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.

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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, -Re, 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 (CHCl3), ethanol (EtOH), and pyridine-d5 (MA, USA) were obtained from SamChun Pure Chemical Co., Korea. Fast atom bombardment mass was conducted using a JEOL JMS-AX505WA mass spectrometer.
(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 1H- and 13C-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 F254 (250–μm silica gel plate (Art. 5715, Merck Co., Darmstadt, Germany), and visualized by a 10% H2SO4 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, &W Scientific, Folsom, CA, USA; injector temperature: 200°C; detector temperature: 220°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 H2O (7 L) was successively partitioned with n-hexane (3 × 7 L), CHCl3 (3 × 7 L), EtOAc (3 × 7 L), and n-BuOH (3 × 7 L) to provide the n-hexane, CHCl3, EtOAc, and n-BuOH-soluble fractions. A portion of the n-BuOH extract (600 g) was subjected to MPLC for separation using CHCl3/MeOH (gradient: 100:0 → 0:100). A total of 13 fractions were obtained by combining those with the same Rf value on the TLC pattern (1 → 13). Fraction 3 was separated on a LiChroprep RP18 column (φ 1.0 × 32 cm) using MeOH/H2O (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) was separated on a LiChroprep RP18 column (φ 1.0 × 32 cm) using MeOH/H2O (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 H2O layer was neutralized with Ag2CO3. Subsequently, the remaining solid was removed by filtration. The residue from filtration and standard sugars were compared through cellulose TLC (C5H5N, 500 MHz)

25. 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 C5D5N, and added to 0.1 mL C5D5N solution of 2 mg L-cysteine methyl ester hydrochloride followed by warming at 60°C for 1 h. The solvent was evaporated under N2 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 H2O to the trimethylsilylated residue. The retention time (tR) of the peak was 22.03 min as α-glucose.

3. Results and discussion

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

**Table 1**

| No. | δH | δC | HMBC |
|-----|----|----|------|
| 1   | 1.55 (2H, m) | 39.7 | C-10,19 |
| 2   | 1.85 (2H, m) | 25.9 | C-13 |
| 3   | 3.27 (1H, dd, 12.0, 4.4) | 89.5 | C-1,12,28,29 |
| 4   | 4.02 | 40.2 | C-28,29 |
| 5   | 0.77 (1H, m) | 56.9 | — |
| 6   | 1.49,1.36 (2H, m) | 36.7 | — |
| 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-7,18,14 |
| 19  | 0.83 (3H, s) | 17.9 | C-15,10 |
| 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) | 272 | — |
| 27  | 1.57 (3H, s) | 189 | — |
| 28  | 1.30 (3H, s) | 286 | C-3,4,29 |
| 29  | 1.19 (3H, s) | 163.5 | C-3,4 |
| 30  | 0.97 (3H, s) | 16.7 | C-8,13,14,15 |
| 3-O-gluc-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, d, 11.6, 6.0) | — |
| 2'-O-gluc-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, d, 11.6, 6.0) | — |

HMBC, Heteronuclear Multiple Bond Correlation; δC 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 $\alpha$-glucose. The chemical shifts of the two anomic carbons in the $^{13}$C-NMR spectrum were recorded at $\delta$ 105.6 and 106.3 (Table 1). Accordingly, the signals of anomic carbon showed two $\beta$-$\alpha$-glucosyl moieties. The significant downfield shift of C-2’ at $\delta$ 79.8 in the inner $\beta$-$\alpha$-glucosyl moiety at C-3 position of aglycone in the $^{13}$C-NMR spectrum of C-2’ at $\delta$ 79.8 indicated the linkage of the terminal $\beta$-$\alpha$-glucosyl moiety to the inner $\beta$-$\alpha$-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( i.e., 5.33) and C-2 (w 25.8 ppm, respectively. From identifi cation of the correlations between H-1 (i.e., $\delta$ 2.22 and 2.54) and C-22 and -23 (i.e., $\delta$ 127.0 and 138.7) and H-23 (i.e., $\delta$ 6.25) and C-25 (i.e., $\delta$ 81.9) by the HMBC [18–24]. Accordingly, Compound 1 is a 20($\beta$)-protopanaxadiol 3-monodesmosyl containing two $\beta$-$\alpha$-glucosyl moieties. Therefore, the structure of 1 was elucidated as ginsenoside Rg12. The isolation was for the fi rst 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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References

