Arch. Pharm. Res. Vol.19, No.5, pp. 551-553, 1996

Ginsenoside Rg_5, A Genuine Dammarane Glycoside from Korean Red Ginseng

Shin Il Kim[1], Jeong Hill Park[2], Jae-Ha Ryu[3], Jong Dae Park[1], You Hui Lee[1], Jae-Hyun Park[3], Tae-Hee Kim[3], Jong Moon Kim[2], and Nam-In Baek[1]

[1]Korea Ginseng & Tobacco Research Institute, Taejeon 305-345, Korea, [2]College of Pharmacy, Seoul National University, Seoul 151-742, Korea, [3]Department of Pharmacy, Sookmyung Women's University, Seoul 140-742, Korea

(Received June 20, 1996)

A genuine dammarane glycoside, named ginsenoside Rg_5, has been isolated by repeated column chromatography and preparative HPLC from the MeOH extract of Korean red ginseng (*Panax ginseng* C.A. Meyer). The chemical structure of ginsenoside Rg_5 was determined as 3-O-[β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl] dammar-20(22),24-diene-3β,12β-diol by spectral and chemical methods. The stereostructure of a double bond at C-20(22) of ginsenoside Rg_5 was characterized as (E) from the chemical shift of C-21 in the ^{13}C-NMR and a NOESY experiment in the ^{1}H-NMR.

Key words : *Panax ginseng*, Red ginseng, Novel saponin, Ginsenoside Rg_5, Stereostructure, NMR data

INTRODUCTION

In our search for the new saponins from *Panax ginseng* (Araliaceae), a new dammarane glycoside has been isolated from the EtOH extract of Korean red ginseng and named ginsenoside Rh_4 (Baek *et al.*, 1996). Continuing studies led to an isolation of another new dammarane glycoside from the MeOH extract of Korean red ginseng. Its chemical structure including stereostructure was determined by spectral and chemical methods. In this paper, we are reporting the procedures for isolation and structure determination of the compound.

MATERIALS AND METHODS

Plant material

Red ginseng was prepared from six-year old fresh ginseng (*Panax ginseng* C. A. Meyer), and identified by Prof. Yong Pyo Lim, Dept. of Horticulture, College of Agriculture, Chungnam National University. A voucher specimen has been deposited at the Herbarium of Korea Ginseng and Tobacco Research Institute (No. KG-9524).

Correspondence to: Shin Il Kim, Korea Ginseng & Tobacco Research Institute Yuseong P.O. Box 7, Shinseong-Dong 302, Yuseong-Ku, Taejeon 305-345 Korea

Instruments

Melting Points were determined on a Fisher-John Apparatus and are uncorrected. ^{1}H-(400 MHz) and ^{13}C-NMR (100 MHz) spectra were measured with a Bruker AMX 400 Spectrometer. FAB Mass spectra were taken on a VG-VSEQ(EBqQ type)/VG Analytical Spectrometer. Optical rotation was measured on a JASCO DIP-370 Digital Polarimeter. Perkin-Elmer Model 240C was used for elemental analysis. IR spectra were taken with a Perkin-Elmer Model 599B Spectrometer.

Isolation of ginsenoside (1) Rg_5

The powder of red ginseng (1 kg) was extracted with methanol (2.5 l × 3) at room temperature overnight. The methanol extract (250 g) was partitioned between water (700 ml) and *n*-BuOH (500 ml × 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 $CHCl_3$-MeOH-H_2O (10 : 3 : 1→9 : 3 : 1) to afford ginsenoside Rg_2, Rg_3 and a subfraction. The fraction was rechromatographed with silica gel column using n-BuOH-EtOAc-H_2O (10 : 10 : 0.5) as eluent to yield a dammarane glycoside (**1**, 250 mg). It was further purified by preparative HPLC (CLC-ODS, 10×

250 m/m, CH_3CN-H_2O=4:6, 2.5 ml/min, Rt 39'02").

Compound 1 : white powder (MeOH), m.p; 188-192°C, $[\alpha]_D$: +4.7° (c, 1.2, MeOH), IR (KBr) ν_{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+$CDCl_3$ (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 (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 partitioned with EtOAc and iced water. The organic phase was treated with 5% aq. HCl, saturated aq. $NaHCO_3$, brine and magnesium sulfate anhydrous, successively. The obtained acetate mixture 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 (x 2), 2.022, 2.016, 2.01, 1.99 (each 3H, all s, acetylmethyl × 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), ^{13}C-NMR (Table 1)

RESULTS AND DISCUSSION

Repeated column chromatography and preparative HPLC of the MeOH extract of Korean red ginseng gave rise to a purified unknown glycoside compound (**1**).

From the IR spectrum, compound **1** was supposed to contain double bond (1646 cm^{-1}) and hydroxy group (3460 cm^{-1}). In the ^{1}H-NMR spectrum (400 MHz, d_5-Py) 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, d, *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 observed. All these data suggested that compound **1** be a triterpenoidal-diglycoside with double bonds. In the ^{13}C-NMR spectrum (100 MHz, d_5-Py), the chemical shifts of two anomeric (δ 105.00, 105.91) and other sugar moiety signals (δ 83.31, 78.13, 71.50, 77.82, 62.58, 77.00, 78.21, 71.53, 77.98, 62.73) 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 except for the chemical shifts of four olefinic carbon signals, indicating it to be dammarane glycoside. And two quaternary carbon signals were observed at 131.16 and 140.06. 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

Table I. ^{13}C-NMR chemical shifts of ginsenoside Rg_3 (**1**) and its acetate (**1a**) (100 Hz)

