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Protopanaxatriol-Type Ginsenosides from the Root of *Panax ginseng*

GUO-YUAN ZHU, YING-WEI LI, DESMOND KWOK-PO HAU, ZHI-HONG JIANG, ZHI-LING YU, AND WANG-FUN FONG*

Center for Cancer and Inflammation Research, School of Chinese Medicine, Hong Kong Baptist University, Kowloon, Hong Kong Special Administrative Region (SAR), China

Ginseng, the root of *Panax ginseng* C. A. Meyer (Araliaceae), is one of the most important traditional medicines and functional foods. A detailed phytochemical investigation on the roots of *P. ginseng* led to the isolation of 6 new natural protopanaxatriol (PPT)-type ginsenosides, ginsenosides Re_1-Re_6 (compounds **1**−**6**), along with 10 known PPT-type ginsenosides. Their structures were elucidated on the basis of chemical and spectroscopic analyses, including high-resolution mass spectrometry (HRMS) and 1D and 2D nuclear magnetic resonance (NMR). The unusual α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl sugar chain, as found in compounds **1** and **2**, is reported in the genus *Panax* for the first time.

KEYWORDS: ***Panax ginseng*; Araliaceae; ginsenosides Re_1-Re_6; protopanaxatriol; ginsenoside**

INTRODUCTION

Ginseng, the root of *Panax ginseng* (Araliaceae), is one of the most well-known traditional Chinese medicines and functional foods. Ginseng has attracted extensive interest because of its diverse pharmacological and therapeutic effects on the central nervous system, cardiovascular system, immune-modulating function, diabetes, inflammation, aging, and cancer (*1*−*3*). Earlier phytochemical investigations of *P. ginseng* have led to the isolation of many dammarane-type triterpene glycosides, known as ginsenosides, which can be classified into protopanaxadiol (PPD) or protopanaxatriol (PPT) types and are believed to be the primary biologically active constituents of ginseng (*4*−*8*). As part of continued chemical and biological activity studies of ginseng and ginsenosides (*9*−*11*), the dried root of *P. ginseng* was subjected to detailed phytochemical analysis and resulted in the isolation of 6 new (compounds **1**−**6**) and 10 known PPT-type ginsenosides. We report herein the isolation and structural elucidation of these new PPT-type ginsenosides.

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were obtained on a Jasco P-1010 polarimeter (Na 589 nm); ultraviolet (UV) spectra were obtained on a Jasco V-530 spectrophotometer; and infrared (IR) spectra were obtained on a PerkinElmer Spectrum One Fourier transform infrared (FTIR) spectrometer (KBr). Nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker DMX-400 NMR spectrometer using standard Bruker pulse programs. Samples were dissolved in pyridine-d_5, and the NMR spectra were recorded using the lowest field signals of pyridine-d_5 (^{1}H, δ 8.71; ^{13}C, δ 149.9) as an internal reference. Ultra-performance liquid chromatography−high-resolution electrospray ionization mass spectrometry (UPLC−HRESIMS) was performed on an Acquity ultra-performance LC-Bruker micrOTOF mass spectrometer system, and ESIMS was run on a Thermo Finnigan LCQ Advantage mass spectrometer. Thin-layer chromatography (TLC) was performed on plates precoated with silica gel 60 F_{254} (Merck) and reversed-phase RP18 F_{254} (Merck), and spots were visualized by spraying the plates with 10% H_2SO_4 ethanol solution, followed by heating. Semi-preparative high-performance liquid chromatography (HPLC) was carried out on a Perkin-Elmer series 200 separation system with an autosampler, an IC pump, a 253C diode array detector (DAD), and a YMC-Pack ODS-A semi-preparative column (10 μm, 250 × 10 mm), at an elution flow rate of 5.0 mL/min. Column chromatography (CC) was performed with silica gel (200−300 mesh, Qingdao Haiyang Chemical Group Co., Qingdao, Shandong Province, People's Republic of China) and reversed-phase RP18 (45 μm, Merck).

Chemicals. HPLC-grade MeOH and MeCN, AR-grade EtOAc, EtOH, *n*-BuOH, MeOH, $CHCl_3$, HCl, and pyridine were purchased from Labscan Asia (Bangkok, Thailand). L-Cysteine methyl ester hydrochloride, phenyl isothiocyanate, formic acid, L-glucose, D-arabinose, L-arabinose, and MeONa were obtained from Sigma Aldrich (St. Louis, MO). D-Glucose was purchased from Alfa Aesar (Lancashire, U.K.).

Plant Material. The root of *P. ginseng* C. A. Meyer was collected from Jilin Province, People's Republic of China, in October 2007. The species was identified by Professor Zhong-Zhen Zhao of the School of Chinese Medicine, Hong Kong Baptist University. A voucher specimen (PG-0710) was deposited at the Research and Development Division, School of Chinese Medicine, Hong Kong Baptist University.

