Four New Dammarane-Glycosides, Ginsenosides Rg_5, Rh_4, Rs_3, and Rf_2, from Korean Red Ginseng, the Root of *Panax ginseng* C. A. Meyer

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ABSTRACT

Four new dammarnae-glycosides named ginsenosides Rg_5, Rh_4, Rs_3 and Rf_2 have been isolated from Korean red ginseng, the root of *Panax ginseng* C. A. Meyer (Araliaceae) and their chemical structures have been elucidated by chemical and spectroscopic methods, including ^{1}H-^{1}H COSY, HMQC, HMBC, NOESY, as 3-O-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl] dammar-20(22), 24-diene-3β, 12β-diol (ginsenoside Rg_5), 6-O-β-D-glucopyranosyl-dammar-20(22), 24-diene-3β, 6β, 12β-triol (ginsenoside Rh_4), 3-O-[6″-O-acetyl-D-glucopyranosyl(1→2)- -D-glucopyranosyl] 20(S)-protopanaxadiol (ginsenoside Rs_3) and 6-O-[α-L-rhamno-pyranosyl(1→2)-β-D-glucopyranosyl] dammarane -3β, 6α, 12β, 20(R), 25-pentol(ginsenoside Rf_2). The absolute stereostructure of a double bond at C-20(22) was determined as entgegen type by applying NOESY.

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[1], while 20(R)-ginsenoside Rg_2, 20(S)-ginsenoside Rg_3, 20(R)-ginsenoside Rh_1 and ginsenoside Rh_2 were saponins found only in red ginseng[2]. In addition, two minor saponins were also isolated only from red ginseng, two being designated as ginsenosides Rs_1 and Rs_2[1]. Both of these had an acetoxyl group and afforded ginsenosides Rb_2 and Rc on alkaline saponification, respectively. Since ginsenoside Rh_2, 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[2,3], differentia-

tion activity in F9 teratocarcinoma stem cell[4] and also, 20(S)-ginsenoside Rg_3, reversal effects of multidrug resistance[5], many researcher focussed on the minor ginsenosides of red ginseng, regarding them as biologically active compounds. Recently, 20(E)-ginsenoside F_4 has been isolated and elucidated from red ginseng by Ryu *et al.*[6] These rsults led us to isolate further new minor ginsenosides from Korean red ginseng. We deal herein with the isolation and structure elucidation of ginsenosides Rg_5(**1**), Rh_4(**2**), Rs_3(**3**) and Rf_2(**5**) from Korean red ginseng.

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; ^{1}H-(400MHz) and ^{13}C-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.

Extraction and Isolation

The powder of red ginseng (1 kg) was extracted with methanol (2.5 l x 3) at room temperature overnight. The methanol extract (250 g) was partitioned between water (700 ml) and *n*-BuOH (500 ml x 2). The *n*-BuOH phase was taken and evaporated under vacuum to yield the *n*-BuOH extract (114 g). The extract was applied to a silica gel (350 g) column eluting with CHC_{13}-MeOH-H_2O (20:3:1 → 15:3:1→ 10:3:1) to afford eight fractions. The each fraction was rechromatographed with silica gel column using *n*-BuOH-EtOAc (1:3) and *n*-BuOH-EtOAc-H_2O (15:1:4) as eluent to yield compounds **1** (250 mg), **2** (860 mg), **3** (45 mg) and **5** (16 mg). They were further purified by Rp-18 reversecolumn chromatography using MeOH/H_2O (6 : 4), followed by semi-preparative HPLC (Maxil C_{18} 250 × 10 mm, CH_3CN/H_2O = 4 : 6).

Compound **1** : white powder (MeOH), m.p; 188-192℃, $[\alpha]_D$: +4.7 (C, 1.2, MeOH), IR (KBr) V_{max} ; 3460, 3124, 2985, 1646 cm^{-1}; pos. FAB-MS : *m/z* = 767 $(M+1)^+$, Anal. Calcd. for $C_{42}H_{70}O_{12}$: C,

