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Four New Acetylated Ginsenosides from Processed Ginseng (Sun Ginseng)

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Four new acetylated ginsenosides were isolated from the processed ginseng (SG, sun ginseng). Their structures were determined to be 3β,12β-dihydroxydammar-20(22),24-diene-3-O-β-D-glucopyranosyl(1→2)-β-D-6"-O-acetylglucopyranoside; 3β,12β-dihydroxydammar-20(21),24-diene-3-O-β-D-glucopyranosyl(1→2)-β-D-6"-O-acetylglucopyranoside; 3β, 6α,12β-trihydroxydammar-20(22),24-diene-6-O-β-D-6'-O-acetylglucopyranoside and 3β,6α,12β-trihydroxydammar-20(21),24-diene-6-O-β-D-6'-O-acetylglucopyranoside based on spectroscopic evidences. The compounds were named ginsenoside Rs_4, Rs_5, Rs_6 and Rs_7, respectively.

Key words: *Panax ginseng*, Acetylated ginsenoside, Processed ginseng, Sun ginseng

INTRODUCTION

Ginseng (*Panax ginseng* C. A. Meyer, Araliaceae) is one of the most popular herbal medicines in the Orient (Han, 1988). Thousands of papers have reported its chemical constituents, biological activities, and cultivation. The most well-known chemical constituent of ginseng is ginsenoside, which is a dammarane glycoside. More than 30 ginsenosides have been reported from ginseng so far (The society for Korean Ginseng, 1995). Ginsenosides Rb_1, Rb_2, Rc, Rd, Rg_1, Rg_2, and Re are major constituents of white and red ginsengs, while ginsenosides Rh_1, Rh_2, Rg_3, Rg_5, Rg_6, Rs_1, Rs_2 and Rs_3 are known to be unique constituents of red ginseng (Kim *et al.*, 1996, Kitagawa *et al.*, 1983, Ryu *et al.*, 1997). Ginsenosides Rh_1, Rh_2, and Rg_3 are deglycosylated ginsenosides of ginsenosides Rg_2, Rg_3, and Rd, while ginsenosides Rg_5 and Rg_6 are dehydrated ginsenoside of Rg_3 and Rg_2, respectively. Ginsenoside Rs_1, Rs_2, and Rs_3 are acetylated ginsenosides of ginsenoside Rb_2, Rc, and Rg_3, respectively. Recently, we reported a new type of processed ginseng, named sun ginseng (SG), with increased radical scavenging, vasodilating, and anti-tumor promoting activities (Kim *et al.*, 2000; Keum *et al.*, 2000). Recently, we reported three new dammarane glycosides from SG (Park *et al.*, 2002). Further study on the chemical constituents of SG led us to the isolation of four new acetylated ginsenosides.

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MATERIALS AND METHODS

^{1}H-NMR and ^{13}C-NMR spectra were recorded on AMX 500 NMR spectrometer (Bruker, Germany) or Lambda 300 spectrometer (Jeol, Tokyo, Japan). AX 505WA double-focusing mass spectrometer (Jeol, Tokyo, Japan), DIP-360 polarimeter (Jasco, Tokyo, Japan), and 1710 IR spectrometer (Perkin-Elmer, Beaconsfield, U.K.) were used. Ag-impregnated TLC plate was prepared by spraying 3% $AgNO_3$ in MeOH on a precoated TLC plate (Merck Art. 5717, Darmstadt, Germany).

Isolation of ginsenosides

Dried rootlet of ginseng (3 kg) was steamed at 120°C for 3 hours in an autoclave. Steamed ginseng was extracted with MeOH (10 L) three times under reflux for 2 hr. The solvent was removed in vacuo to yield 0.4 kg of MeOH extract, which was suspended in water (5 L) and extracted with CH_2Cl_2 (10 L). The remaining aqueous layer was extracted with water-saturated *n*-BuOH (10 L) three times. The *n*-BuOH fraction was concentrated in vacuo to yield 0.3 kg of BuOH fraction, which was subjected to

silica gel column chromatography. Five fractions were obtained using stepwise gradient elution (EtOAc : MeOH : H_2O = 40 : 1 : 1 → 10 : 1 : 1) (Park *et al.*, 2002).