Extraction and Isolation. Air-dried, milled root powder of *P. ginseng* C. A. Meyer (7.0 kg) was extracted with 70% EtOH (21 L × 3) under reflux, and the extracts were combined and evaporated to afford a brown residue (∼1.8 kg). The residue was dissolved in water (7 L) and partitioned successively with petroleum ether (7 L × 3), EtOAc (7 L × 3), and *n*-BuOH (7 L × 3) to give the petroleum-ether-soluble (110 g), EtOAc-soluble (120 g), and *n*-BuOH-soluble (185 g) fractions. The *n*-BuOH extract (180 g) was subjected to CC eluted with a $CHCl_3$/MeOH gradient (10:1 → 3:7). Fractions were combined according to their TLC behavior to obtain nine fractions (A → I). Fraction A (3 g) was repeatedly chromatographed on a silica gel column eluted with EtOAc/MeOH/H_2O (50:5:1)

*To whom correspondence should be addressed. Telephone: +852-3411 2928. Fax: +852-3411 2902. E-mail: wffong@hkbu.edu.hk.

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and a reversed-phase RP18 column eluted with $MeCN/H_2O$ (20:80 → 35:65) and purified by HPLC eluted with $MeCN/H_2O$ (30:70) to yield compound **6** (4 mg), yesanchinoside D (8 mg), and koryoginsenoside R_1 (23 mg). Fraction B (15 g) was subjected to CC on a silica gel column eluted with $EtOAc/MeOH/H_2O$ (50:5:1) and a reversed-phase RP18 column eluted with $MeCN/H_2O$ (20:80 → 35:65) and finally isolated on HPLC eluted with $MeCN/H_2O$ (20:80 → 35:65) to afford ginsenoside Rg_1 (220 mg), ginsenoside Rg_2 (72 mg), and notoginsenoside R_2 (180 mg). Fraction C (13 g) was rechromatographed on a silica gel column eluted with $EtOAc/MeOH/H_2O$ (50:6:1) and a RP18 column eluted with $MeCN/H_2O$ (25:75) to give ginsenoside Rf (150 mg). Fraction D (17 g) was submitted to CC on a normal-phase silica gel column eluted with $EtOAc/MeOH/H_2O$ (50:8:1) and a reversed-phase RP18 column eluted with $MeCN/H_2O$ (20:80 → 40:60) and finally purified by HPLC eluted with a gradient of $MeCN/H_2O$ (18:82 → 20:80) to obtain compound **1** (18 mg), compound **2** (65 mg), compound **3** (30 mg), compound **4** (10 mg), compound **5** (8 mg), ginsenoside Re (300 mg), 20-glucoginsenoside Rf (150 mg), notoginsenoside R_1 (11 mg), and notoginsenoside N (22 mg).

Ginsenoside Re_1 (Compound 1). White amorphous powder. $[\alpha]_D^{25}$ +74.7 (*c* 0.9, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3400, 2931, 2878, 1642, 1550, 1455, 1385, 1307, 1258, 1202, 1147, 1076, 1031, 925, 891, 842, 771, 619, 534. ^1H and ^{13}C NMR spectra data: see **Tables 1** and **2**. HRESIMS (*m*/*z*): $[M - H]^-$ 961.5349 (calcd for $C_{48}H_{81}O_{19}$, 961.5378), $[M + HCOO]^-$ 1007.5414 (calcd for $C_{49}H_{83}O_{21}$, 1007.5432).

Ginsenoside Re_2 (Compound 2). White amorphous powder. $[\alpha]_D^{25}$ +66.1 (*c* 0.8, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3400, 2931, 2879, 1642, 1455, 1386, 1310, 1259, 1202, 1147, 1076, 1039, 925, 891, 843, 771, 640, 616, 559. ^1H and ^{13}C NMR spectra data: see **Tables 1** and **2**. HRESIMS (*m*/*z*): $[M - H]^-$ 961.5346 (calcd for $C_{48}H_{81}O_{19}$, 961.5378), $[M + HCOO]^-$ 1007.5390 (calcd for $C_{49}H_{83}O_{21}$, 1007.5432).

Ginsenoside Re_3 (Compound 3). White amorphous powder. $[\alpha]_D^{25}$ +63.6 (*c* 1.2, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3400, 2931, 2879, 1642, 1551, 1456, 1385, 1307, 1259, 1201, 1147, 1075, 1028, 926, 891, 841, 780, 640, 616, 534. ^1H and ^{13}C NMR spectra data: see **Tables 1** and **2**. HRESIMS (*m*/*z*): $[M - H]^-$ 961.5340 (calcd for $C_{48}H_{81}O_{19}$, 961.5378).

Ginsenoside Re_4 (Compound 4). White amorphous powder. $[\alpha]_D^{25}$ −5.8 (*c* 0.2, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3400, 2931, 2879, 1640, 1552, 1456, 1385, 1308, 1259, 1204, 1076, 1040, 891, 862, 841, 780, 646, 616, 534. ^1H and ^{13}C NMR spectra data: see **Tables 1** and **2**. HRESIMS (*m*/*z*): $[M - H]^-$ 931.5260 (calcd for $C_{47}H_{79}O_{18}$, 931.5272), $[M + HCOO]^-$ 977.5292 (calcd for $C_{48}H_{81}O_{20}$, 977.5327).

Ginsenoside Re_5 (Compound 5). White amorphous powder. $[\alpha]_D^{25}$ +6.9 (*c* 0.3, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3391, 2934, 2877, 1641, 1551, 1456, 1376, 1307, 1257, 1076, 1029, 891, 863, 825, 666, 619, 534. ^1H and ^{13}C NMR spectra data: see **Tables 1** and **2**. HRESIMS (*m*/*z*): $[M - H]^-$ 815.4785 (calcd for $C_{42}H_{71}O_{15}$, 815.4798), $[M + HCOO]^-$ 861.4826 (calcd for $C_{43}H_{73}O_{17}$, 861.4853).