65.75 H, 9.20 ; Found : C, 65.61 H, 9.31, ^{1}H-NMR (400 MHz, d_5-Py) δ ; 5.49 (1H, t, J = 7.0 Hz, H-22), 5.33 (1H, d, J = 7.6 Hz, H-1″), 5.21 (1H, t, J = 6.9 Hz, H-24), 4.90 (1H, d, J = 7.4 Hz, H-1′), 4.21 (1H, dd-like, H-2″), 4.10 (1H, dd-like, H-2″), 3.90 (1H, br. s, H-12), 3.26 (1H, dd, J = 4.0, 11.6 Hz, H-3), 2.77 (2H, dd, J = 7.0, 6.9 Hz, H-23), 1.81 (3H, s, H-21), 1.62 (3H, s, H-26), 1.58 (3H, s, H-27), 1.27 (3H, s, H-28), 1.09 (3H, s, H-18), 1.01 (3H, s, H-19), 0.95 (3H, s, H-30), 0.81 (3H, s, H-29); [400 MHz, CD_3OD + CDC_{13} (10:1)] : δ= 5.30 (1H, t, J = 6.9 Hz, H-22), 5.07 (1H, t, J = 6.9 Hz, H-24), 4.67 (1H, d, J = 7.7 Hz, H-1′), 4.43 (1H, d, J = 6.6 Hz, H-1′), 3.25 (1H, br. s, H-12), 3.17 (1H, dd-like, H-3), 2.67 (2H, dd, J = 6.9, 6.9 Hz, H-23), 1.67 (3H, s, H-21), 1.64 (3H, s, H-26), 1.62 (3H, s, H-27), 1.07 (3H, s, H-28), 1.04 (3H, s, H-18), 0.92 (3H, s, H-19), 0.91 (3H, s, H-30), 0.86 (3H, s, H-29). ^{13}C-NMR: see Table 1.

Acetylation of ***1***

In the pyridine (2 ml) solution of compound **1** (10 mg), acetic anhydride (2 ml) was added dropwise in iced water bath. After stirring for 15 h at room temperature, the reaction mixture was worked up in a usual manner to give a product, which was purified by silica gel column chromatography eluting with hexane-acetone (1:1) to yield heptaacetate of **1** (**1a**). Compound **1a**: white powder (benzene-methanol), ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 5.07 (1H, t-like, H-22), 4.91 (1H, t-like, H-24), 4.70 (1H, d, J = 8.0 Hz, H-1″), 4.45 (1H, d, J = 7.6 Hz, H-1′), 3.80 (1H, dd, J = 8.5, 7.6 Hz, H-2″), 3.68 (1H, br. s, H-12), 3.08 (1H, dd, J = 4.0, 11.4 Hz, H-3), 2.66 (2H, dd-like, H-23), 2.10, 2.07 (x2), 2.022, 2.016, 2.01, 1.99 (each 3H, all s, acetyl-methyl x 7), 1.67 (3H, s, H-21), 1.61 (3H, s, H-26), 1.57 (3H, s, H-27), 1.23 (3H, s, H-28), 1.03 (3H, s, H-18), 1.03 (3H, s, H-19), 0.87 (3H, s, H-30), 0.80 (3H, s, H-29).

Compound ***2*** : colorless fine crystals (MeOH-H_2O); m.p. 160-161℃, $[\alpha]_D$ + 28.2℃ (1.0, MeOH); IR KBr, Vmax cm^{-1} : 3450, 3121, 2984, 1645; pos. FAB MS *(m/z)* : 621 $(M+1)^+$; Anal. Calcd. for $C_{36}H60O_8$: C, 69.64 H, 9.74; Found : C, 69.58 H 9.80; ^{1}H-NMR (400 MHz, d_5-Py., δ) : 5.35 (1H, br. t, J = 7.4, H-22), 5.01 (1H, br. t, J = 7.4, H-24), 4.91 (1H, d, J = 7.7, H-1′), 4.26 (1H, m, H-12), 4.23 (1H, m, H-6), 3.97 (1H, dd, J = 8.0, 8.0, H-2′), 3.42 (1H, dd, J = 4.0, 10.8, H-3), 2.66 (2H, br. dd, J = 7.4, 7.4, H-23), 1.96 (3H, s, CH_3-29), 1.70 (3H, s, CH_3-21), 1.58, 1.49 (each 3H, each s, CH_3-26, -27), 1.46 (3H, s, CH3-30), 1.11 (3H, s, CH_3-28), 0.93 (3H, s, CH_3-18), 0.71 (3H, s, CH_3-19); ^{13}C-NMR : see Table 1.

Acid hydrolysis of ***2***

Compound **2** (20 mg) was dissolved in 9% HCl/MeOH and refluxed for 2 hrs. The reaction mixture was neutralized with Ag_2CO_3, filtered and evaporated *in vacuo*. A portion of the residue was used for identification of sugar and the other portion was purified with silica gel column chromatography eluting with hexane-ethylacetate (2:1) to afford the aglycone of **2** (**2b**, 6.3 mg). **2b** : white

powder (MeOH-H_2O); m.p. 131-132℃, $[\alpha]_D$ +40.0° (c 0.2, MeOH), IR KBr, max, cm^{-1} : 3634, 3119, 2980, 1642 ; EI/MS *(m/z)* : 458 (M^+), HR EI/MS : 458.3755 (Anal. Calcd. for $C_{30}H_{50}O_3$: 458.3762); ^{1}H-NMR (400 MHz, d_5-Py, δ) : 5.39 (^{1}H, br. t, *J* = 7.1, H-22), 5.19 (^{1}H, br. t, *J* = 7.1, H-24), 4.33 (^{1}H, m, H-12), 3.86 (^{1}H, m, H-6), 3.44 (^{1}H, dd, *J* = 5.0, 11.3, H-3), 2.68 (2H, dd, *J* = 7.1, 7.1, H-23), 1.92 (3H, s, CH_3-29), 1.73 (3H, s, CH_3-21), 1.52, 1.48 (each 3H, each s, CH_3-26, -27), 1.36 (3H, s, CH_3-30), 1.06 (3H, s, CH3-28), 0.92 (3H, s, CH_3-18), 0.87 (3H, s, CH_3-19); ^{13}C-NMR : see Table 1.

