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Research article

Ginsenoside Rg12, a new dammarane-type triterpene saponin from *Panax ginseng* root

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ABSTRACT

Background: *Panax ginseng* has been used as Korean medicine for various diseases. It has antioxidant, hypotensive, sedative, analgesic, and endocrine activities. Dammarane-type triterpenes from the plant have various beneficial effects.
Methods: A dammarane-type triterpene saponin was isolated from *P. ginseng* root through chromatography such as repeated column chromatography and medium pressure liquid chromatography.
Results and conclusion: New dammarane-type triterpene saponin was isolated for the first time from nature. The structure was elucidated as ginsenoside Rg12 (**1**) based on spectral data. There may be good materials from *P. ginseng* for the development of industrial applications such as nutraceutical, pharmaceutical, and cosmeceutical purposes.

1. Introduction

Panax ginseng (Araliaceae plant) has been used as Korean medicine for several years to treat various diseases [1,2]. Dried ginseng has been used as medicine because it has various pharmacological effects on the central nervous and cardiovascular systems. It is also used for treating diabetes, inflammation aging, fatigue, oxidative damage, mutagenicity, and cancer. Finally, it is used as an antioxidant, hypotensive, sedative, analgesic, and endocrine [3–14].

The majority of *P. ginseng* contains protopanaxadiols (PPDs) and protopanaxatriols (PPTs) as dammarane-type triterpene saponins [15]. The PPDs are ginsenosides-Rb1, -Rb2, -Rd, -Rc, and -Rg3 at the C-3 position sugar moieties, whereas the PPTs are ginsenosides-Rg1, -Re, and -Rg2 at the C-6 position [16].

There have been many recent reports on the conversion of major dammarane-type triterpene saponins to more active minor dammarane-type triterpene saponins, which are in small quantities in ginseng. Current studies demonstrate the beneficial effects of these ginsenosides in a wide range of pathological activities [16,17]. In our continued chemical investigation on *P. ginseng* and dammarane-type triterpene saponins, we isolated and identified phytochemicals from *P. ginseng* root. The compound is purified through repeated column chromatography (CC) and medium pressure liquid chromatography (MPLC).

2. Materials and methods

2.1. Plant materials

The plant of *P. ginseng* Meyer was obtained at Geumsan region, Korea in 2014. A voucher specimen (No. LEE 2011-03) of this plant was deposited at our department.

2.2. Apparatus and chemicals

n-Hexane, *n*-butanol (*n*-BuOH), ethyl acetate (EtOAc), chloroform ($CHCl_3$), ethanol (EtOH), and pyridine-d_5 (MA, USA) were obtained from SamChun Pure Chemical Co., Korea. Fast atom bombardment mass was conducted using a JEOL JMS-AX505WA

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(Jeol, Japan), mass spectrometer. A high-resolution LC/MS/MS analysis was done in a Xevo G2 Q-TOF LC/MS/MS system (Waters, USA) using an ACQUITY UPLC I Class system (Dionex). The ^{1}H- and ^{13}C-NMR spectra were checked with a Bruker Avance 500 NMR spectrophotometer (Bremen, Germany) with trimethylsilane (TMS), the internal standard. Thin-layer chromatography (TLC) was conducted on Kiesel gel 60 F_{254} (250-μm) silica gel plate (Art. 5715, Merck Co., Darmstadt, Germany), and visualized by a 10% H_2SO_4 spraying in a methanol (MeOH) solution. Accordingly, CC was performed with a LiChroprep RP-18 (40-63 μm, Merck Co.). An MPLC system (Biotage, Uppsala, Sweden), which was equipped with cartridges (KP-SIL, 39 mm × 225 mm), was used. The sugar determinations were conducted with an HP 5890 series II GC (Hewlett-Packard, Avondale, PA, USA) using an HP-5 capillary column (30 m × 0.32 mm i.d., 0.25-μm film thickness; Agilent, J&W Scientific, Folsom, CA, USA; injector temperature: 200°C; detector temperature: 200°C; column temperature: 230°C; and flow rate of He gas: 1 mL/min).