Ginsenoside Re_6 (Compound 6). White amorphous powder. $[\alpha]_D^{25}$ +40.4 (*c* 0.1, MeOH). UV (MeOH) λ_{max}, nm (log ε): 211 (3.89), 252 (3.02). IR (KBr) ν_{max} (cm^{-1}): 3400, 2934, 2879, 1715, 1656, 1445, 1385, 1313, 1191, 1076, 1031, 970, 926, 892, 838, 647, 618, 530. ^1H and ^{13}C NMR spectra data: see **Tables 1** and **2**. HRESIMS (*m*/*z*): $[M + HCOO]^-$ 913.5190 (calcd for $C_{47}H_{77}O_{17}$, 913.5166).

Determination of the Absolute Configuration of Sugars. The absolute configuration of sugars of new gisenosides was determined according to the reported protocol (*12*). Briefly, compounds **1**−**5** (1 mg each) were dissolved in 1 N HCl (0.1 mL) and then heated to 80 °C for 4 h. The solvent was removed under N_2. The residue was dissolved in pyridine (0.1 mL) containing L-cysteine methyl ester hydrochloride (0.5 mg) and heated at 60 °C for 1 h. A 0.1 mL solution of phenyl isothiocyanate (0.5 mg/mL) in pyridine was added to the mixture, which was heated at 60 °C for a further 1 h. The reaction mixture (2 μL) was directly analyzed by HPLC under the following conditions: Waters Symmetry C18 column (5 μm, 4.6 × 250 mm); DAD detection, 250 nm; column temperature, 35 °C; mobile phase, 25% MeCN in 0.1% H_3PO_4; and flow rate, 0.8 mL/min. From the acid hydrolysates of compounds **1**−**3** and **5**, D-glucose was confirmed by a comparison of the retention time values (15.46, 15.45, 15.44, and 15.42 min, respectively) of their derivatives to those of derivatized authentic sugars. L-Arabinose (t_R, 17.65 min) and D-glucose (t_R, 15.41 min) were confirmed from the acid hydrolysate of compound **4**. Treated in the same way, standard D-glucose (Alfa Aesar), L-glucose (Sigma), D-arabinose (Sigma), and L-arabinose (Sigma) gave peaks at t_R (min) of 15.41, 14.56, 19.16, and 17.70, respectively.

Alkaline Hydrolysis of Compound 6. A methanol solution of compound **6** (0.2 mg/mL) was hydrolyzed by MeONa (20 mmol) for 12 h at room temperature. The reaction mixtures (compound **6a**) were neutralized with formic acid (20 mmol) and then subjected to UPLC−HRESIMS analysis under the following conditions: Acquity UPLC BEH C_{18} column (1.7 μm, 2.1 × 100 mm); gradient elution with 0.1% formic acid in MeCN (solvent A) and 0.1% formic acid in water (solvent B) from 10 to 40% solvent A over 6 min at a flow rate of 0.35 mL/min; and negative HRESIMS (nebulizer, 2.0 bar; dry heater, 180 °C; dry gas, 8.0 L/min; capillary, 4000 V; end plate offset, −500 V; and collision cell RF, 550.0 Vpp). The desacyl saponin (compound **6a**; t_R, 3.005 min; HRESIMS, 845.4891) was identified by comparing its retention time and HRMS to those of authentic sample ginsenoside Rg_1 (t_R, 3.005 min; HRESIMS, $[M + HCOO]^-$ *m*/*z* 845.4897; $C_{43}H_{73}O_{16}$, calcd 845.4904).

RESULTS AND DISCUSSION

The *n*-BuOH-soluble fraction of *P. ginseng* ethanolic extract was chromatographed repeatedly on silica gel and reversed-phase RP18 columns and finally purified by HPLC to yield 6 new PPT-type ginsenosides, ginsenosides Re_1−Re_6 (compounds **1**−**6**) (**Figure 1**), and 10 known PPT-type ginsenosides (**Figure 1**).

The known PPT-type ginsenosides were identified as ginsenosides Rg_1 (*13*), Rg_2 (*13*), Re (*13*), and Rf (*14*), 20-glucoginsenoside Rf (*15*), notoginsenosides R_1 (*13*), R_2 (*13*), and N (*16*), yesanchinoside D (*17*), and koryoginsenoside R_1 (*7*) by comparison of their MS and NMR spectroscopic data to published data. Among them, notoginsenoside N and yesanchinoside D were isolated from *P. ginseng* for the first time.