Compound **3** : white powder (MeOH-EtOH), $[\alpha]_D$ +17.9° (c=0.8, MeOH), IR (KBr) V_{max} : 3465, 3150, 2990, 1725, 1640 cm^{-1}; pos. FAB-MS : *m/z*=849 $(M+Na)^+$, 621, 605; Anal. Calcd. for $C_{44}H_{74}O_{14}$: C, 63.90, H, 9.02; Found : C, 63.99, H, 8.97; ^{1}H-NMR (400 MHz, d_5-Py.) 5.33 (1H, d, J=7.7, H-1"), 5.31 (1H, t, J=7.0, H-24), 4.96 (1H, dd, *J*=4.5, 11.7, H-6"$_a$), 4.92 (1H, d, *J*=7.6, H-1'), 4.80 (1H, dd, *J*=4.9, 11.7, H-6"$_b$), 4.02 (1H, ddd, *J*=4.5, 4.9, 9.5, H-5"), 3.94 (1H, m, H-12), 3.27 (1H, dd, *J*=4.5, 11.6, H-3), 2.04 (3H, s, acetyl-methyl), 1.64 (3H, s, H-26), 1.61 (3H, s, H-27), 1.42 (3H, s, H-21), 1.34 (3H, s, H-28), 1.12 (3H, s, H-19), 0.96 (3H, s, H-30), 0.95 (3H, s, H-18), 0.81 (3H, s, H-29). ^{13}C-NMR : see Table 1.

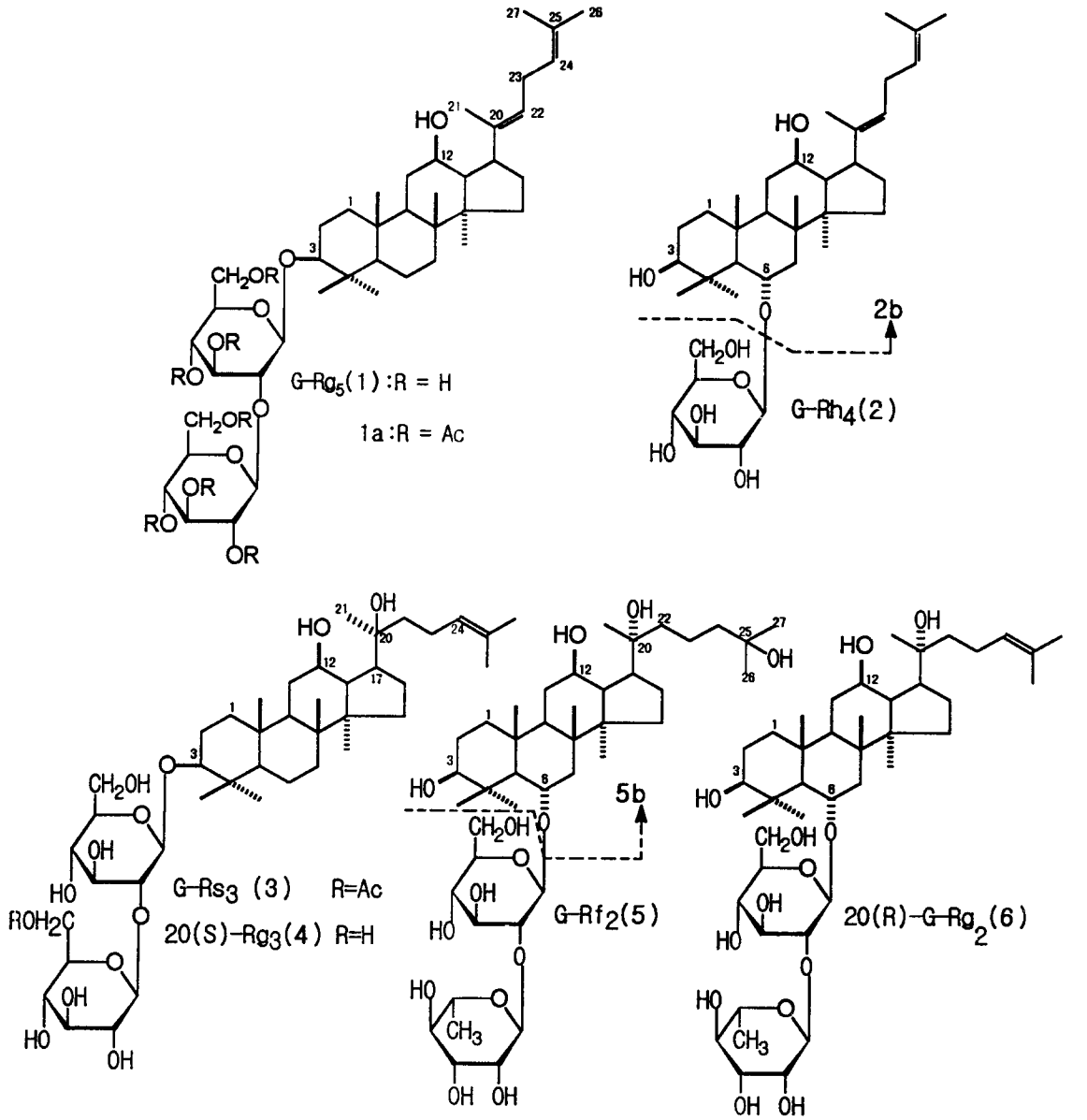
Compound **5**: white powder (MeOH-H_2O); m.p. 182-184 °C, $[\alpha]_D$: -12.5° (c 0.1, MeOH) ; IR(KBr): V_{max} = 3410, 2950, 1460, 1380, 1060 cm^{-1} ; pos. FAB-MS: *m/z* = 804 (M+1)$^+$; Anal. Calcd. for $C_{42}H_{74}O_{14}$ · H_2O : C, 61.44 H, 9.32 ; Found: C, 61.36 H 9.40 ; 1H-NMR (400 MHz, d_5-py) 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, CH_3-21), 1.62, 1.60 (each 3H, each s, CH_3-26, 27), 1.36 (3H, d, *J*=4.2 Hz, CH_3-6″), 1.30 (3H, s, CH_3-30), 1.24 (3H, s, CH_3-28), 0.98 (3H, s, CH_3-18), 0.92 (3H, s, CH_3-19); ^{13}C-NMR : see Table 1.

