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Ginsenoside-Rg_6, a Novel Triterpenoid Saponin from the Stem-Leaves of *Panax ginseng* C. A. Mey.

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Abstract: A novel dammarane-type triterpene oligoglycoside, named ginsenoside-Rg_6 **3**, was isolated from the stem-leaves of *Panax ginseng* C. A. Mey., together with two known ones, 20(*S*)-ginsenoside-Rg_2 **1** and 20(*R*)-ginsenoside-Rg_2 **2**. On the basis of chemical and physicochemical evidence , the structure of ginsenoside-Rg_6 have been elucidated as 6-O-α-L-rhamnosyl-(1→2)-β-D-glucopyranosyl-dammarane-(*E*)-20(22), 24-diene-3β, 6α, 12β-triol.

Keywords: *Panax ginseng* C. A. Mey., stem-leaves, triterpenoid, ginsenoside-Rg_6.

There is growing evidence in the literature that the root of *Panax ginseng* C. A. Mey. (ginseng radix), the well known traditional herbal remedy used in Chinese medicine for thousands of years, possesses an array of interesting pharmacological actions, such as cardioprotection, vasorelaxant, antistress, a stimulating activity of the central nervous system with effects on memory, learning and behavious. The biologically active constituents of ginseng radix have been pursued extensively and in recent years various ginsenosides, the dammarane-type triterpene oligoglycosides, have been characterized as the principal ingredients[1-8]. The stem-leaves of *P. ginseng* have been also recorded in "The Pharmacopoeia of Chinese People's Republic"(edited 2000)[9]. As a part of eluci-dating the biologically active principles of the stem-leaves of *P. ginseng*, we describe the structural determination of a novel dammarane-type triterpene oligoglycoside named as ginsenoside-Rg_6 **3**.

The H_2O extract of the dried stem-leaves of *Panax ginseng* was salting-out by NaCl to give a precipitate. The precipitate was dissolved in H_2O and then subjected to adsorption resin column eluting in a stepwise manner with EtOH / H_2O mixture to afford the total ginsenosides. The total ginsenosides was rechromatographed by reverse phase HPLC on C_{18} column eluting with MeOH / H_2O to get 20(*S*)-ginsenoside Rg_2 **1**, 20(*R*)-ginsenoside Rg_2 **2** and compound **3**, respectively.

3 was isolated as white powder. It was positive to Liebermann-Burchard and Molish reactions. Acid hydrolysis of **3** yielded an aglycone and two sugars which were

identified as glucose and rhamnose by PC comparison with authentic samples. The IR spectrum displayed strong absorption bands at 3420 and 1080 cm^{-1} suggestive of the oligoglycosidic structure. The electro-spray ionization mass spectrometry (ESI-MS) showed the fragment ion peaks at *m/z* 767 $[M + H]^+$ and 620$[M – rhamnosyl]^+$. Its molecular formula $C_{42}H_{71}O_{12}$ was determined by the high-resolution secondary-ion (HR-SI)-MS *m/z* 767.4951 $[M + 1]^+$ (calcd for $C_{42}H_{71}O_{12}$ 767.4946).

Detailed analysis of the ^1H (**Table 1**) and ^{13}C (**Table 2**) NMR spectra of **3** by ^1H-^1H COSY, ^1H-^{13}C COSY, HMQC and HMBC suggested the presence of eight methyl, one methyl doublet, two olefinic, and two anomeric proton signals. All these data suggested that **3** to be a dammarane-type triterpenoidal diglycoside with double bonds. The ^{13}C NMR spectral data of **3** was similar to that of 20 (*S*)- ginsenoside Rg_2 **1** and 20 (*R*)-ginsenoside Rg_2 **2** except for the signals assigned to C_{17} side-chain. These results indicated that **3** may be a compound closely related to **1** and **2** (see **Scheme 1**). Further comparison of the ^1H and ^{13}C NMR data of **3** with those of ginsenoside Rh_4 **4**[4], ginsenoside Rg_5 **5**[5-7], ginsenoside Rg_4 **6**[10], and ginsenoside Rh_3 **7**[11] showed that the signals of C_{17} side-chain were similar to those of **4** and **5** (see **Table** 2) but different from those of **6** and **7** (see **Table** 2). The stereochemistry of the double bond at C-20 (22) was supposed to be entgegen (E) from the fact that C_{21}-methyl signal was observed at δ 13.0[4, 12] in the ^{13}C NMR spectrum, nuclear Overhauser effect (NOE) correlation between C_{21}-Me and H-23 was observed, but no NOE correlation between C_{21}-Me and H–22 could be observed. These findings led us to conclude the structure of **3** as 6-O-α-L-rhamnosyl- (1→2)-β-D-glucopyranosyl-dammarane- (*E*)-20 (22), 24-diene-3β, 6α, 12β-triol (**Sche- me 1**), a novel compound, named ginsenoside-Rg_6.