Isolation of compound 1 and 2 (ginsenoside Rs_4 and Rs_5)

Fraction 3 was chromatographed over silica gel using EtOAc : MeOH : H_2O = 25 : 1 :1 solvent. Compound **1** and compound **2** rich fractions were obtained, which were further purified on Ag-impregnated preparative TLC using EtOAc : MeOH : H_2O = 15 : 1 : 1 solvent. The bands were visualized by spraying water. Compounds **1** and **2** were collected from the band of Rf = 0.3 and Rf = 0.25, respectively. They were further purified over semi-preparative HPLC using a reverse-phase column (LiChrospher 100 RP-18, 250 mm × 10 mm i.d.) with 60% CH_3CN eluent to isolate compound **1** (20 mg) and compound **2** (13 mg).

Compound **1** : Amorphous powder, $C_{44}H_{72}O_{13}$, mp: 161-162 °C, $[\alpha]_D$: +2.54° (MeOH, c = 0.2%, 20); IR ν_{max} (KBr, cm^{-1}): 3400, 2950, 1740, 1450 Mass (FAB^+, 6 kV, Xe, glycerol): 831 ($[M+Na]^+$). ^{1}H-NMR (500 MHz, C_5D_5N, ppm): 0.85 (3H, s, H-29), 0.97 (3H, s, H-30), 1.05 (3H, s, H-19), 1.13 (3H, s, H-18), 1.34 (3H, s, H-28), 1.59 (3H, s, H-27), 1.63 (3H, s, H-26), 1.82 (3H, s, H-21), 2.05 (3H, s, CH_3CO), 3.28 (1H, dd, *J* = 4.3, 11.6 Hz, H-3), 3.92 (1H, m, H-12), 4.89 (1H, d, *J* = 7.3 Hz, H-1'), 5.23 (1H, t, *J* = 6.9 Hz, H-24), 5.31 (1H, d, J=7.7 Hz, H-1"), 5.51 (1H, t, J=7.0 Hz, H-22). ^{13}C-NMR (125 MHz, C_5D_5N, ppm) : Table I.

Compound **2** : $C_{42}H_{70}O_{12}$, amorphous powder, mp: 140-142 °C, $[\alpha]_D$: +13.5° (MeOH, c = 0.4%, 10), IR ν_{max} (KBr, cm^{-1}): 3411, 2947, 1741, 1637, 1450, 1078. Mass (FAB^+, 6 kV, Xe, glycerol): 837 ($[M+Na]^+$). ^{1}H-NMR (500 MHz, C_5D_5N, ppm, D_2O exchanged): 0.67 (1H, d, *J* = 11.86 Hz, H-5), 0.77 (3H, s, H-19), 0.93 (3H, s, H-30), 0.99 (3H, s, H-18), 1.05 (3H, s, H-29), 1.26 (3H, s, H-28), 1.57 (3H, s, H-27), 1.62 (3H, s, H-26), 2.02 (3H, s, CH_3CO), 2.79 (1H, m, H-17), 3.24 (1H, dd, *J* = 11.58, 4.43 Hz, H-3), 3.90 (2H, m, H-12, 5'), 4.84 (1H, d, *J* = 7.36 Hz, H-1'), 4.88 (1H, br. s, H-21_a), 5.14 (1H, br. s, H-21_b), 5.24 (2H, br. d, *J* = 7.75 Hz, H-1", H-24). ^{13}C-NMR (125 MHz, C_5D_5N, ppm): Table I.

Isolation of Compounds 3 and 4 (ginsenoside Rs_6 and Rs_7)

Fraction 2 was chromatographed over silica gel using *n*-Hexane : Isopropyl alcohol = 6 : 1 solvent to give compound **3** and compound **4** rich fractions. The fractions were further purified by semi-preparative HPLC using a reverse-phase column (LiChrospher 100 RP-18, 250 mm ×10 mm i.d.) with 50% CH_3CN eluent to yield compound **3** (10 mg) and compound **4** (9 mg).