Methanolysis of ***5***

Compound **5** (4 mg) in 9% HCl-MeOH (1 ml) was heated under reflux for 2h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 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 **5** (**5b**, 1.1 mg). white powder; ^{1}H-NMR(400 MHz, d_5-py) : δ= 4.42 (1H, ddd, *J*=4.2, 10.4, 10.4 Hz, H-6), 3.93(^{1}H, 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, CH_3-29), 1.70(3H, s, CH_3-21), 1.60(3H, s, CH_3-26), 1.58(3H, s, CH_3-27), 1.28(3H, s, CH_3-30), 1.20(3H, s, CH_3-28), 0.96(3H, s, CH_3-18), 0.90(3H, s, CH_3-19).

Results and Discussion

Compound **1** was supposed to contain double bond (1646 cm^{-1}) and hydroxy group (3460 cm^{-1}) in the IR spectrum. In the ^{1}H-NMR spectrum of **1**, two olefinic [δ 5.49 (1H, t, J=7.0 Hz), 5.21(1H, t, J=6.9 Hz)], two anomeric [δ 5.33 (1H, t, J=7.6 Hz), 4.90 (1H, d, J=7.4 Hz)], eight singlet methyl [δ 1.81, 1.62, 1.58, 1.27, 1.09, 1.01, 0.95, 0.81 (each 3H, all, s)], and lots of oxy-methine (δ 3.88 - 4.45) proton signals were obserbed. All these data suggested that compound **1** be a triterpenoidal dammarane diglucoside with double bonds. In the ^{13}C-NMR spectrum, the chemical shifts of two anomeric (δ105.00, 105.91) and other sugar moiety signals (δ 83.3, 78.1, 71.5, 77.8, 62.6, 77.0, 78.2, 71.5, 78.0, 62.7) revealed compound **1** to have a sophorosyl [β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl] moiety in the molecule. The ^{13}C-NMR spectral data of **1** was also quite similar to that of ginsenoside Rg_3**(4)** except for the chemical shifts of four olefinic carbon signals, indicating it to be dammarane glycoside. And two quaternary carbon signals were obserbed at 131.6 and 140.6 ppm. Two olefinic protons and two quaternary carbons were from two double bonds and this was confirmed from the ^{13}C-NMR data using CD_3OD-$CDCl_3$ (10 :1). They showed olefinic two quaternary (δ 132.4, 140.8) and two methine (124.2, 125.0) carbon signals. Four olefinic carbon signals (δ 122.9, 123.4, 130.9, 137.3) observed in the ^{13}C-NMR spectrum of the acetate compound**(1a)** and two cross peaks observed in the HMQC of **1** due to two olefinic protons and methine carbon signals (δ 123.2, 123.5) confirmed the presence of two double bonds in the molecule of **1**. Additionally, the molecular weight, which was determined to be 766 from positive ion FAB MS [m/z 767$(M+1)^+$], and other NMR data led compound **1** to be a sophorosyl glycoside of a dammarane triterpenoid with oxymethine carbons and two double bonds. The position of the two double bonds of compound **1** was determined to be C-20(22) and C-24(25) in the dammarane skeleton from the fact that two olefinic proton signals were coupled with a common methylene proton signal(H-23) obserbed at 2.77 ppm in the ^{1}H-^{1}H COSY of **1.** In addition, an olefinic proton signal at δ 5.49 (H-22) and another one at δ 5.21 (H-24) were coupled through long-range *(J_4)* with a singlet methyl signal (δ 1.81, H-21) and two singlet methyl signals (δ 1.58, 1.62, H-26, 27), respectively. In the ^{13}C-NMR of **1**, C-3 (δ 88.82) showed downfield shifts relative to other 3-oxy dammarane triterpenoid by about 10 ppm. Accordingly, the linkage position of D-sophorose in compound **1** was determined to be C-3 hydroxy group. And the configuration of the upper anomeric carbon (C-1′) was established to be β on the basis of the coupling constant (J = 7.4Hz) of the anomeric proton signal in the ^{1}H-NMR of 1. From the