

Ginsenoside Rf₂, a New Dammarane Glycoside from Korean Red Ginseng (*Panax ginseng*)

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A new dammarane glycoside named ginsenoside Rf₂ has been isolated from Korean red ginseng (*Panax ginseng*) and its chemical structure has been elucidated as 6-O-[α -L-rhamnopyranosyl (1 \rightarrow 2) β -D-glucopyranosyl]dammarane-3 β , 6 α , 12 β , 20(R), 25-pentol by chemical and spectral methods.

Key words : *Panax ginseng*, Araliaceae, Red ginseng, Ginsenoside Rf₂

INTRODUCTION

The root of ginseng has been traditionally used as a precious medicine in oriental countries, such as Korea, China and Japan for more than 5,000 years. The root of *Panax ginseng* (Araliaceae) is steamed and dried to prepare red ginseng, while the peeled roots dried without steaming are designated as white ginseng. The commercially available ginseng roots are classified into two forms, red and white ginseng. Ginseng saponins isolated from the root have been reported to be main effective ingredients responsible for the pharmacological and biological activities. It was reported that all of the saponins found in white ginseng were isolated in similar yields from red ginseng (Kasai *et al.*, 1983), while 20(R)-ginsenoside Rg₂, 20(S)-ginsenoside Rg₃, 20(R)-ginsenoside Rh₁ and ginsenoside Rh₂ were saponins found only in red ginseng (Kitagawa *et al.*, 1983). In addition, two minor saponins were also isolated only from red ginseng, two being designated as ginsenosides Rs₁ and Rs₂ (Kasai *et al.*, 1983). Both of these had an acetoxyl group and afforded ginsenosides Rb₂ and Rc on alkaline saponification, respectively. Since ginsenoside Rh₂, found in minor amount only in red ginseng, has been recently reported to show potent cytotoxicities against several cancer cells such as Lewis lung, Morris hepatoma, B16 and HeLa cells (Kitagawa *et al.*, 1983; Kitagawa, 1984), differentiation activity in F9 teratocarcinoma stem cell (Lee *et al.*, 1995) and also, 20(S)-ginsenoside Rg₃, reversal effects of multi-drug resistance (Park *et al.*, 1996), many researcher focussed on the minor ginsenosides of red ginseng, regar-

ding them as biologically active compounds. These results led us to isolate three kinds of new minor ginsenosides from Korean red ginseng. We have previously reported several new dammarane glycosides, ginsenosides Rg₅ (Kim *et al.*, 1996), Rh₄ (Baek *et al.*, 1996) and Rs₃ (Baek *et al.*, 1997) and also 20(E)-ginsenoside F₄ (Ryu *et al.*, 1996) has been recently isolated from Korean red ginseng. In a continuation of our chemical studies on the plant, we have further isolated a new dammarane glycoside and named ginsenoside Rf₂.

MATERIALS AND METHODS

Materials

Red ginseng used, prepared from six year old fresh ginseng (*Panax ginseng* C. A. Meyer), was provided by Korea Tobacco and Ginseng Corporation.

Instruments

Melting point were determined on a Fisher-John Apparatus and are uncorrected; ¹H-(400 MHz) and ¹³C-NMR (100 MHz) were recorded on a Bruker AMX 400 spectrometer. Chemical shift values are expressed as ppm downfield from tetramethylsilane used as an internal standard. FAB Mass spectra were measured with a VG-VSEQ (EBqQ type/VG Analytical) spectrometer. Optical rotation were measured with a JASCO DIP-370 automatic polarimeter. Elemental analysis were taken with a Perkin-Elmer Model 240C instrument. IR spectra were obtained using a Perkin-Elmer Model 599B spectrometer. For column chromatography, Kiesel gel 60 (230~400 mesh, Merck) were used and for TLC, silica gel 60F-254 (Merck). HPLC was carried out with a Shimadzu RID-6A detector, Shimadzu LC-10AD pump and Shimadzu SCL system controller.

