Ginsenoside Rs_3, A Genuine Dammarane-Glycoside from Korean Red Ginseng

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A genuine dammarane-glycoside, named as ginsenoside Rs_3, was isolated from the MeOH extracts of Korean red ginseng (*Panax ginseng* C.A. Meyer) through repeated silica gel column chromatographies and its chemical structure was determined as (20*S*)-protopanaxadiol 3-*O*-[6"-*O*-acetyl-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside on the basis of several spectral and physical evidences including HMBC and FAB-MS.

Key words : *Panax ginseng*, Genuine saponin, Red ginseng, Ginsenoside Rs_3, HMBC

INTRODUCTION

On the course of our efforts to search for new saponins from *Panax ginseng* (Araliaceae), two new dammarane glycosides, ginsenoside Rh_4 (Baek *et al.*, 1996) and Rg_5 (Kim *et al.*, 1996), have been isolated from Korean red ginseng. Continuing studies led to isolation of another new dammarane-glycoside and its chemical structure was elucidated through the spectral and physical data. Especially, the acetylated position in the chemical structure of the compound was confirmed from its HMBC and FAB-MS Spectra.

MATERIALS AND METHODS

Red ginseng

Red ginseng was manufactured from six-year-old fresh ginseng by Korea Tobacco & Ginseng Corporation and deposited at the Herbarium of Korea Ginseng & Tobacco Research Institute (N-KG-9527).

Instruments

^{1}H- (400 MHz), ^{13}C-NMR (100 MHz) and HMBC (d_6 value=100 ms): Bruker ARX 400, FAB Mass: VG-VSEQ (EBqQ type)/VG Analytical, Optical rotation: JASCO DIP-370, Elemental analysis: Perkin-Elmer 240C, IR: Perkin-Elmer 599B.

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Isolation of ginsenoside Rs_3 (1)

The red ginseng (1 kg) was milled and extracted in methanol (4 L×3) at room temperature overnight, and filtered through NO. 2 Whatman filter paper. The combined filtrates were evaporated under vacuum to give the extracts (256 g), which was dissolved in 2 L of water and defatted with *n*-hexane (1 L×3). The aqueous layer was extracted with *n*-BuOH (1 L×3), and the combined *n*-BuOH layer was evaporated under vacuum to afford the extracts (180 g). The 45 g of the extracts was applied on silica gel columns eluting with $CHCl_3$-MeOH-H_2O (15:3:1, lower phase), *n*-BuOH-EtOAc-H_2O (15:1:4, upper phase) and $CHCl_3$-MeOH-H_2O (12:3:1, lower phase), repeatedly, to give rise to a unknown compound, named ginsenoside Rs_3 (**1**, 45 mg).

Ginsenoside Rs_3 (**1**): white powder (MeOH-EtOH), $[\alpha]_D$ +17.9° (c=0.8, MeOH), IR (KBr) ν_{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.) δ 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, acetylmethyl), 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 (Table I).

RESULTS AND DISCUSSION

The *n*-BuOH fraction of methanolic extracts obtained from Korean red ginseng (*P. ginseng* C.A. Meyer) was subjected to silica gel column chromatography eluting various solvent systems to give a unknown component (**1**) as white powder (MeOH-EtOH). 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 **1** (400 MHz, d_5-Py.), one olefinic (δ 5.31, 1H, t, *J*=7.0), several oxy-methine or oxy-methylene (δ 4.96-3.57), two hemiacetal methine [(δ 5.33, 1H, d, *J*=7.7), (δ 4.92, 1H, d, *J*=7.6)], 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 guessed due to acetyl-methyl, proton signals were observed. The above results suggested compound **1** to be a triterpenoid-glycoside containing two sugars, one double bond, and one acetyl group. The ^{13}C-NMR data (100 MHz, d_5-Py., Table I)

Table I. ^{13}C-NMR Data of (20*S*)-ginsenoside Rs_3 (**1**) & Rg_3 (**2**) (100 MHz, d_5-Py., δ_C)

No of C	1	2	No of C	1	2
1	38.99	38.51	23	22.93	22.39
2	26.78	26.46	24	126.25	126.95
3	89.14	88.30	25	130.69	130.16
4	39.94	39.37	26	25.75	25.24
5	56.34	55.74	27	17.62	17.39
6	18.40	18.32	28	27.95	27.51
7	35.81	36.29	29	15.77	15.73
8	36.87	37.34	30	16.39	16.31
9	50.33	49.76	1'	104.84	104.52
10	39.67	39.09	2'	84.24	82.80
11	31.97	31.43	3'	78.07	77.80
12	70.94	70.40	4'	71.30	70.99
13	48.50	47.94	5'	77.87	77.33
14	51.64	51.10	6'	62.75	62.05
15	31.27	30.72	1"	106.13	105.45
16	26.71	26.24	2"	76.68	76.55
17	54.75	54.19	3"	78.49	78.21
18	16.94	16.45	4"	70.94	72.36
19	16.30	16.26	5"	75.29	77.52
20	72.89	72.40	6"	64.70	62.21
21	27.01	26.12	acetyl	170.96	
22	35.10	35.27		20.85	

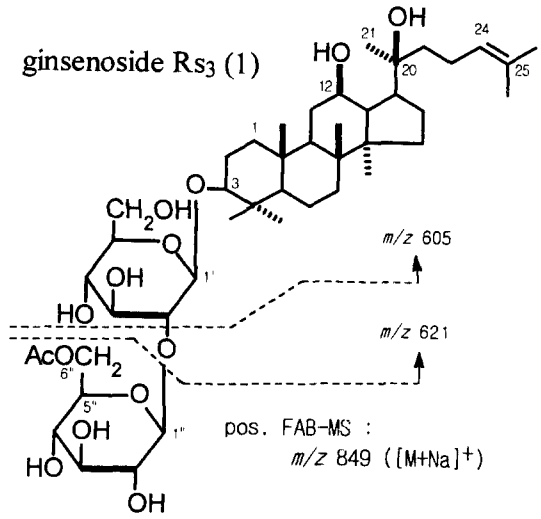

Fig. 1. Chemical structure of ginsenoside Rs_3 (**1**) and its positive FAB-MS data.