Compound **1** was obtained as a white amorphous powder. The HRESIMS of compound **1** showed two negative quasi-molecular ion peaks at *m*/*z* 961.5349 $[M - H]^-$ and 1007.5414 $[M + HCOO]^-$, demonstrating the molecular formula of compound **1** to be $C_{48}H_{82}O_{19}$. The IR spectrum of compound **1** showed strong absorption bands at 3400 and 1076 cm^{-1}, suggestive of an alcohol moiety, together with an absorption band at 1642 cm^{-1} because of a double bond. Acid hydrolysis of compound **1** with 1 N HCl only liberated glucose, whose absolute configuration was determined to be the D form by HPLC analysis of chiral derivatives (*12*). The ^1H and ^{13}C NMR spectra (**Table 1** and **2**) of compound **1** showed three anomeric signals at δ_H 5.00 (d, J = 8.0 Hz), 5.08 (d, J = 8.0 Hz), 5.86 (d, J = 3.6 Hz), and δ_C 106.0, 98.2, and 102.0 and signals assignable to an aglycone part [8 methyl proton singlets at δ_H 0.75−2.05, 1 olefinic proton signal at δ_H 5.22 (t, J = 6.8 Hz, H-24), and 30 carbon resonances, including 8 methyl, 8 methylene, 8 methine, and 6 quaternary carbons]. The aglycone carbon signals of compound **1** were superimposable with those of ginsenosides Rg_1 and Re (*13*), which indicated that compound **1** is also a 20(*S*)-protopanaxatriol 6,20-bisdesmoside, containing three glucosyl moieties. The resonances of the sugar residues were assigned starting from the anomeric protons by means of 2D NMR experiments ($^1H-^1H$ COSY, HSQC, HMBC, and NOESY), which led to the determination of sequences of the three glucosyl moieties and the location of their linkages to the aglycone. The key $^1H-^1H$ COSY, HMBC, and NOESY correlations of compound **1** are shown in **Figures 2** and **3**. The correlations in the HMBC spectrum between the anomeric proton at δ_H 5.00 (d, J = 8.0 Hz, H-1′) and the carbon signal at δ_C 80.2 (C-6) and between H-1″ at δ_H 5.08 (d, J = 8.0 Hz) and C-20 at δ_C 83.6 indicated that two β-glucopyranosyl units are attached at C-6 and C-20 positions of the aglycone, respectively. The significant downfield shift of C-3″ (δ 88.5) in the inner β-glucopyranosyl moiety at C-20 of aglycone in the ^{13}C NMR spectrum together with the HMBC correlation from H-1‴ (δ_H 5.86, d, J = 3.6 Hz) to C-3″ (δ_C 88.5) showed that the terminal α-glucopyranosyl is linked to the inner

Table 1. ^{1}H NMR (400 MHz, C_5D_5N) Spectroscopic Data for Compounds **1**−**6**[a]

position	1	2	3	4	5	6
1a	1.58	1.69	1.71	1.71	1.65	1.71
1b	0.92	0.96	0.98	0.97	0.96	0.96
2a	1.89	1.89	1.92	1.92	1.86	1.92
2b	1.78	1.81	1.84	1.81	1.80	1.84
3	3.49 dd (11.2, 4.4)	3.45 dd (10.8, 4.4)	3.50 dd (11.2, 4.4)	3.49 dd (11.2, 4.4)	3.47 brd (10.8)	3.50 dd (10.8, 4.4)
5	1.37 d (10.4)	1.36 d (10.4)	1.39 d (10.4)	1.39 d (10.4)	1.37 d (10.8)	1.40 d (10.4)
6	4.38 t (11.6)	4.34	4.39	4.40 t (10.4)	4.28	4.41
7a	2.46	2.41	2.47	2.46	2.41	2.47
7b	1.89	1.87	1.91	1.91	1.93 t (11.6)	1.92
9	1.48	1.48	1.50	1.51	1.50	1.50
11a	2.00	2.05	2.05	2.06	2.08	2.03
11b	1.44	1.47	1.48	1.50	1.49	1.48
12	3.99	4.11	4.11	4.13	3.85	4.17
13	1.92	1.96	1.95 t (10.8)	1.97 t (10.8)	2.01	1.97 t (10.4)
15a	1.59	1.60	1.62	1.63	1.61	1.62
15b	1.03 t (10.8)	1.01	1.04	1.03	1.11	1.06
16a	1.75	1.72	1.73	1.73	1.76	1.79
16b	1.26	1.27	1.26	1.25	1.24	1.27
17	2.39	2.46	2.45	2.46	2.26	2.51
18	1.11 s	1.13 s	1.13 s	1.15 s	1.15 s	1.14 s
19	0.97 s	0.99 s	1.01 s	1.01 s	0.95 s	1.01 s
21	1.52 s	1.58 s	1.55 s	1.59 s	1.34 s	1.56 s
22a	2.32 t (13.2)	2.37	2.34	2.35	2.06	2.37 t (12.4)
22b	1.80	1.79	177	1.80	1.67	1.77
23a	2.41	2.47	2.44	2.56	2.68	2.56
23b	2.19	2.21	2.19	2.34	2.42	2.26
24	5.22 t (6.8)	5.24 t (6.8)	5.21 t (10.8)	5.31 t (6.8)	5.47 t (7.2)	5.28 t (6.0)
26	1.60 s	1.59 s	1.59 s	1.61 s	1.99 s	1.61 s
27a	1.60 s	1.59 s	1.57 s	1.65 s	4.55 d (12.0)	1.63 s
27b					4.46	
28	2.05 s	1.96 s	2.05 s	2.05 s	2.08 s	2.06 s
29	1.58 s	1.49 s	1.59 s	1.59 s	1.46 s	1.60 s
30	0.75 s	0.77 s	0.78 s	0.79 s	0.79 s	0.81 s
	6-Glc	6-Glc	6-Glc	6-Glc	6-Glc	6-Glc
1′	5.00 d (8.0)	4.92 d (8.0)	5.00 d (8.0)	5.01 d (8.0)	4.92 d (7.2)	5.02 d (8.0)
2′	4.08 t (8.0)	4.02	4.07 t (8.0)	4.08	4.46	4.09 t (8.0)
3′	4.23	4.21	4.24	4.24	4.35	4.23 t (8.0)
4′	4.20	4.27	4.21	4.21	4.12 t (8.8)	4.21
5′	3.92	3.83	3.93 t (8.0)	3.93	3.83	3.94
6′a	4.51	4.41	4.49	4.51 d (10.4)	4.47	4.52 d (11.6)
6′b	4.34	4.26	4.35	4.34	4.29	4.36 dd (11.6, 5.2)
	20-Glc	3′-Glc	20-Glc	20-Glc	2′-Glc	20-Glc
1″	5.08 d (8.0)	5.89 d (4.0)	5.05 d (8.0)	5.10 d (7.6)	5.92 d (7.2)	5.10 d (8.0)
2″	3.89	4.19	3.93 t (8.0)	3.94	4.19	3.97
3″	4.18	4.59 t (9.2)	4.27	4.17	4.23 t (8.8)	4.17 t (8.8)
4″	4.19	4.20	4.23	3.96	4.16	3.96
5″	3.80	4.76	3.72 dt (2.8, 8.8)	3.98	3.94	3.99
6″a	4.37	4.46	4.38	4.66 d (10.0)	4.46	5.03 d (11.6)
6″b	4.20	4.28		4.08	4.34	4.73 dd (11.6, 6.4)
	3″-Glc	20-Glc	4″-Glc	6″-Ara		6″-Acyl
1‴	5.86 d (3.6)	5.16 d (8.0)	5.84 d (3.6)	5.65 s		
2‴	4.17	3.97 t (8.0)	4.14	4.86		5.96 dt (15.6, 2.0)
3‴	4.57 t (8.8)	4.21	4.58 t (9.2)	4.79 t (4.8)		7.03 dq (15.6, 6.8)
4‴	4.16	4.17	4.13	4.73		1.65 d (6.8)
5‴	4.77	3.90	4.54	4.30		
6‴a	4.49	4.47	4.53	4.19		
6‴b	4.26	4.31	4.31			

