max} 3448, 2922, 1637, 1262, 1054 cm⁻¹; ^1H -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 C₃₆H₆₂O₁₁Na: 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 H₂O (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 H₂O.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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References

1. Park, J. D.; Rhee, D. K.; Lee, Y. H. *Phytochem. Rev.* **2005**, *4*, 159.
2. Shibata, S. *J. Korean Med. Sci.* **2001**, *16*, 28.
3. Yahara, S.; Kaji, K.; Tanaka, O. *Chem. Pharm. Bull.* **1979**, *27*, 88.
4. Yoshikawa, M.; Sugimoto, S.; Nakamura, S.; Sakumae, H.; Matsuda, H. *Chem. Pharm. Bull.* **2007**, *55*, 1034.
5. Sun, L. K.; Yoshii, Y.; Yamashiro, K.; Tomiyama, N.; Ishida, A.; Mukawa, J.; Jin, Y. R.; Wu, G. X. *J. Brain Sci.* **1999**, *25*, 63.
6. Tung, N. H.; Song, G. Y.; Park, Y. J.; Kim, Y. H. *Chem. Pharm. Bull.* **2009**, *57*, 1412.
7. Tung, N. H.; Song, G. Y.; Kim, J. A.; Hyun, J. H.; Kang, H. K.; Kim, Y. H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 309.
8. Park, I. H.; Kim, N. Y.; Han, S. B.; Kim, J. M.; Kwon, S. W.; Kim, H. J.; Park, M. K.; Park, J. H. *Arch. Pharm. Res.* **2002**, *25*, 428.
9. Ko, S. R.; Choi, K. J.; Suzuki, K.; Suzuki, Y. *Chem. Pharm. Bull.* **2003**, *51*, 404.
10. Ryu, J. H.; Park, J. H.; Kim, T. H.; Sohn, D. H.; Kim, J. M.; Park, J. H. *Arch. Pharm. Res.* **1996**, *19*, 335.
11. Yang, X. W.; Li, L. Y.; Tian, J. M.; Zhang, Z. W.; Ye, J. M.; Gu, W. F. *Chin. Chem. Lett.* **2000**, *11*, 909.
12. Ryu, J. H.; Park, J. H.; Eun, J. H.; Jung, J. H.; Sohn, D. H. *Phytochemistry* **1997**, *44*, 931.
13. Fujioka, N.; Kohda, H.; Yamasaki, K.; Kasai, R.; Shoyama, Y.; Nishioka, I. *Phytochemistry* **1989**, *28*, 1855.
14. Teng, R. W.; Ang, C. S.; McManus, D.; Armstrong, D.; Mau, S.; Bacic, A. *Tetrahedron Lett.* **2003**, *44*, 5661.
15. Bae, E. A.; Choo, M. K.; Park, E. K.; Park, S. Y.; Shin, H. Y.; Kim, D. H. *Biol. Pharm. Bull.* **2002**, *25*, 743.
16. Park, I. H.; Han, S. B.; Kim, J. M.; Piao, L.; Kwon, S. W.; Kim, N. Y.; Kang, T. L.; Park, M. K.; Park, J. H. *Arch. Pharm. Res.* **2002**, *25*, 837.
17. Zhao, P.; Liu, Y. Q.; Yang, C. R. *Phytochemistry* **1996**, *41*, 1419.
18. Teng, R. W.; Li, H. Z.; Chen, J. T.; Wang, D.; He, Y.; Yang, C. R. *Magn. Reson. Chem.* **2002**, *40*, 483.
19. Nakamura, S.; Sugimoto, S.; Matsuda, H.; Yoshikawa, M. *Chem. Pharm. Bull.* **2007**, *55*, 1342.
20. Takaku, T.; Kameda, K.; Matsuura, Y.; Sekiya, K.; Okuda, H. *Planta Med.* **1990**, *56*, 27.
21. Lee, S. D.; Okuda, H. *Korean J. Ginseng Sci.* **1990**, *14*, 67.
22. Do, J. H.; Lee, H. O.; Lee, S. K.; Noh, K. B.; Lee, S. D.; Lee, K. S. *Korean J. Ginseng Sci.* **1993**, *17*, 145.