Table 1 ^1H NMR Spectral Data (in C_5D_5N) of Compound **3**

H	δ_H; J/Hz	H	δ_H; J/Hz	H	δ_H; J/Hz	H	δ_H; J/Hz
1 α	0.83 m	13 α	1.91 m	24	5.18 t (6.5)	G-4	4.18 t (8.5)
1 β	1.41 m						
2 α	1.74 m	15 α	1.47 m	26	1.59 s	G-5	3.95 m
2 β	1.81 m	15 β	1.64 m				
3 α	3.45 dd (5.0, 11.3)	16 α	1.56 m	27	1.51 s	G-6a	4.35 dd (4.5, 10.0)
		16 β	1.83 m			G-6b	4.50 dd (4.5, 10.0)
5 α	1.38 d (9.5)	17 α	2.69 m	28 α	2.09 s	R-1	6.46 br s
6 β	4.65 dd (3.0, 9.5)	18 β	0.94 s	29 β	0.92 s	R-2	4.77 m
7 α	1.99 dd (2.5, 10.8)	19 β	1.22 s	30 α	1.33 s	R-3	4.69 m
7 β	2.25 dd (2.5, 10.8)						
9 α	1.52 d (10.5)	21	1.77 s	G-1	5.24 d (7.0)	R-4	4.29 m
11 α	1.36 m	22	5.43 t (6.5)	G-2	4.33 m	R-5	4.94 m
11 β	1.96 m						
12 α	3.89 ddd (5.0, 8.8, 10.0)	23 α	1.85 t (6.5)	G-3	4.31 m	R-6	1.76 d (6.0)
		23 β	2.74 t				

(6.5)

In HMBC spectrum of **3**, the obvious correlations between δ_C 50.0 (C-17) and δ_H 1.77 (Me-21) 5.43 (H-22), 2.74 (H-23), 1.91 (H-13), δ_C 140.0 (C-20) and δ_H 2.74 (H-23), 2.69 (H-17), 1.91 (H-13), 1.77 (Me-21), 1.56 (H-16α), 1.83 (H-16β), δ_C 123.0 (C-22) and δ_H 5.18 (H-24), 2.74 (H-23), 2.69 (H-17), 1.77 (Me-21), δ_C 123.7 (C-24) and δ_H 5.43 (H-22), 1.59 (Me-26), 1.51 (Me-27), δ_C 131.2 (C-25) and δ_H 2.74 (H-23), 1.59 (Me-26), 1.51 (Me-27), δ_C 101.7 (Glc-C-1) and δ_H 4.65 (H-6), 4.33 (Glc-H-3), and δ_C 101.8 (Rha- C-1) and δ_H 4.31 (Glc-H-2), 4.69 (Rha-H-3) also supported the proposed structure (**Scheme 1**).

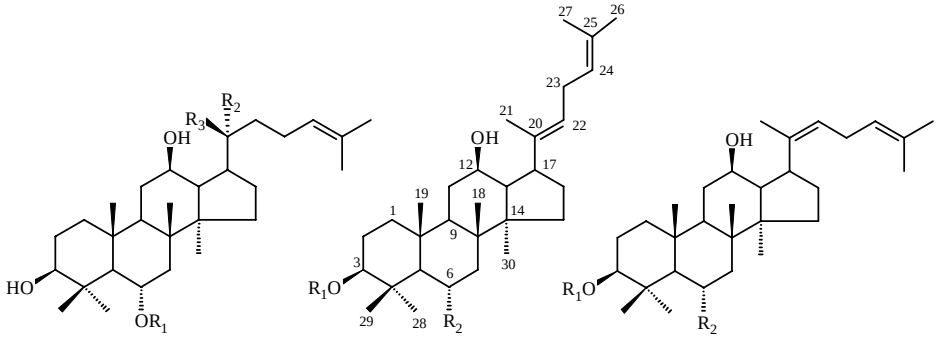
Table 2 ^{13}C NMR Spectral Data (in C_5D_5N) of Compounds **1** – **7**