[1] Chau CF, Wu SH. The development of regulations of Chinese herbal medicines for both medicinal and food uses. Trends Food Sci Technol 2006;17:313–23.
[2] Coates PM, Blackman MR, Cragg GM, Levine M, Moss J, White JD. Encyclopedia of dietary supplements. New York: Marcel Dekker; 2005.
[3] Kim SH, Park KS. Effects of Panax ginseng extract on lipid metabolism in humans. Pharmacol Res 2003;48:511–3.
[4] Sun BS, Gu LJ, Fang ZM, Wang CY, Wang Z, Lee MR, Li Z, Li JJ, Sung CK. Simultaneous quantifi cation of 19 ginsenosides in black ginseng developed from Panax ginseng by HPLC-ELSD. J Pharm Biomed Anal 2009;50:15–22.
[5] Chen CF, Chiou WF, Zhang JT. Comparison of the pharmacological effects of Panax ginseng and Panax quinquefolium. Acta Pharm Sin 2008;29:1103–8.
[6] Yue PY, Mak NK, Cheng YK, Leung KW, Ng TB, Fan DT, Yeung HW, Wong RN. Pharmacogenomics and the Yin/Yang actions of ginseng: antitumor, angiogenesis, and steroid-like activities of ginsenoside. Chin Med 2007;2:1–21.
[7] Hofseth LJ, Wargovich MJ. Inflammation, cancer, and targets of Ginseng. J Nutr 2007;137:183–5.
[8] Jung CH, Seog HM, Choi IW, Cho HY. Antioxidant activities of cultivated and wild Korean ginseng leaves. Food Chem 2005;92:535–40.
[9] Rai D, Bhatia G, Sen T, Palit GJ. Antistress effects of Ginkgo biloba and Panax ginseng: a comparative study. Pharmacol Sci 2003;93:458–64.
[10] Sun JY, Na HK, Lee JT, Keum YS. Molecular mechanisms underlying antitumor promoting activities of heat-processed Panax ginseng C. Korean Med Sci 2001;16:38–41.
[11] Choi S. Epidermis proliferative effect of the Panax ginseng ginsenoside Rb2. Arch Pharm Res 2002;25:71–6.
[12] Chang LP, Whitaker DC. The impact of herbal medicines on dermatologic surgery. Dermatol Surg 2001;27:579–63.
[13] Keum YS, Park KK, Lee JM, Chun KS, Park JH, Lee SK, Kwon H, Suh YJ. Antioxidant and antitumor promoting activities of the methanol extract of heat-processed ginseng. Cancer Lett 2000;150:41–8.
[14] Attelle AS, Wu JA, Yuan C. Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pharmacol 1999;58:1685–93.
[15] Hong HD, Choi SY, Kim YC, Lee YC, Cho CW. Rapid determination of ginsenosides Rb 1, Rf, and Rg 1 in Korean ginseng using HPLC. J Ginseng Res 2009;33:8–12.
[16] Sun J, Hu S, Song X. Adjuvant effects of protopanaxadiol and protopanaxatriol saponins from ginseng roots on the immune responses to ovalbumin in mice. Vaccine 2007;25:1114–20.
[17] Wang JR, Yamasaki Y, Tanaka T, Kouno I, Jiang ZH. Dammarane-type triterpene saponins from the flowers of Panax notoginseng. Molecules 2009;14:2087–94.
[18] Kim DS, Chang YJ, Zedk U, Zhao P, Liu YQ, Yang CR. Dammarane-type saponins from Panax ginseng. Phytochemistry 1995;40:1493–7.
[19] Ping Z, Liu YQ, Yang CR. Minor dammarane-type saponins from Panax notoginseng. Phytochemistry 1996;41:1415–22.
[20] Baek SH, Bae ON, Park JH. Recent methodology in ginseng analysis. J Ginseng Res 2012;36:119–34.
[21] Cho JC, Lee MK, Lee JW, Park HJ, Lee DY, Lee YH, Yang DC, Baek NI. Physicochemical characterization and NMR assignments of ginsenosides Rb1, Rb2, Rc, and Rd isolated from Panax ginseng. J Ginseng Res 2010;34:113–21.
[22] Yang CM, Seo DS, Hong SH, Kim CH, Lee KR. Ginsenosides from the roots of Korean cultivated-wild ginseng. Nat Prod Sci 2008;14:171–6.
[23] Ahmad VU, Bashia A. Spectroscopic data of saponins: the triterpenoid glycosides. Florida: CRC Press; 2000. p. 664–717.
[24] Wang DQ, Feng BS, Wang XB, Yang CR, Zhou J. Further study on dammarane saponins of leaves of Panax japonicus var. major collected in Quinling Mountains China. Yao Xue Xue Bao 1989;24:633–8.