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Extraction and isolation

The powder of red ginseng (0.5 kg) was exhaustively extracted with methanol (2 l) at room temperature overnight and the solvent was evaporated off under reduced pressure to give the MeOH extract (108 g). The MeOH extract was partitioned into Ether and *n*-BuOH, successively. The *n*-BuOH soluble phase was taken and concentrated under reduced pressure to give the *n*-BuOH soluble fraction (14.2 g). The *n*-BuOH soluble fraction (14.0 g) was chromatographed by silica gel column chromatography with CHCl₃/MeOH/H₂O (10:3:1, lower phase) to afford eight fractions. The fourth fraction (0.81 g) was repeatedly separated by silica gel column chromatography with *n*-BuOH/H₂O/EtOAc (15:1:4, upper phase) to give six fractions, among which fourth fraction was further subjected to Rp-18 reverse column chromatography using MeOH/H₂O (6:4), followed by semi-preparative HPLC (Maxil C₁₈ 250×10 mm, CH₃CN/H₂O=4:6) to furnish compound **1** (8.3 mg). Compound **1**: white powder (MeOH-H₂O); m.p. 182~184°C, [α]_D: -12.5° (c 0.1, MeOH); IR (KBr): ν_{max}=3410, 2950, 1460, 1380, 1060 cm⁻¹; pos. FAB-MS: m/z=804 (M+1)⁺; Anal. Calcd. for C₄₂H₇₄O₁₄·H₂O: C, 61.44 H, 9.32; Found: C, 61.36 H 9.40; ¹H-NMR (400 MHz, d₅-py): δ=5.24 (1H, d, *J*=7.2 Hz, H-1"), 4.92 (1H, d, *J*=7.8 Hz, H-1'), 4.71 (1H, m, H-6), 3.98 (1H, m, H-12), 3.49 (1H, m, H-3), 2.06 (3H, s, CH₃-29), 1.68 (3H, s, CH₃-21), 1.62, 1.60 (each 3H, each s, CH₃-26, 27), 1.36 (3H, d, *J*=4.2 Hz, CH₃-6"), 1.30 (3H, s, CH₃-30), 1.24 (3H, s, CH₃-28), 0.98 (3H, s, CH₃-18), 0.92 (3H, s, CH₃-19); ¹³C-NMR: see Table I.

Methanolysis of compound 1

Compound **1** (4 mg) in 9% HCl-MeOH (1 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag₂CO₃ powder and the precipitate was removed by filtration. A part of the solvent was identified as methyl-L-rhamnopyranoside and methyl-D-glucopyranoside by TLC comparison with authentic samples. The remaining solvent was evaporated off under reduced pressure from the filtrate to give a product (1.8 mg). Purification of the product by silica gel column chromatography (Benzene-Acetone=5:1) to afford the aglycone of **1** (**3**, 1.1 mg), white powder; ¹H-NMR (400 MHz, d₅-py): δ=4.42 (1H, ddd, *J*=4.2, 10.4, 10.4 Hz, H-6), 3.93 (1H, ddd, *J*=2.4, 9.8, 9.8 Hz, H-6m, H-12), 3.52 (1H, dd, *J*=4.2, 8.8 Hz, H-3), 2.02 (3H, s, CH₃-29), 1.70 (3H, s, CH₃-21), 1.60 (3H, s, CH₃-26), 1.58 (3H, s, CH₃-27), 1.28 (3H, s, CH₃-30), 1.20 (3H, s, CH₃-28), 0.96 (3H, s, CH₃-18), 0.90 (3H, s, CH₃-19).

RESULTS AND DISCUSSION

The *n*-BuOH soluble fraction obtained from red gin-

seng was subjected to silica gel column chromatography, followed by reverse phase column chromatography and HPLC to afford compound **1**. Compound **1**, mp 182~184°C, [α]_D -12.5°, showed a positive fast atom bombardment (FAB) ion at *m/z* 804 for (M+H)⁺, indicating its molecular weight to be 803, 18 mass units larger than that of ginsenoside Rg₂ and absorption bands due to hydroxyl (3410 cm⁻¹) group and glycosidic bond (1060 cm⁻¹) in the IR spectrum. As shown in Table I, the ¹³C-NMR spectrum of **1** was quite similar to that of 20(R)-ginsenoside Rg₂ (**2**) except for the chemical shifts of C-23 (-4.2 ppm), C-26 (+4.1 ppm) and C-27 (+13.3 ppm) and also a comparative investigation with that of **2** revealed that signals due to the C-24 and C-25 were shifted upfield from 126.3 and 130.7 ppm in **2** to 46.1 and 69.4 ppm in **1**, respectively (**4**).