2.3. Extraction and isolation

The extraction of *P. ginseng* root (10.0 kg) was performed with EtOH (3 × 21 L) under reflux. The concentration of the combined extracts was proceeded to have a brown residue (139 g). And then, the residue melted in H_2O (7 L) was successively partitioned with *n*-hexane (3 × 7 L), $CHCl_3$ (3 × 7 L), EtOAc (3 × 7 L), and *n*-BuOH (3 × 7 L) to provide the *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH-soluble fractions. A portion of the *n*-BuOH extract (600 g) was subjected to MPLC for separation using $CHCl_3$/MeOH (gradient: 100:0 → 0:100). A total of 13 fractions were obtained by combining those with the same R_f value on the TLC pattern (1 → 13). Fraction 3 was separated on a LiChroprep RP18 column (φ 1.0 × 32 cm) using MeOH/H_2O (gradient: 1:3 → 1:0) to obtain 9 fractions (WGB 3.1–3.9). A portion of the combined fractions (WGB 3.8 and WGB 3.9) were separated on a LiChroprep RP18 column (φ 1.0 × 32 cm) using MeOH/H_2O (gradient: 1:2 → 1:0) to obtain 16 fractions (WGB 3.9.1–3.9.16) yielding Compound **1** (WGB 3.9.14).

2.4. Acidic hydrolysis of Compound 1

Compound **1** (10 mg) was heated under reflux with a 5% HCl in 60% aqueous dioxane (10 mL) mixture for 2 h. Under reduced pressure, the mixed solution was concentrated. The residue was then extracted with ether. The H_2O layer was neutralized with Ag_2CO_3. Subsequently, the remaining solid was removed by filtration. The residue from filtration and standard sugars were compared through cellulose TLC (C_5H_5N:EtOAc:HOAc:H_2O, 36:36:7:21). The sugars were elucidated as D-glucoside.

2.5. Absolute configuration of sugars in Compound 1

Compound **1** (10 mg) was tested as in the above method. The sugar mixture was melted in 0.1 mL C_5H_5N, and added to 0.1 mL C_5H_5N solution of 2 mg L-cysteine methyl ester hydrochloride followed by warming at 60°C for 1 h. The solvent was evaporated under N_2 gas. The residue was then dried *in vacuo* and was trimethylsilylated with TMS-HT (0.1 mL) at 60°C for 30 min. The *n*-hexane layer was separated and analyzed by GC after adding *n*-hexane and H_2O to the trimethylsilylated residue. The retention time (t_R) of the peak was 22.03 min as D-glucoside.

3. Results and discussion

The *n*-BuOH fraction was chromatographed by CC and MPLC to yield Compound **1** (Fig. 1).

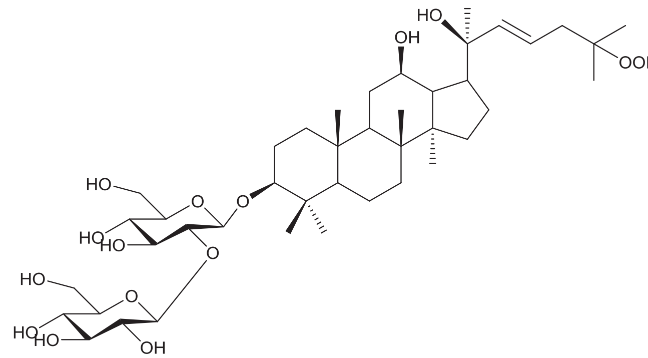

Fig. 1. Structure of Compound **1**.

Compound **1** was gained as a white powder that has a molecular ion peak at *m/z* 815 $[M]^-$ in the negative LC-MS. Compound **1** was corresponded to a molecular formula of $C_{42}H_{72}O_{15}$ in HRLC-MS [*m/z* 861.4843 $(M + HCOO)^-$]. The calculated value of **1** was *m/z* 861.4848. The ^{1}H-NMR spectrum indicated two olefinic (i.e., δ 6.16 and 6.25) and two anomeric (i.e., δ 4.92 and 5.33) proton signals.