Compound **3** : Amorphous powder, $C_{38}H_{62}O_9$, mp: 165-166 °C, $[\alpha]_D$: +18.8° (MeOH, c = 0.5%, 10); IR ν_{max} (KBr, cm^{-1}): 3400, 2926, 1734, 1370, 1246, 1031 Mass (FAB^+, 6 kV, Xe, glycerol): 635 ($[M+Na]^+$). ^{1}H-NMR (300 MHz, C_5D_5N, ppm) : 0.95 (3H, s, H-30), 1.05 (3H, s, H-19), 1.29 (3H, s, H-18), 1.55 (3H, s, H-29), 1.56 (3H, s, H-27), 1.61 (3H, s, H-26), 1.83 (3H, s, H-21), 2.05 (3H, s, H-28), 2.06 (3H, s, CH_3CO), 2.49 (1H, br. d, J=9.99 Hz, H-7_a), 2.77 (3H, m, H-23, 17), 3.50 (1H, dd, *J* = 11.5, 4.5 Hz, H-3), 4.01 (1H, m, H-12), 4.41 (1H, m, H-6), 5.21 (1H, br. t, *J* = 6.8 Hz, H-24), 5.48 (1H, br. t, *J* = 7.4 Hz, H-22). ^{13}C-NMR (75 MHz, C_5D_5N, ppm) : Table I.

Compound **4** : Amorphous powder, $C_{42}H_{70}O_{12}$, mp: 110-112 °C, $[\alpha]_D$: + 21.1° (MeOH, c = 0.4%, 10); IR ν_{max} (KBr, cm^{-1}): 3400, 2929, 1735, 1368, 1034 Mass (FAB^+, 6 kV, Xe, glycerol): 685 ($[M+Na]^+$). ^{1}H-NMR (500 MHz, C_5D_5N, ppm) : 0.98 (3H, s, H-30), 1.08 (3H, s, H-19), 1.31 (3H, s, H-18), 1.55 (3H, s, H-29), 1.61 (3H, s, H-27), 1.67 (3H, s, H-26), 2.02 (3H, s, H-28), 2.06 (3H, s, CH_3CO), 2.82 (1H, m, H-17), 2.97 (1H, m, H-23), 3.51 (1H, br. d, *J* = 11.4 Hz, H-3), 3.93 (1H, m, H-12), 4.43 (1H, m, H-6), 4.92 (1H, br. s, H-21_a), 5.04 (1H, d, *J* = 7.7 Hz, H-1'), 5.16 (1H, br. s, H-21_b), 5.29 (1H, m, H-24). ^{13}C-NMR (125 MHz, C_5D_5N, ppm) : Table I.

RESULTS AND DISCUSSION

Compound 1 (ginsenoside Rs_4)

Compound **1** was isolated as amorphous powder. This compound was not separated from compound **2** on a normal silica gel TLC plate or HPLC using an amino column. Compound **1** was separated from compound **2** on an $AgNO_3$-impregnated silicagel TLC plate and a reverse-phase HPLC. The molecular weight of compound **1** was 808, which suggested that compound **1** is a monoacetylated ginsenoside Rg_5 (MW = 766). The difference of molecular weight of 42 suggested an acetyl group. The ^{1}H- and ^{13}C-NMR patterns of compound **1** were very similar to those of ginsenoside Rg_5, except for the signals arising from one acetyl group (Table I). δ_H 2.09 in its ^{1}H-NMR spectrum and δ_C 170.97 and δ_C 20.90 in its ^{13}C-NMR spectrum showed the characteristic peak of the acetyl group ($C\underline{\mathbf{H}}_3C=O$, $\underline{\mathbf{C}}=O$, and $\underline{\mathbf{C}}H_3C=O$, respectively) and 5", 6" carbon of sugar appeared at δ_C 75.36, 64.74, respectively. The carbonyl carbon at δ_C 170.97 showed a connection with 6" proton (δ_H 4.78) of sugar in a heteronuclear multiple bond connection (HMBC) spectrum, which suggested the acetylation of 6" carbon of compound **1**. Two anomeric carbon signals at 104.90 and 106.17, and signals between 60-85 ppm suggested that compound **1** is a protopanaxadiol type ginsenoside with two sugar moieties. Four olefinic carbon signals at δ_C 140.19, 131.26, 123.80, and 123.51 suggested two double bonds in the molecule. Therefore, it was concluded that compound **1** is a monoacetylated ginsenoside with two double bonds. Thus, the structure of compound **1** was

Table I. ^{13}C-NMR chemical shift of compound **1**, **2**, **3**, **4** and ginsenoside Rg_5 (Kim *et al.*, 1996), Rk_1, Rh_4, Rk_3