The ¹H-NMR spectrum of **1** exhibited a similar signal pattern to that of **2** except for the disappearance of signals due to a double bond of side chain. These findings suggested that the chemical structure of **1** may possess a hydroxyl group in place of a double bond of C-24. This was further supported by the facts that the proton signals at 1.83 ppm (H-23), 1.78 ppm (H-24), 1.62 ppm (CH₃-26) and 1.60 ppm (CH₃-27) showed long-range couplings with the carbon signal (C-25) at 69.4 ppm in the HMBC spectrum of **1**. The ¹H-NMR spectrum of **1** showed the presence of two anomeric proton signals at 4.92 (1H, d, *J*=7.8 Hz, H-1') and 5.24 (1H, d, *J*=7.2 Hz, H-1"), which were found to correspond to the anomeric carbon signals at 101.7 and 101.9 ppm in the ¹³C-NMR spectrum of **1**, respectively. On methanolysis with 9% HCl-MeOH, **1** liberated the aglycone **3**, dammarane-3β, 6α, 12β, 20(R), 25-pentol and two methyl glycoside, which were identified as methyl-D-glucopyranoside and methyl-L-rhamnopyranoside by comparison of TLC. In the ¹H-NMR spectrum of **3**, glycosidation shifts of the proton signal was observed for H-6 (-0.29 ppm) as compared with that of **1**, so that sugar moiety was linked to the C-6 hydroxyl group of **1**. The configuration of C-20 has been determined to be 20(R) form from the fact

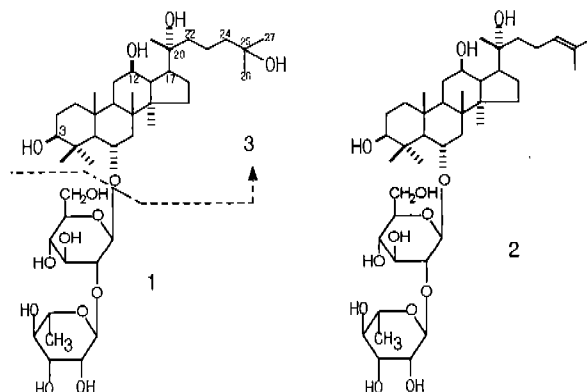


Table I. ¹³C NMR data for compounds 1 and 2³ (pyridine-d₅, 100 MHz)

Carbon	1	2	Carbon	1	2
1	39.4	39.3	23	18.7	22.9
2	27.8	27.6	24	46.1	126.3
3	79.4	79.4	25	69.4	130.7
4	40.0	39.9	26	29.8	25.7
5	60.9	60.8	27	30.4	17.1
6	78.4	78.5	28	31.7	32.1
7	46.1	46.0	29	16.8	16.9
8	41.2	41.1	30	17.4	17.1
9	50.2	50.1	Glc		
10	39.8	39.6	1'	101.9	101.9
11	32.6	32.2	2'	79.4	79.5
12	71.1	71.0	3'	78.4	78.3
13	50.2	49.3	4'	72.5	72.3
14	51.7	51.7	5'	78.4	78.4
15	31.2	31.3	6'	63.2	63.1
16	27.2	26.8	Rham		
17	51.7	51.0	1"	101.8	101.7
18	17.6	17.6	2"	72.6	72.6
19	17.6	17.6	3"	72.4	72.4
20	72.8	72.9	4"	74.2	74.3
21	22.5	22.9	5"	69.4	69.4
22	43.6	43.4	6"	18.8	18.8

^aAssignments were made by comparison with literature data (Kitagawa *et al.*, 1983; Han *et al.*, 1982) and DEPT, HMQC, HMBC spectra.

that chemical shifts characteristic of 20(R) form were observed in the ¹³C-NMR spectrum of 1 for C-17 (51.7 ppm) and C-22 (43.6 ppm) signals as compared with those of 20(R)-ginsenoside Rg₂ (Kitagawa *et al.*, 1983). From the above results, compound 1 was characterized as 6-O-[α-L-rhamnopyranosyl (1→2)β-D-glucopyranosyl]dammarane-3β, 6α, 12β, 20(R), 25-pentol and named ginsenoside Rf₂. It should be, interestingly, noted that on mild acidic hydrolysis with 0.1 N-HCl at 37°C, ginsenoside Re gave a decomposition product, which was proved to be a C-20 (R&S) epimeric mixture of compound 1, Re-prosapogenin II (Han *et al.*, 1982). This compound contained only in red ginseng may possess some biological and pharmacological activities. So, several biological investigations on this compound are now in progress.

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