[a] Data were assigned on the basis of heteronuclear single-quantum coherence (HSQC), heteronuclear multiple-bond correlation (HMBC), H−H correlation spectroscopy (COSY), and nuclear Overhauser effect spectrometry (NOESY) experiments. The chemical shifts are in parts per million (ppm), and coupling constants [*J* in hertz (Hz)] are in parentheses. Overlapped signals are reported without designated multiplicities.

β-glucopyranosyl at C-20 by a specific 1 → 3 linkage (*18*). These linkages were further confirmed by NOESY correlations between H-1′ and H-6 and between H-1‴ and H-3″. On the basis of the above results, the structure of compound **1** was determined as 6-*O*-β-D-glucopyranosyl-20-*O*-[α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-20(*S*)-protopanaxatriol and named ginsenoside Re_1.

Table 2. ^{13}C NMR (100 MHz, C_5D_5N) Chemical-Shift Assignments for Compounds **1**−**6**

position	1	2	3	4	5	6
1	39.4	39.5	39.5	39.4	39.4	39.4
2	27.9	27.9	28.0	28.0	27.8	28.0
3	78.7	78.7	78.7	78.7	78.7	78.7
4	40.4	40.3	40.4	40.4	40.2	40.4
5	61.4	61.4	61.4	61.4	61.4	61.4
6	80.2	80.4	80.2	80.1	79.7	80.2
7	45.1	45.1	45.2	45.1	45.1	45.1
8	41.1	41.2	41.2	41.1	41.2	41.1
9	49.9	50.0	50.1	50.0	50.2	50.0
10	39.7	39.7	39.7	39.7	39.7	39.7
11	30.9	31.0	31.0	30.9	32.1	31.0
12	70.3	70.3	70.2	70.3	71.1	70.1
13	49.1	49.2	49.2	49.2	48.3	49.2
14	51.4	51.4	51.4	51.4	51.7	51.4
15	30.7	30.7	30.7	30.6	31.2	30.7
16	26.7	26.7	26.6	26.6	27.0	26.7
17	51.9	51.6	51.5	51.6	54.7	51.6
18	17.5	17.6	17.6	17.6	17.7	17.6
19	17.6	17.6	17.6	17.6	17.5	17.6
20	83.6	83.4	83.5	83.4	73.0	83.5
21	22.4	22.4	22.2	22.3	26.8	21.9
22	36.1	36.2	36.1	36.2	36.2	36.1
23	23.3	23.3	23.2	23.2	22.6	23.0
24	125.9	126.0	125.9	126.1	128.0	126.1
25	131.0	131.0	131.0	131.1	136.2	131.0
26	25.8	25.8	25.8	25.8	21.9	25.8
27	17.8	17.8	17.8	17.9	60.9	17.8
28	31.8	31.8	31.8	31.8	32.1	31.8
29	16.4	16.4	16.4	16.4	16.8	16.4
30	17.1	17.2	17.2	17.2	16.8	17.2
	6-Glc	6-Glc	6-Glc	6-Glc	6-Glc	6-Glc
1′	106.0	105.8	106.0	106.0	103.9	106.0
2′	75.5	73.8	75.5	75.5	79.9	75.5
3′	79.7	89.3	79.7	79.7	80.0	79.7
4′	71.9	71.0	71.9	71.9	71.8	71.9
5′	78.2	77.6	78.2	78.2	78.1	78.2
6′	63.1	62.4	63.1	63.1	63.0	63.1
	20-Glc	3′-Glc	20-Glc	20-Glc	2′-Glc	20-Glc
1″	98.2	102.2	98.1	98.1	103.9	98.0
2″	73.5	74.3	74.6	75.1	76.1	75.0
3″	88.5	75.6	78.7	79.3	78.5	79.3
4″	70.7	72.0	81.2	72.2	72.4	71.6
5″	77.7	74.8	76.6	76.6	77.9	75.0
6″	62.3	62.6	62.1	68.6	63.4	64.6
	3″-Glc	20-Glc	4″-Glc	6″-Ara		6″-Acyl
1‴	102.0	98.3	103.0	110.2		166.4
2‴	74.3	75.2	74.5	83.4		123.2
3‴	75.7	79.4	75.5	78.9		144.8
4‴	72.0	71.7	71.9	86.0		17.8
5‴	74.7	78.3	75.3	62.7		
6‴	62.5	62.9	62.7			