C No.	Rg_5	Compound 1 (Rs_4)	Rk_1	Compound 2 (Rs_5)	Rh_4	Compound 4 (Rs_6)	Rk_3	Compound 3 (Rs_7)
1	39.17	39.29	39.30	39.29	39.44	39.48	39.50	39.58
2	28.00	26.79	26.75	26.79	27.80	27.89	27.92	27.94
3	88.82	89.20	88.95	89.21	78.52	78.51	78.56	78.61
4	40.14	39.75	39.72	39.74	40.27	40.49	40.37	40.32
5	56.29	56.46	56.43	56.47	61.36	61.40	61.44	61.49
6	18.33	18.48	18.45	18.48	79.97	79.69	80.05	79.72
7	35.24	35.47	35.36	35.37	45.22	45.60	45.31	45.66
8	39.60	40.29	40.21	40.22	41.25	41.42	41.26	41.46
9	50.66	50.79	48.23	50.88	50.50	50.55	50.64	50.70
10	36.91	37.07	37.03	37.06	39.66	39.75	39.71	39.82
11	32.10	32.20	32.60	32.60	32.18	32.31	32.73	32.80
12	72.49	72.61	72.47	72.48	72.51	72.49	72.42	72.49
13	50.33	51.04	52.49	52.48	50.59	50.69	52.07	52.21
14	50.91	50.90	51.21	51.21	50.77	50.89	51.13	51.27
15	32.54	32.63	32.67	32.66	32.47	32.69	32.50	32.71
16	26.64	28.83	30.77	30.76	28.74	28.77	30.71	30.76
17	50.80	50.44	50.86	48.24	50.32	50.39	48.27	48.22
18	16.35	15.84	16.45	15.82	17.31	17.36	17.33	17.41
19	16.49	16.45	15.80	16.43	17.67	17.72	17.73	17.78
20	140.06	140.19	155.55	155.55	140.01	140.02	155.42	155.47
21	13.07	13.16	108.15	108.15	13.07	13.16	108.11	108.24
22	123.21	123.51	33.89	33.87	123.42	123.17	33.70	33.99
23	27.35	27.45	27.08	27.08	27.38	27.43	27.02	27.12
24	123.54	123.80	125.33	125.33	123.78	123.83	125.33	125.37
25	131.16	131.26	131.21	131.20	131.18	131.22	131.18	131.25
26	25.60	25.67	25.74	25.74	25.64	25.68	25.74	25.77
27	17.66	17.71	17.74	17.74	17.67	17.70	17.33	17.78
28	28.73	28.01	28.11	28.01	31.63	31.54	31.70	31.59
29	15.72	16.45	16.58	16.43	16.27	16.50	16.34	16.51
30	16.92	17.03	16.98	16.98	16.73	16.96	16.73	17.00
1'	105.00	104.90	105.09	104.89	105.87	105.90	106.00	105.92
2'	83.31	84.29	83.45	84.26	75.34	75.34	75.45	75.41
3'	78.13	78.07	78.19	78.06	79.50	79.20	79.65	79.22
4'	71.50	71.02	71.65	71.02	71.71	71.37	71.82	71.47
5'	77.82	77.92	77.96	77.92	77.98	75.08	78.12	75.17
6'	62.58	62.84	62.76	62.84	62.96	62.12	63.06	65.17
1	105.91	106.17	106.01	106.14				
2	77.00	76.73	77.08	76.71				
3	78.21	78.52	78.34	78.52				
4	71.53	71.42	71.72	71.42				
5	77.98	75.36	78.06	75.35				
6	62.73	64.74	62.87	64.74				
C**O**		170.97		170.96		170.88		170.86
C**H**$_3$CO		20.90		20.88		20.93		20.93

elucidated to be 3β,12β-dihydroxydammar-20(22),24-diene-3-O-β-D-glucopyranosyl(1→2)-β-D-6"-O-acetylglucopyranoside. Since the compound has not been reported yet, we named it ginsenoside Rs_4.