above results, compound **1** was characterized as 3-O-[β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl] dammar-20(22), 24-diene-3β,12β-diol and named ginsenoside Rg_5. The stereochemistry of the double bond at C-20(22) was supposed to be (E) from the fact that C-21 was observed at 13.1 ppm in the ^{13}C-NMR[7] of **1**, while the methyl carbon of the (Z) structure was usually observed at lower field around 20-30 ppm[8]. Besides, NOE was not observed between H-21 methyl and H-22 olefinic protons but observed between H-21 and H-23 methylenes in the NOESY spectrum of ginsenoside Rg_5 **(1)**.



Flg. 1. Chemical structures of compounds 1 - 6

Compound **2** was supposed to be a glycoside composed of a D-glucopyranose and an aglycone with three hydroxyls and two pairs of double bonds from IR, ^{1}H-, ^{13}C-NMR, and DEPT spectra. The ^{13}C-NMR data and the molecular weight, which was determined as 620 from $(M+1)^+$ ion peak (*m/z* 621) in the pos. FAB MS, suggested that compound **2** be composed of one more double bond and one less hydroxyl group than ginsenoside Rh_1 isolated previously from *Panax ginseng*[9]. In the ^{1}H-NMR of **2**, a broad doublet-doublet methylene signal (δ 2.66, 2H) was revealed to be coupled with both olefinic proton signals [δ 5.35 (1H, br. t, J = 7.4), δ 5.01 (1H, br. t, J = 7.4)] from the ^{1}H-^{1}H COSY of **2.** Therefore, the methylene proton and two olefinic protons signals were assignable to H-23, H-22, and H-24, respectively. The above fact and comparison of NMR data with those of ginsenoside Rh_1[9] made clear two pairs of double bonds in compound **2** be formed at C-20(22) and C-24(25). Methanolysis of compound **2** with 9% HCl/MeOH afforded methyl-D-glucopyranoside and compound **2b** as the aglycone, whose structure was established as dammar-20(22),24-diene-3β, 6α,12β-triol from speculation of several spectral data and comparision with those of protopanaxatriol[10]. In the ^{13}C-NMR of **2,** C-6 (δ 78.0) showed downfield shifts relative to the aglycone, **2b**, by 4.9 ppm. Accordingly, D-glucopyranose was revealed to be binded at C-6 in compound **2**. The configuration of the anomeric carbon was determined to be β on the basis of the coupling constant (J = 7.7 Hz) of the anomeric proton signal in the ^{1}H-NMR of **2**. From the above results, compound **2** was characterized as 6-O-β-D-glucopyranosyl-dammar- 20(22),24-diene-3β, 6α, 12β-triol and named ginsenoside Rh_4. By the way, ginsenoside Rh_3[11], isolated from ginseng leaves, has same partial structure in the unit from C-20 to C-27 as that of ginsenoside Rh_4 (**2**). However, C-21 methyl signal of ginsenoside Rh_3 was reported to be observed at 27.3 ppm in the ^{13}C-NMR spectrum[10], whereas that of **2** was been at 12.5 ppm. To confirm the chemical shift of C-21 in compound **2**, we first assigned the chemical shift of H-21 methyl to be 1.70 ppm in the ^{1}H-NMR from the fact that the signal was coupled with H-22 olefinic signal through long-range (J_4) in the ^{1}H-^{1}H COSY. And then C-21 was assignable to the signal observed at 12.5 ppm in the ^{13}C-NMR, which was directly coupled with H-21 in HMQC. The stereostructure of the double bond between C-20 and C-22 of ginsenoside Rh_4 was determined as (E) from the fact that NOE was not observed between H-21 methyl and H-22 olefinic, but between H-21 methyl and H-23 methylene in NOESY of the aglycone (2). Whilest, that of ginsenoside Rh_3 was reported to be (Z)[11].