C-No	1 *	1**	1a***	C-No	1 *	1**	1a***
1	39.17	40.49	38.92	1'	105.00	104.68	100.41
2	28.00	28.58	27.45	2'	83.31	81.35	76.93
3	88.82	91.42	90.89	3'	78.13	78.33	74.33
4	40.14	41.37	39.86	4'	71.50	71.65	68.16
5	56.29	59.69	56.07	5'	77.82	77.69	71.73
6	18.33	19.40	17.95	6'	62.58	62.98	61.90
7	35.24	36.27	34.72	1"	105.91	105.51	103.27
8	39.60	40.74	39.28	2"	77.00	76.40	71.30
9	50.66	51.51	49.16	3"	78.21	78.53	75.13
10	36.91	38.19	36.86	4"	71.53	72.00	68.91
11	32.10	32.29	29.58	5"	77.98	77.94	73.03
12	72.49	74.41	71.20	6"	62.73	63.27	62.40
13	50.33	51.41	46.81	acetyl			20.95
14	50.91	52.02	50.92				20.81
15	32.54	33.61	31.97				20.72
16	26.64	27.41	25.90				20.65
17	50.80	51.90	50.23				20.47
18	16.35	16.94	15.82				20.47
19	16.49	17.12	16.02				20.31
20	140.06	140.75	1 3 7				170.54
21	13.07	13.22	29				170.49
22	123.21	124.15	12.15				170.16
23	27.35	28.12	122.92				169.91
24	123.54	124.95	27.10				169.67
25	131.16	132.44	123.36				169.40
26	25.60	26.16	130.85				169.26
27	17.66	18.11	25.56				
28	28.73	29.34	17.60				
29	15.72	16.45	28.25				
30	16.92	17.50	15.51				

*d_5-Py
**CD_3OD+$CDCl_3$=10:1
***$CDCl_3$

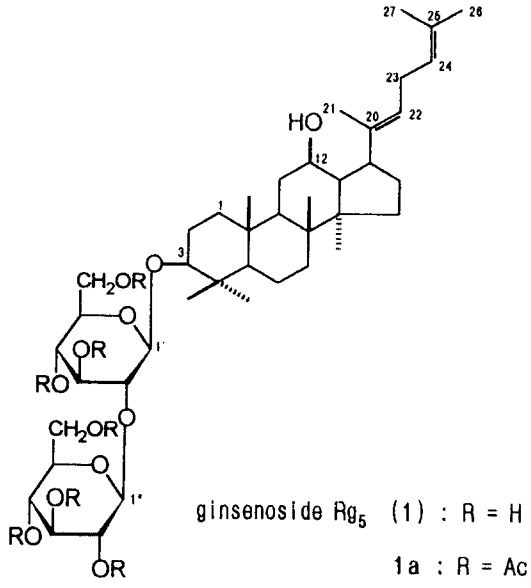

Fig. 1. Chemical Structure of ginsnoside Rg_5 (**1**) and its acetate (**1a**)

olefinic two quaternary (δ 132.44, 140.75) and two methine (δ 124.15, 124.95) carbon signals. In the d_5-pyridine solution, the two olefinic methine carbon signals of 1 might lie on the pyridine-d_5 signals. Four olefinic carbon signals (δ 122.92, 123.36, 130.85, 137.29) observed in the ^{13}C-NMR spectrum (400 MHz, $CDCl_3$) of the acetate compound (**1a**) and two cross peaks observed in the HMQC (d_5-Py) of 1 due to two olefinic protons and methine carbons signals (δ 123.21, 123.54) 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 two 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) observed 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=7.4 Hz) 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 13.07 ppm in the ^{13}C-NMR (Kim *et al.*, 1995) of **1**, while the methyl carbon of the (Z) structure was usually observed at lower field around 20-30 ppm (Chen *et al.*, 1987). 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**).

Acetylation of ginsenoside Rg_5 (**1**) with acetic anhydride in pyridine afforded the heptaacetate of **1** (**1a**). Seven singlet methyl signals [δ 2.08, 2.04 (x2), 1.992, 1.986, 1.98, 1.96] in the ^{1}H-NMR spectrum (400 MHz, $CDCl_3$), and seven acetyl carbonyls (δ 169.26, 169.40, 169.67, 169.91, 170.16, 170.49, 170.54) and methyls [δ 20.31, 20.47 (x2), 20.65, 20.72, 20.81, 20.95] in the ^{13}C-NMR spectrum (100 MHz, $CDCl_3$) from seven acetyls of **1a** were observed. Steric hindrance might prevent from introduction of acetyl to 12-hydroxy group in **1**. In the ^{1}H-NMR spectrum of 1a, H-2' was observed at 3.77 ppm without low-field shift, which indicated terminal D-glucopyranose was bound to 2-hydroxy group of innermost D-glucopyranose.

All the NMR data of **1** and its acetate (**1a**) were fully assigned from DEPT, ^{1}H-^{1}H COSY, NOESY, HMQC, and HMBC.

REFERENCES CITED

Baek, N.I., Kim, D.S., Lee, Y.H, Park, J.D., Lee, C.B, and Kim, S.I., Ginsenoside Rh_4, a genuine dammarane glycoside from Korean red ginseng. *Planta Medica*, 62(1), 86-88 (1996).

Chen, Y., Su, S., Ma, Q., Pei, Y., Xie, H. and Yao, X., Studies on new minor saponins isolated from leaves of *Panax ginseng* C.A. Meyer. Acta *Pharmaceutica Sinica*, 22(9), 685-689 (1987).

Kim, D. S., Baek, N.-I., Lee, Y.H., Park, J.D. and Kim, S.I., Preparation and structure determination of a new glycoside, (20E)-ginsenoside Rh_3, and its isomer from diol-type ginseng saponins. *Yakhak Hoeji*, 39(1), 85-93 (1995).