Compound 2 (ginsenoside Rs_5)

Compound **2** was isolated as amorphous powder. The molecular weight of compound **2** was 808, which is the same as that of compound **1**. This suggests that compound **2** is a monoacetylated ginsenoside Rk_1 (MW 766) (Park *et al.*, 2002). The ^{1}H- and ^{13}C-NMR patterns of compound **2** were very similar to those of ginsenoside Rk_1, with the exception of signals arising from one acetyl group (Table I). δ_H 2.07 ppm in its ^{1}H-NMR spectrum and δ_C 170.96 and δ_C 20.88 in its ^{13}C-NMR spectrum showed the characteristic peak of an acetyl group (C**H**$_3$C = O, **C** = O, and **C**H$_3$C = O, respectively) and 5", 6" carbon of sugar appeared at δ_C 75.35, 64.74, respectively. δ_C 170.96 (**C** = O), which showed a connection with proton signals at δ_H 4.78 (C-6") in the HMBC spectrum, also suggested the acetylation of 6" carbon of sugar. Two anomeric carbon signals at 104.89 and 106.14, plus signals between 60-85 ppm in its ^{13}C-NMR spectrum suggests that compound **2** is a protopanaxadiol type ginsenoside with two sugar moieties. Four olefinic carbon signals at δ_C 155.55, 131.20, 125.33, and 108.15 suggest that there are two double bonds in the molecule. Therefore, it was concluded that compound **2** is an acetylated ginsenoside with two double bonds. Thus, the structure of compound **2** was elucidated to be 3β,12β-dihydroxydammar-20(21),24-diene-3-O-β-