Compound **3** was obtained as white powder. From the IR spectrum, it was supposed to have hydroxyl (3465 cm^{-1}) and ester (1725 cm^{-1}). In the ^{1}H-NMR spectrum of **3**, one olefinic (δ 5.31, 1H, t, J=7.0 Hz), several oxy methine or oxy-methylene (δ 4.96 - 3.57), two hemiacetal methine [(δ 5.33, 1H, d, J=7.7 Hz), (δ 4.92, ^{1}H, d, J=7.6 Hz)], and nine singlet methyl (δ 2.04, 1.64, 1.61, 1.42, 1.34, 1.12, 0.96, 0.95, 0.81), one of which (δ 2.04) was gussed due to acetyl-methyl, proton signals were observed. The above results suggested compound **3** to be a triterpenoidal dammarane glycoside containing two sugars, one double bond, and one acetyl group. The ^{13}C-NMR data of **3** were very similar

Table 1. ^{13}C-NMR data for compounds **1, 1a, 2, 2b, 3, 4, 5** and **6** (Pyridine-d$_5$, 100 MHz)

Carbon	1	2	2b	3	4	5	6
1	39.2	38.9	38.9	39.0	38.5	39.4	39.3
2	28.0	27.3	28.0	26.8	26.5	27.8	27.6
3	88.8	79.5	78.6	89.1	88.3	79.4	79.4
4	40.1	39.8	39.3	39.9	39.4	40.0	39.9
5	56.3	60.9	61.2	56.3	55.7	60.9	60.8
6	18.3	78.0	73.1	18.4	18.3	78.4	78.5
7	35.2	44.7	47.2	35.8	36.3	46.1	46.0
8	39.6	40.8	41.3	36.9	37.3	41.2	41.1
9	50.7	50.0	49.8	50.3	49.8	50.2	50.1
10	36.9	39.2	39.3	39.7	39.1	39.8	39.6
11	32.1	31.1	30.9	32.0	31.4	32.6	32.2
12	72.5	71.2	68.6	70.9	70.4	71.1	71.0
13	50.3	49.8	49.8	48.5	47.9	50.2	49.3
14	50.9	50.3	50.3	51.6	51.1	51.7	51.7
15	32.5	32.0	32.4	31.3	30.7	31.2	31.3
16	26.6	26.9	27.0	26.7	26.2	27.2	26.8
17	50.8	50.1	50.1	54.8	54.2	51.7	51.0
18	16.4	17.2	17.7	16.9	16.5	17.6	17.6
19	16.5	17.2	17.3	16.3	16.3	17.6	17.6
20	140.1	139.5	140.0	72.9	72.4	72.8	72.9
21	13.1	12.5	12.5	27.0	26.1	22.5	22.9
22	123.2	122.9	122.1	35.1	35.3	43.6	43.4
23	27.4	29.4	29.7	22.9	22.4	18.7	22.9
24	123.5	124.7	124.9	126.3	127.0	46.1	126.3
25	131.2	130.7	132.1	130.7	130.2	69.4	130.7
26	25.6	25.1	22.4	25.8	25.2	29.8	25.7
27	17.7	16.8	17.2	17.6	17.4	30.4	17.1
28	28.7	31.1	30.9	28.0	27.5	31.7	32.1
29	15.7	15.8	15.5	15.8	15.7	16.8	16.9
30	16.9	16.2	16.7	16.4	16.3	17.4	17.1
1′	105.0	105.4		104.8	104.5	101.9	101.9
2′	83.3	74.8		84.2	82.8	79.4	79.5
3′	78.1	79.0		78.1	77.8	78.4	78.3
4′	71.5	72.0		71.3	71.0	72.5	72.3
5′	77.8	77.5		77.9	77.3	78.4	78.4
6′	62.6	62.5		62.8	62.1	63.2	63.1
1″	105.9			106.1	105.5	101.8	101.7
2″	77.0			76.7	76.6	72.6	72.6
3″	78.2			78.5	78.2	72.4	72.4
4″	71.5			70.9	72.4	74.2	74.3
5″	78.0			75.3	77.5	69.4	69.4
6″	62.7			64.7	62.2	18.8	18.8
	acetyl				171.0		
				20.9			