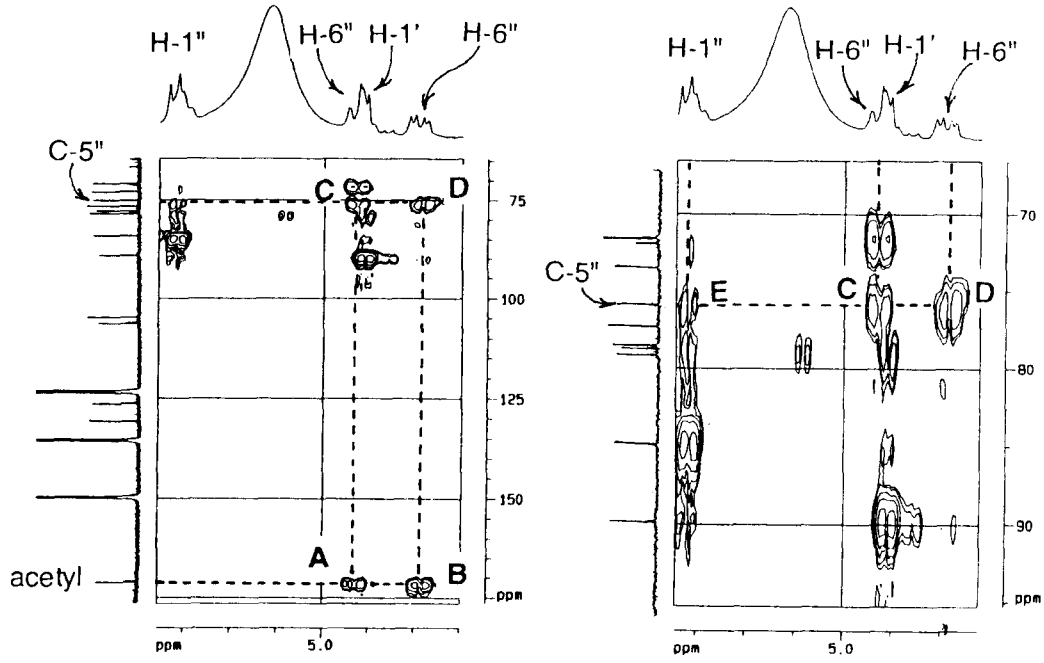

Fig. 2. HMBC spectra of ginsenoside Rs_3 (**1**).

of the glycoside (**1**) were very similar to those of (20*S*)-ginsenoside Rg_3 (**2**), previously isolated from *Panax ginseng,* only except that compound **1** showed additional one methyl and one carbonyl signals (δ_C 20.85, 170.96) 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 (Tanaka *et al.*, 1985) or malonyl (Kitagawa *et al.*, 1987) residues have been isolated from *P. ginseng,* from which compound **1** 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 (**2**) and compound **1** were carefully compared. In the ^{1}H-NMR spectrum of **1**, two proton signals [(δ 4.96, 1H, dd, *J*=4.5, 11.7), (δ 4.80, 1H, 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 **1** showed the most variances indicated the acetyl group was introduced to primary hydroxy of terninal D-glucopyranose moiety (C-6"). Moreover, such fragment ion signals as *m/z* 621 and *m/z* 605 in the positive FAB-MS (Fig. 1) 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, cabonyl-C and H-6"$_a$ (peak A), cabonyl-C and H-6"$_b$ (peak B), C-5" and H-6"$_{a,b}$ (peak C and D), C-5" and H-1" (peak E), respectively, (Fig. 2) indicating the position of acetyl to be C-6" hydroxy group without quarrels. Finally, the chemical structure of compound **1** was determined to be (20*S*)-protopanaxadiol 3-*O*-[6"-*O*-acetyl-β-*D*-glucopyranosyl (1→2)-β-*D*-glucopyranoside, named ginsenoside Rs_3.

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 Med.*, 62, 86-87 (1996).

Kim, S. I., Park, J. H., Ryu, J.-H., Park, J. D., Lee, Y. H., Park, J.-H., Kim, T.-H., Kim, J. M. and Baek, N.-I., Ginsenoside Rg_5, A Genuine Dammarane Glycoside from Korean Red Ginseng, *Arch. Pharm. Res.*, 19-5, 551-553 (1996).

Tanaka, O., Morita, T., Kasai, R., Kinouchi, J., Sanada, S., Ida, Y. and Shoji, J., Study on saponins of rhizomes of *Panax pseudo-ginseng subsp. himalaicus* collected at Tzatogang and Pari-la, Bhutan-Himalaya, *Chem. Pharm. Bull.*, 33, 2323-2330 (1985).

Kitagawa, I., Taniyama, T., Shibuya, H., Noda, T. and Yoshikawa, M., Chemical studies on crude drug processing. V. On the constituents of ginseng radix rubura (2): Comparison of the constituents of white ginseng and red ginseng prepared from the same *Panax ginseng root, Yakugaku zasshi,* 107, 495-505 (1987).