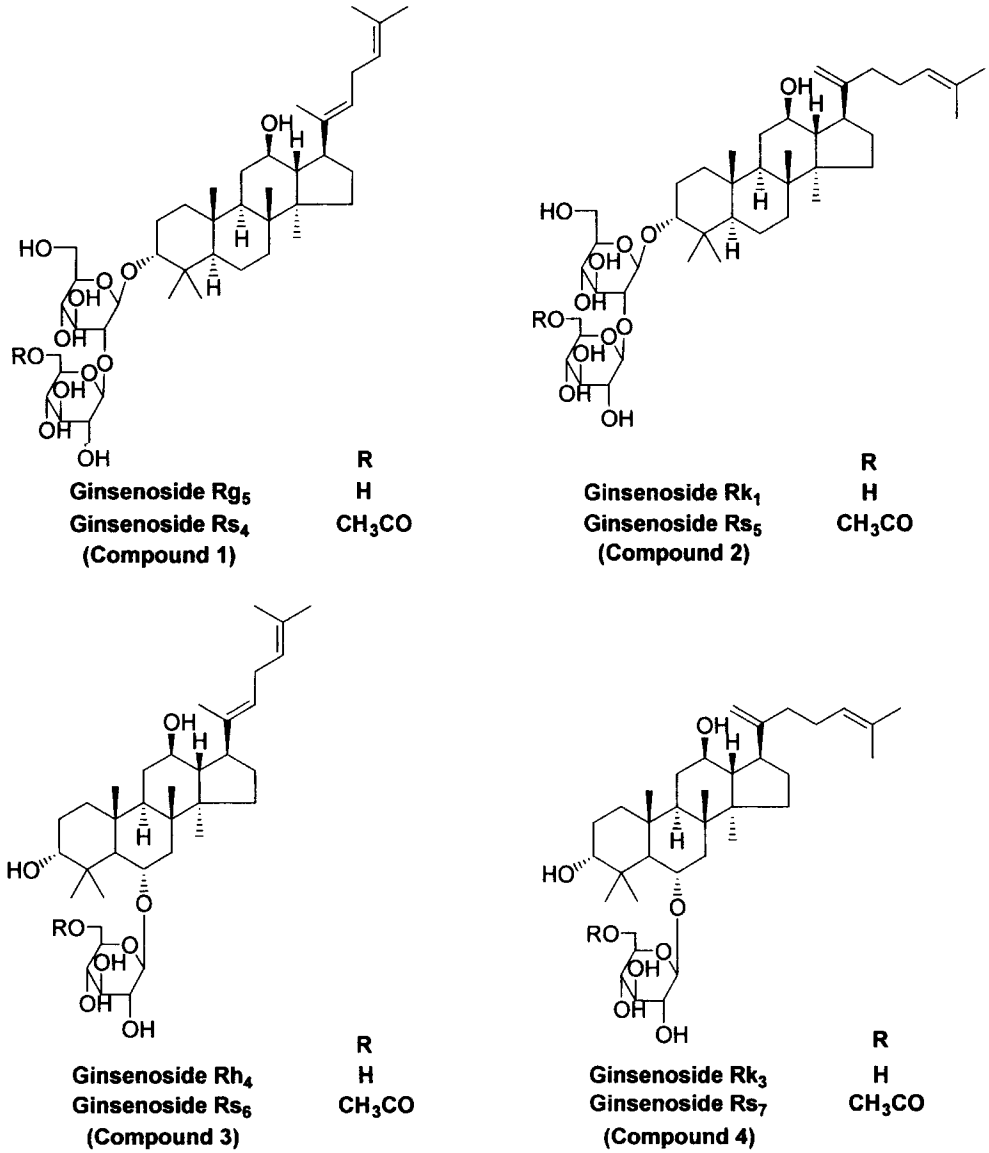

Fig. 1. Structure of ginsenoside Rg_5, Rs_4, Rk_1, Rs_5, Rh_4, Rs_6, Rk_3,Rs_7

D-glucopyranosyl(1→2)-β-D-6"-O-acetylglucopyranoside. Since the compound is not reported yet, we named it ginsenoside Rs_5.

Compound 3 (ginsenoside Rs_6)

Compound **3** was isolated as amorphous powder. This compound was not separated from compound **4** on a normal silica gel TLC plate or HPLC using an amino column. Compound **3** was separated from compound **4** using semi-preparative reverse-phase HPLC. The molecular weight of compound **3** was 662, which suggested that compound **3** is a monoacetylated ginsenoside Rh_4 (MW 620), i.e., protopanaxatriol type ginsenoside with one sugar moiety. A signal at δ_C 79.69 arising from oxygenated carbon at C-6 in ^{13}C-NMR supported the assumption. ^{1}H- and ^{13}C-NMR patterns of compound **3** were very similar to those of ginsenoside Rh_4 except for the signals arising from one acetyl group (Table I). δ_C 170.88 and δ_C 20.93 displayed the characteristic peak of an acetyl group (**C** = O, **C**H_3C = O), and 5', 6' carbons of sugar appeared at δ_C 75.08, 65.12, respectively. Carbonyl carbon at δ_C 170.88 showed a connection with 6' proton of sugar in the HMBC spectrum, which suggested the acetylation of 6' carbon of compound **3**. One anomeric carbon signal at δ_C 105.90 and signals between δ_C 65-80 suggested that compound **3** has one sugar moiety. Four olefinic carbon signals at δ_C 140.02, 131.22, 123.83, and 123.17 suggested two double bonds at 20(22) and 24 (25). These results also suggested that compound **3** has an acetyl group and two double bonds. Thus, the structure of compound **3** was elucidated to be 3β,6α,12β-trihydroxydammar-20(22),24-diene-6-O-β-D-6'-O-acetylglucopy-

ranoside. Since the compound has not been reported yet, we named it ginsenoside Rs_6.

Compound 4 (ginsenoside Rs_7)

Compound **4** was isolated as amorphous powder. The molecular weight of compound **4** was 662, which is identical to that of compound **3**, and which suggests that it is a monoacetylated ginsenoside Rk_3 (MW 620) (Park *et al.*, 2002). ^{1}H- and ^{13}C-NMR signals of compound **4** were quite similar to those of ginsenoside Rk_3, except for the chemical shift of an acetyl group (Table I). δ_C 170.86 and δ_C 20.93 showed the characteristic peak of an acetyl group (**C** = O, **C**H_3C = O), and 5', 6' carbons of sugar appeared at δ_C 75.17, 64.17, respectively. δ_C 170.86 (**C** = O) showed a connection with a proton signal at δ_H 5.08 (C-6') in the HMBC spectrum also suggested the acetylation of 6' carbon of sugar. One anomeric carbon signal at δ_C 105.92 and signals between δ_C 65-80 ppm suggest that compound **4** has one sugar moiety. Four olefinic carbon signals at δ_C 155.47, 131.25, 125.37, and 108.24 suggested two double bonds in the molecule. Therefore, it was concluded that compound **4** has an acetyl group and two double bonds. Thus, the structure of compound **4** was elucidated to be 3β,6α,12β-trihydroxydammar-20(21),24-diene-6-O-β-D-6'-O-acetylglucopyranoside. Since the compound has not been reported yet, we named it ginsenoside Rs_7.

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