* Assignments were made by comparison with literature data [2,7,8,11,14] and DEPT, HMQC, HMBC spectra

to those of 20(S)-ginsenoside Rg_3(**4**), previously isolated from red ginseng, except for additional one methyl and one carbonyl signals (δ 20.9, 171.0) owing to a acetyl group. It was confirmed from the fact that the molecular ion peak in the positive FAB-MS (m/z 849 : $[M+Na]^+$). Some glycosides containing ester form in the structures by combining with acetyl or malonyl residues have been isolated from *Panax ginseng*[12,13]), from which compound **3** might be created through hydrolysis. In order to determine the position for a acetyl to be introduced, the ^{1}H-NMR data of 20(S)-ginsenoside Rg_3 (**4**) and compound **3** were carefully compared. In the ^{1}H-NMR spectrum of 3, two proton signals [(δ 4.96, 1H, dd, *J*=4.5, 11.7), (δ 4.80, ^{1}H, dd, *J*=4.9, 11.7)], which were clearly due to hydroxymethylene, were observed by down-field shift owing to acetylation effect. And the signals showed correlation with a oxy-methine proton (δ 4.02, 1H, ddd, *J*=4.5, 4.9, 9.5), which was thought to be the signal of H-5′ or H-5″, in the ^{1}H-^{1}H COSY. Accordingly, the acetyl was supposed to be introduced to the primary hydroxy of sugar moiety (C-6′ or C-6″). The fact that chemical shifts of C-6″ and its neighbourings in the ^{13}C-NMR of **3** showed the big variances indicated the acetyl group was introduced to primary hydroxy of terminal D-glucopyranose moiety (C-6″). Moreover, such fragment ion signals as *m/z* 621 and *m/z* 605 in the positive FAB-MS made it confirmed. In the HMBC operated with adjusting d_6 value as 50 ms, it was observed no correlation between C-6″ and acetyl proton or carbon signals. While, HMBC operated with adjusting the value as 100 ms apparently showed the cross peaks between C-6″ and acetyl residue, that is, carbonyl-C and H-6″$_a$, carbonyl-C and H-6″$_b$, C-5″ and H-6″$_{a,b}$, C-5" and H-1″, respectively, indicating the position of acetyl to be C-6″ hydroxy group without quarrels. Finally, the chemical structure of compound 3 was determined to be 20(S)-protopanaxadiol 3-O-[6″-O-acetyl-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside, named ginsenoside Rs_3.

Compound **5**, mp 182 - 184℃, $[\alpha]_D$ -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 Rg_2 and absorption bands due to hydroxyl (3410 cm^{-1}) group and glycosidic bond(1060 cm^{-1}) in the IR spectrum. As shown in Table 1, the ^{13}C-NMR spectrum of **5** was quite similar to that of 20(R)-ginsenoside Rg_2 (**6**) 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 **6** revealed that signals due to the C-24 and C-25 were shifted upfield from 126. 3 and 130.7 ppm in **6** to 46.1 and 69.4 ppm in **5**, respectively. The ^{1}H-NMR spectrum of **5** exhibited a similar signal pattern to that of **6** except for the disappearance of signals due to a double bond of side chain. These findings suggested that the chemical structure of **5** 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(CH_3-26) and 1.60 ppm (CH_3-27) showed long-range couplings with the carbon signal (C-25) at 69.4 ppm in the HMBC spectrum of **5** (Fig. 2). The ^{1}H-NMR spectrum of **5** showed the presence of two