Compound **2** showed the same molecular formula $C_{48}H_{82}O_{19}$ as compound **1** determined by HRESIMS (*m/z* 961.5346 [M − H]$^-$, calcd 961.5378; *m/z* 1007.5390 [M + HCOO]$^-$, calcd 1007.5432). Acid hydrolysis of compound **2** also gave D-glucose. The ^{1}H and ^{13}C NMR data (**Tables 1** and **2**) are very similar to those of compound **1**, including signals assignable to the aglycone [20(*S*)-protopanaxatriol] part and three sugar moieties (a terminal α-D-glucopyranosyl unit and two *β*-D-glucopyranosyl units). The terminal α-glucopyranosyl was confirmed to be attached to the inner glucose by a 1 → 3 linkage, as evidenced by the correlation from H-1″ (δ_H 5.89, d, J = 4.0 Hz) to C-3′ (δ_C 89.3) in the HMBC spectrum of compound **2**. However, the HMBC correlation from H-1′ (δ_H 4.92, d, J = 8.0 Hz) to C-6 (δ_C 80.4) suggested that the disaccharide moiety is connected to aglycone at C-6. Another *β*-D-glucopyranosyl unit should be linked to C-20, which was confirmed by the HMBC correlation between H-1‴ (δ_H 5.16) and C-20 (δ_C 83.5). Therefore, the structure of compound **2** was elucidated as 6-*O*-[α-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl]-20-*O*-*β*-D-glucopyranosyl-20(*S*)-protopanaxatriol and named ginsenoside Re$_2$. Compound **2** was also identified in the root of *P. ginseng* by LC−MS analysis (data not shown), and therefore, it is by definition a new natural product, despite the

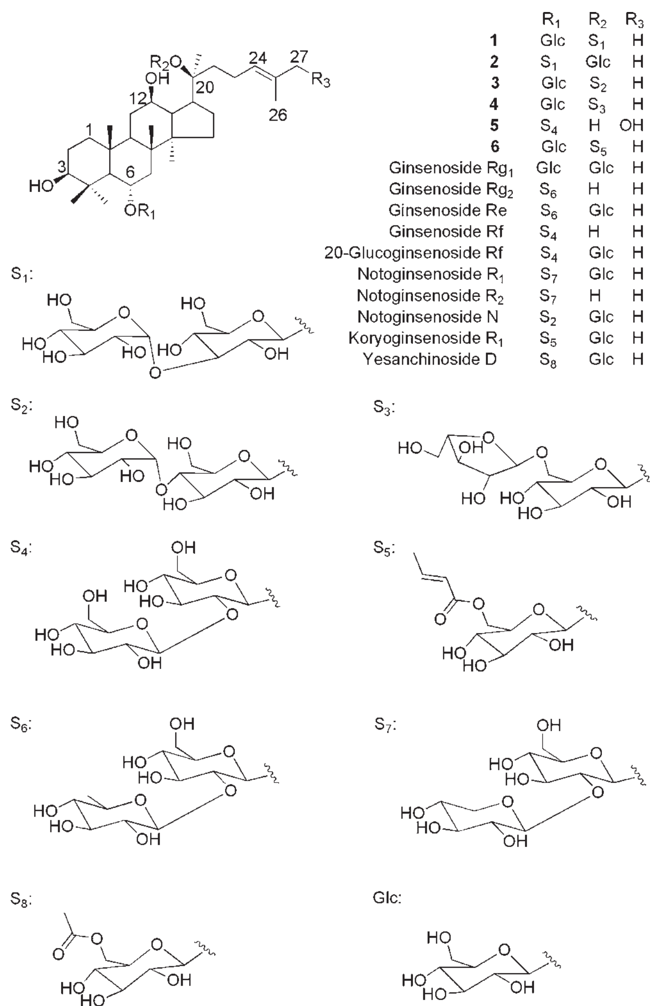

Figure 1. Structures of new and known PPT-type ginsenosides from the root of *P. ginseng*.