Table 1
^{1}H- and ^{13}C-NMR spectral data for Compound **1** (C_5D_5N, 500 MHz)

No.	δ_H	δ_C	HMBC
1	1.55 (2H, m)	39.7	C-3,10,19
2	1.85 (2H, m)	25.9	C-1,3
3	3.27 (1H, dd, 12.0, 4.4)	89.5	C-1′,1,28,29
4	–	40.2	C-28, 29
5	0.77 (1H, m)	56.9	–
6	1.49, 1.36 (2H, m)	18.4	–
7	1.21 (1H, m)	35.6	C-8,14,18
8	–	39.7	C-7,18
9	1.36 (1H, m)	49.9	C-11
10	–	36.7	–
11	1.38 (1H, m)	31.2	C-9
12	3.94 (1H, m)	70.7	C-13
13	1.99 (1H, m)	51.9	C-12
14	–	50.7	C-7
15	1.03, 1.57 (2H, m)	31.3	–
16	1.38, 1.80 (2H, m)	26.3	–
17	2.57 (1H, m)	52.2	C-20
18	0.97 (3H, s)	17.1	C-7,8,14
19	0.83 (3H, s)	17.9	C-1,5,10
20	–	83.8	C-17
21	1.59 (3H, s)	25.8	C-17,20,22
22	6.0 (1H, d, 15.9)	127.1	C-20,21,24
23	6.25 (1H, dd, 15.9, 8.4)	137.9	C-24
24	2.22, 2.54 (2H, m)	39.8	C-20,23
25	–	81.9	–
26	1.62 (3H, s)	27.2	–
27	1.57 (3H, s)	18.9	–
28	1.30 (3H, s)	28.6	C-3,4,5,29
29	1.19 (3H, s)	16.5	C-3,4
30	0.97 (3H, s)	16.7	C-8,13,14,15
3-*O*-glc-1′	4.92 (1H, d, 7.5)	105.6	C-3
2′	4.15 (1H, t)	83.7	C-1″
3′	4.22 (1H, t)	77.6	–
4′	4.05 (1H, t)	72.2	–
5′	3.93 (1H, d)	78.6	–
6′	4.18 (1H, dd, 11.6, 3.2)	63.2	–
	4.36 (1H, dd, 11.6, 6.0)		
2′-*O*-glc-1″	5.13 (1H, d, 7.5)	106.5	C-2′
2″	4.02 (1H, t)	77.6	–
3″	4.14 (1H, t)	78.6	–
4″	4.17 (1H, t)	72.0	–
5″	4.14 (1H, t)	79.3	–
6″	4.42 (1H, dd, 11.6, 3.2)	64.2	–
	4.50 (1H, dd, 11.6, 6.0)		

HMBC, Heteronuclear Multiple Bond Correlation; delta C is ppm of carbon.
Chemical shifts are reported in parts per million (δ), and coupling constants (*J*) are expressed in Hertz.

The acidic hydrolysis of **1** gained D-glucose. The chemical shifts of the two anomeric carbons in the ^{13}C-NMR spectrum were recorded at δ 105.6 and 106.3 (Table 1). Accordingly, the signals of anomeric carbon showed two β-D-glucosyl moieties. The significant downfield shift of C-2′ at δ 79.8 in the inner β-D-glucosyl moiety at C-3 position of aglycone in the ^{13}C-NMR spectrum of C-2′ at δ 79.8 indicated the linkage of the terminal β-D-glucosyl moiety to the inner β-D-glucosyl moiety at C-3. The stark difference of the NMR data between the two isomers was the chemical shift values of C-20 and the stereogenic center in the side chain attached to the PPD scaffold and its adjacent carbons, namely, C-17, and 21. In the NMR spectrum of 20-hydroxy-dammarane derivatives, the C-17 and -21 chemical shift values of 20(*S*)-dammarane derivatives are ~52.2 ppm and ~25.8 ppm, respectively. From identification of the correlations between H-1′ (δ 4.92) and C-3 (δ 89.3) and H-1″ (δ 5.33) and C-2′ (δ 79.8) by the HMBC, it was suggested that monodesmosyl chain was linked to the aglycone C-3. Moreover, the correlations were detected between H-24 (i.e., δ 2.22 and 2.54) and C-22 and -23 (i.e., δ 127.0 and 138.7) and H-23 (i.e., δ 6.25) and C-25 (i.e., δ 81.9) by the HMBC [18–24].

Accordingly, Compound **1** is a 20(*S*)-protopanaxadiol 3-monodesmoside containing two β-D-glucoside moieties. Therefore, the structure of **1** was elucidated as ginsenoside Rg12. The isolation was for the first time from nature. This result will have valuable effects for the industrial development of ginsenosides from *P. ginseng* in diverse applications.

Conflicts of interest

The authors have no conflicts of interest to declare.

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