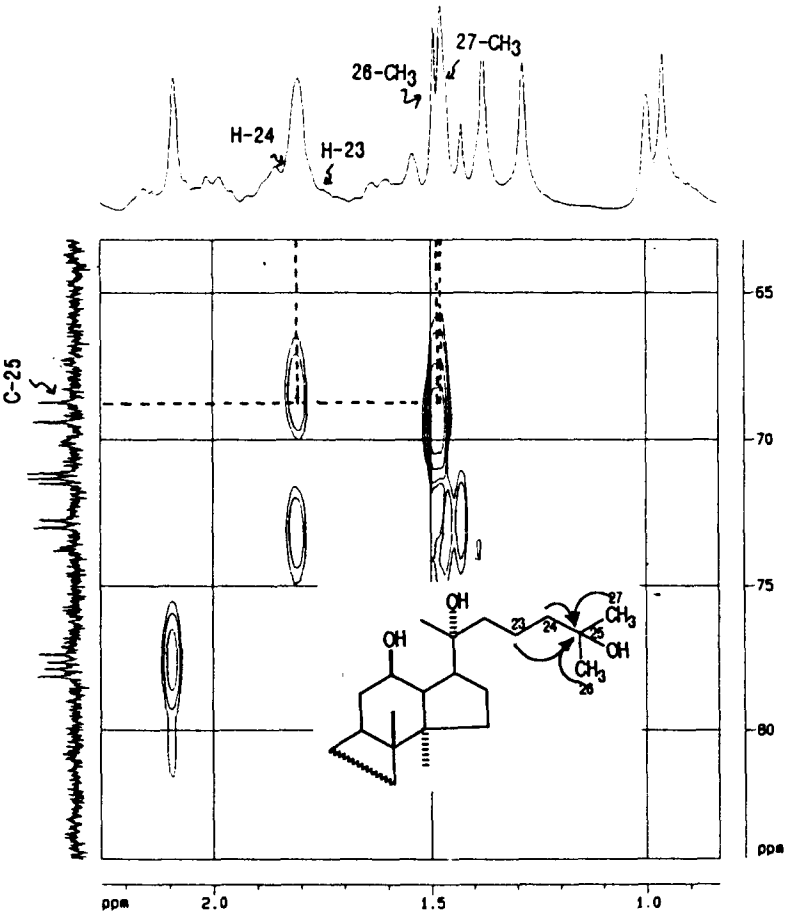


Fig. 2. HMBC spectrum of compound 5

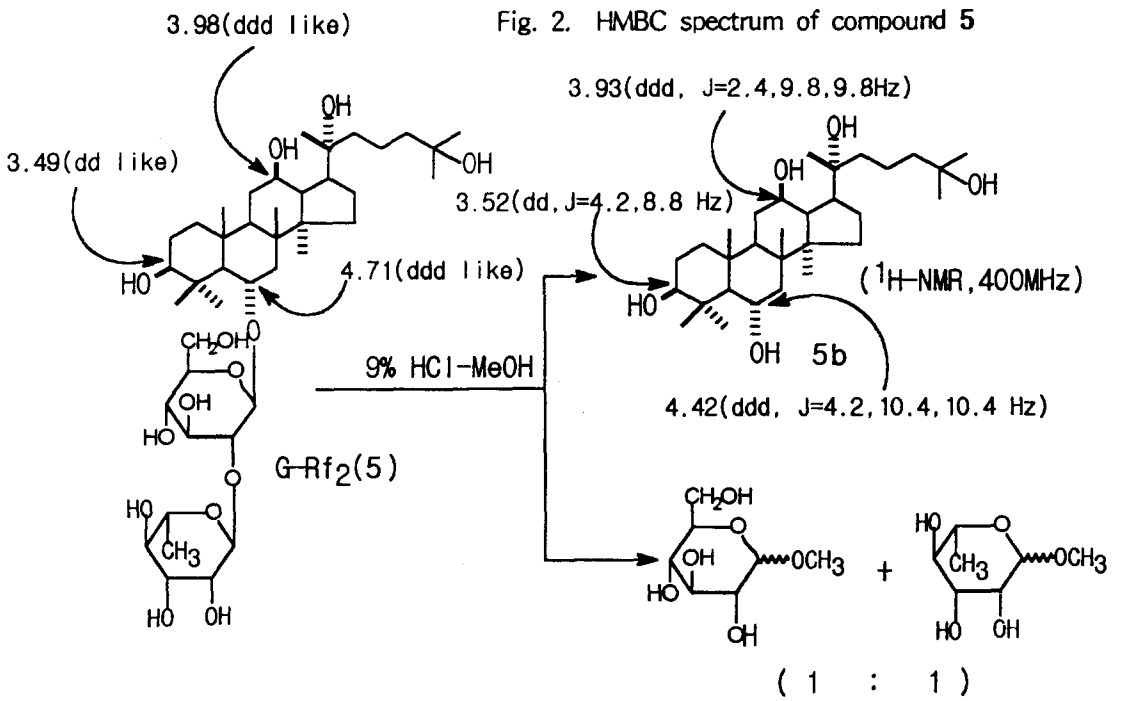


Chart 1

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 ^{13}C-NMR spectrum of **5**, respectively. On methanolysis with 9% HCl-MeOH, **5** liberated the aglycone **5b**, dammarane-3β, 6β, 12β, 20(R), 25-pentol and two methyl glycoside, which were identified as methyl-D-glucopyranoside and methyl-L-rhamnopyranoside by comparison of TLC (Chart 1). In the ^{1}H-NMR spectrum of **5b**, glycosidation shifts of the proton signal was observed for H-6(-0.29 ppm) as compared with that of **5,** so that sugar moiety was linked to the C-6 hydroxyl group of **5.** The configuration of C-20 has been determined to be 20(R) form from the fact that chemical shifts characteristic of 20(R) form were observed in the ^{13}C-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 Rg_2[2]. From the above results, compound **5** was characterized as 6-O-[-L-rhamnopyranosyl (1 → 2)β-D-glucopyranosyl]dammarane-3β, 6β, 12β, 20(R), 25 -pentol and named ginsenoside Rf_2. 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 **5**, Re-prosapogenin II[14]. 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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