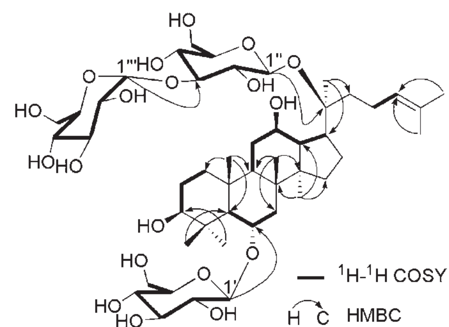

Figure 2. Selected ^{1}H−^{1}H COSY and HMBC correlations of compound **1**.

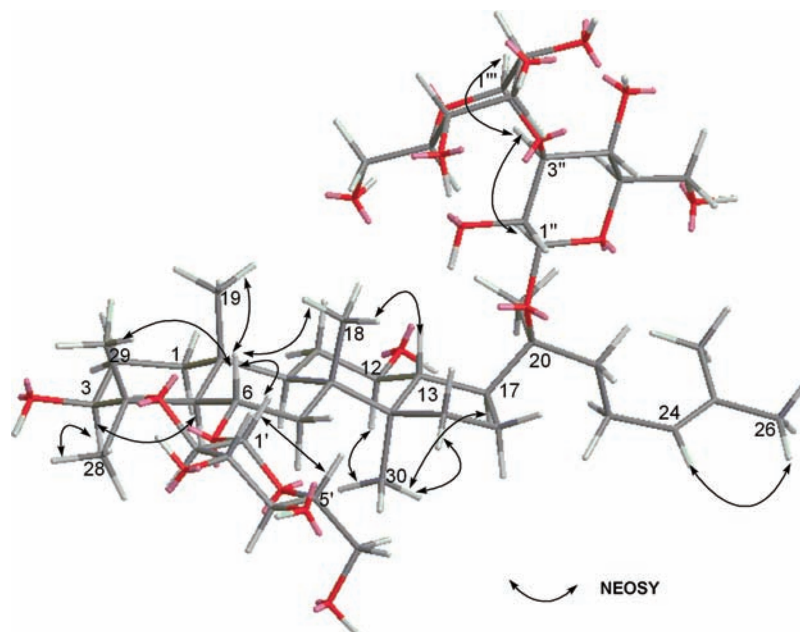

Figure 3. Selected NOESY correlations of compound **1**.

fact that it has been biosynthesized from ginsenoside Rg_1 by cyclomaltodextrin glucanotransferase (*19*). In addition, we reassigned those ambiguous assignments of ^{13}C NMR data in a previous report (*19*) and presented a complete assignment of ^1H and ^{13}C NMR data of compound **2** based on analyses of 2D NMR spectra, including COSY, HSQC, HMBC, and NOESY.

Compound **3** is a white powder with a molecular formula of $C_{48}H_{82}O_{19}$, which is the same as that of compound **1**, compound **2**, and notoginsenoside N (*16*), on the basis of negative-ion HRESIMS data (*m*/*z* 961.5340 [M − H]$^-$, calcd 961.5378). The ^1H and ^{13}C NMR data (**Tables 1** and **2**) of compound **3** are very similar to those of notoginsenoside N (*16*), except for a little difference on anomeric signals, which suggested that the linkage of the sugar part in compound **3** is different from that in notoginsenoside N. In the HMBC spectrum of compound **3**, the anomeric proton of the α-glucopyranosyl group at δ_H 5.84 (d, J = 3.6 Hz, H-1‴) showed a long-range correlation with C-4″ of the inner β-glucopyranosyl unit at δ_C 81.2, of which H-1″ (δ_H 5.05, d, J = 8.0 Hz) in turn correlated to C-20 (δ_C 83.5) of the aglycone. The HMBC correlation from H-1′ (δ_H 5.00, d, J = 8.0 Hz) to C-6 (δ_C 80.2) was also observed. Furthermore, the shifts of signals assignable to C-6-linked β-glucopyranosyl were identical to those in compound **1** rather than compound **2**. D-Glucose was the only monosaccharide identified in the acid hydrolysate of compound **3**. The above data suggested that β-glucopyranosyl was connected to aglycone at C-6 and the disaccharide chain was linked to the C-20 position in compound **3**. Thus, compound **3** was determined to be 6-*O*-β-D-glucopyranosyl-20-*O*-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]-20(*S*)-protopanaxatriol and named ginsenoside Re_3.