C	Compounds						
	1	**2**	**3**	**4**	**5**	**6**	**7**
1	39.5 t	39.6 t	39.9 t	38.9	39.2	39.5 t	39.3
2	27.6 t	27.7 t	27.6 t	27.3	28.0	27.8 t	27.0
3	78.3 d	78.5 d	78.3 d	79.5	88.8	78.3 d	88.3
4	39.9 s	40.0 s	39.6 s	39.8	40.1	40.1 s	40.3
5	60.7 d	60.8 d	60.7 d	60.9	56.3	60.9 d	56.4
6	74.2 d	74.1 d	74.1 d	78.0	18.3	74.4 d	18.5
7	46.0 t	46.0 t	46.1 t	44.7	35.2	46.2 t	35.3
8	39.3 s	39.3 s	41.3 s	40.8	39.6	41.4 s	37.1
9	49.6 d	50.5 d	50.2 d	50.0	50.7	50.1 d	50.9
10	41.0 s	41.1 s	39.4 s	39.2	36.9	40.0 s	39.7
11	31.9 t	32.2 t	32.1 t	31.1	32.1	32.2 t	32.2
12	70.9 d	70.9 d	72.5 d	71.2	72.5	69.5 d	71.9
13	48.0 d	48.8 d	50.6 d	49.8	50.3	50.7 d	50.4
14	51.6 s	51.7 s	50.8 s	50.3	50.9	50.9 s	51.2
15	31.2 t	31.3 t	32.5 t	32.0	32.5	32.6 t	32.6
16	26.7 t	26.6 t	27.6 t	26.9	26.6	27.1 t	26.8
17	54.6 d	49.7 d	50.0 d	50.1	50.8	51.2 d	51.2
18	17.6 q	17.7 q	17.6 q	17.2	16.5	17.8 q	16.8
19	17.5 q	17.7 q	17.6 q	17.2	16.4	17.7 q	16.5
20	72.9 s	73.0 s	140.0 s	139.5	140.1	140.1 s	140.2
21	26.9 q	22.7 q	13.0 q	12.5	13.1	27.5 q	27.4
22	35.7 t	43.2 t	123.0 d	122.9	123.2	123.5 d	123.8
23	22.9 t	22.6 t	27.4 t	29.4	27.4	23.0 t	30.0
24	126.2 d	126.0 d	123.7 d	124.7	123.5	125.4 d	125.4
25	130.7 s	130.7 s	131.2 s	130.7	131.2	131.3 s	131.5
26	25.8 q	25.8 q	25.6 q	25.1	25.6	25.8 q	25.7
27	16.8 q	17.6 q	16.8 q	16.8	17.7	17.6 q	17.7
28	32.1 q	32.2 q	32.1 q	31.1	28.7	32.6 q	28.2
29	17.6 q	17.6 q	17.5 q	15.8	15.7	17.2 q	15.8
30	17.0 q	17.2 q	17.1 q	16.2	16.9	17.0 q	17.0
1′	101.7 d	101.7 d	101.7 d	105.4	105.0	101.8 d	106.9
2′	79.3 d	79.4 d	79.3 d	74.8	83.3	79.5 d	75.7
3′	78.4 d	78.4 d	78.4 d	79.0	78.1	78.4 d	78.7
4′	72.5 d	72.3 d	72.5 d	72.0	71.5	72.6 d	71.9
5′	78.3 d	78.4 d	78.4 d	77.5	77.8	78.4 d	78.3
6′	62.9 t	63.1 t	63.0 t	62.5	62.6	63.2 t	63.1
1″	101.8 d	101.9 d	101.8 d		105.9	102.0 d	
2″	72.2 d	72.6 d	72.2 d		77.0	72.3 d	
3″	72.3 d	72.4 d	72.3 d		78.2	72.4 d	
4″	74.0 d	74.3 d	74.3 d		71.5	74.2 d	

5″	69.4 d	69.4 d	69.4 d		78.0	69.5 d
6″	18.7 q	18.7 q	18.7 q		62.7	18.8 q

4=ginsenoside Rh_4, **5**=ginsenoside Rg_5, **6**=ginsenoside Rg_4, **7**=ginsenoside Rh_3

Scheme 1 The Structures of Compounds **1 - 7**



	R_1	R_2	R_3		R_1	R_2		R_1	R_2
1	-Glc(2-1)rha	Me	OH	**3**	H	-OGlc(2-1)rha	**6**	H	-OGlc(2-1)rha
2	-Glc(2-1)rha	OH	Me	**4**	H	-OGlc	**7**	-Glc	H
				5	-Glc(2-1)Glc	H			

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