Compound **4** has a molecular formula of $C_{47}H_{80}O_{18}$, as deduced from the HRESIMS data (*m*/*z* 931.5260 [M − H]$^-$, calcd 931.5272; *m*/*z* 977.5292 [M + HCOO]$^-$, calcd 977.5327). Acid hydrolysis of compound **4** yielded D-glucose and L-arabinose. The ^1H and ^{13}C NMR data of compound **4** (**Tables 1** and **2**) demonstrated that the aglycone signals were in good agreement with those of 20(*S*)-protopanaxatriol characterized in compounds **1**−**3**. Three sets of signals because of two β-glucopyranosyl units and an α-arabinofuranosyl unit were also observed. The observation of glycosylation-induced downfield shifts in the ^{13}C NMR spectrum at δ_C 80.1 (C-6) and 83.4 (C-20), along with long-range correlations of H-1′/C-6, H-1‴/C-6″, and H-1″/C-20 observed in the HMBC spectrum, suggested that a β-glucopyranosyl unit was connected at C-6 and an α-arabinofuranosyl-(1→6)-β-glucopyranosyl unit was linked to C-20 of protopanaxatriol. Hence, the structure of compound **4** was elucidated as 6-*O*-β-D-glucopyranosyl-20-*O*-[α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxatriol and named ginsenoside Re_4.

Compound **5** showed spectral features closely related to those of ginsenoside Rf (*14*). However, the molecular formula of compound **5** analyzed for $C_{42}H_{72}O_{15}$ by HRESIMS (*m*/*z* 815.4785 [M − H]$^-$, calcd 815.4798; *m*/*z* 861.4826 [M + HCOO]$^-$, calcd 861.4853) has an additional oxygen atom compared to that of ginsenoside Rf (*14*). In comparison of ^1H and ^{13}C NMR data (**Tables 1** and **2**) of compound **5** to those of ginsenoside Rf (*14*), a change in shift because of an oxygenated methylene was observed at δ_H 4.55 (d, J = 12.0 Hz, H-27a), 4.46 (H-27b), and δ_C 60.9 (C-27) and the olefinic carbon signal of C-24 (δ_C 128.0) and the methyl carbon at C-26 (δ_C 21.9) were significantly downfield-shifted, which indicated that an additional hydroxyl group was located at C-27 in compound **5**, which can be confirmed by the HMBC correlations from H-27a and H-27b to C-24 and C-26, respectively. Furthermore, the signals ascribed to aglycone of compound **5** were found to be identical to those of 27-hydroxyl-20(*S*)-protopanaxatriol (*20*), except for the downfield shift of carbon at C-6 because of glycosidation. D-Glucose was identified in the acid hydrolysate of compound **5**. Accordingly, the structure of compound **5** was determined as 6-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-27-hydroxyl-20(*S*)-protopanaxatriol and named ginsenoside Re_5.

Compound **6** was isolated as a white amorphous powder with a molecular formula of $C_{46}H_{76}O_{15}$ based on its HRESIMS at *m*/*z* 913.5190 [M + HCOO]$^-$ (calcd 913.5166). The IR spectrum of compound **6** showed an absorption band at 1710 and 1654 cm^{-1} because of the ester and olefin group. The UV spectrum showed a significant absorption maximum at 253 nm and the usual 210 nm absorption of triterpene compounds. The ^1H and ^{13}C NMR data (**Tables 1** and **2**) of compound **6** revealed it to be a 20(*S*)-protopanaxatriol 6,20-bisdesmoside (*13*) with two β-D-glucopyranosyl units and a butenoyl group (*7*), suggestive by the signals at

δ_H 1.65 (d, J = 6.8 Hz), 7.03 (dq, J = 15.6, 6.8 Hz), and 7.03 (dd, J = 15.6, 2.0 Hz) and δ_C 17.8, 144.8, 123.2, and 166.4. A detailed comparison of NMR data of compound **6** to those of koryoginsenoside R_1 (*7*) and ginsenoside Rg_1 (*13*) clearly indicated that compound **6** is a butenoyl ester of ginsenoside Rg_1 and differed from koryoginsenoside R_1 only in the location of the butenoyl ester group. The functional group was assigned at C-6″ of glucose linked at C-20 of aglycone based on HMBC correlations between protons at δ_H 5.03 (d, J = 11.6 Hz, H-6″a) and 4.87 (dd, J = 6.4, 11.6 Hz, H-6″b) and the carbon signal at δ_C 166.4 (C-1‴), and the carbon signal of C-6″ was downfield-shifted (δ_C 63.0 → 64.6) by comparing it to that of ginsenoside Rg_1 (*13*). Alkaline hydrolysis of compound **6** with NaOMe liberated ginsenoside Rg_1, which further confirmed that compound **6** is a ginsenoside Rg_1 butenoyl ester. On the basis of the above evidence, compound **6** was elucidated as 6-*O*-*β*-D-glucopyranosyl-20-*O*-(*β*-D-6-*O*-*E*-2-butenoyl-glucopyranosyl)-20(*S*)-protopanaxatriol and named ginsenoside Re_6.

PPT-type ginsenosides, such as Rg_1 and Re, have previously shown extensive bioactivities, including anti-diabetes (*21*), neuroprotective effects (*22*), estrogen and glucocorticoid receptor activation (*23*, *24*), and cardiovascular protective effects (*25*). Along with those known PPT-type ginsenosides, 6 new PPT-type ginsenosides (compounds **1**−**6**) were isolated from the root of *P. ginseng*. Compounds **1** and **2** are the first example of ginsenosides containing α-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl moiety isolated from the genus *Panax*. These new PPT-type ginsenosides further illustrate the interesting chemodiversity of *P. ginseng*.

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Supporting Information Available: NMR (1D and 2D) spectra of compounds **1**−**6** (Figures S1−S37). This material is available free of charge via the Internet at http://pubs.acs.org.

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