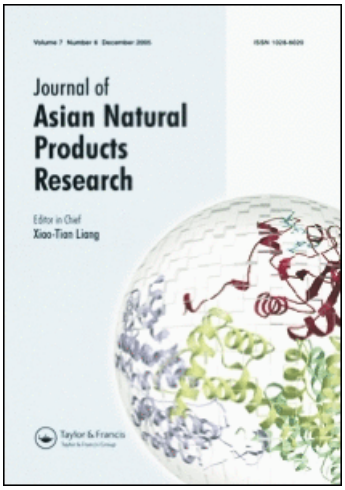

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Isoginsenoside-Rh_3, a new triterpenoid saponin from the fruits of Panax ginseng C. A. Mey

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ISOGINSENOSIDE-Rh_3, A NEW TRITERPENOID SAPONIN FROM THE FRUITS OF *PANAX GINSENG* C. A. MEY

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A new dammarane-type triterpene monoglucoside, named isoginsenoside-Rh_3, has been isolated from the fruits of *Panax ginseng* C. A. Mey, together with eight known analogs, ginsenoside-Rb_1, -Rb_2, -Rc, -Rd, -Re, -Rg_1, -Rh_1, -Rh_2. On the basis of chemical and physicochemical evidence, the structure of isoginsenoside-Rh_3 has been elucidated as 3-*O*-β-D-glucopyranosyl-dammarane-(*E*)-20(22),24-diene-3β,12β-diol (**1**).

Keywords: *Panax ginseng* C. A. Mey; Fruits; Triterpenoid; Isoginsenoside-Rh_3

INTRODUCTION

There is growing evidence in the literature that the fruits of *Panax ginseng* C. A. Mey, the well-known traditional herbal remedy used in Chinese medicine, possess 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 behaviour. The biologically active constituents of ginseng fruits have been studied extensively and various ginsenosides, dammarane-type triterpene oligoglycosides, have been characterized as the principal ingredients [1–3]. As a part of elucidating the biologically active principles of the fruits of *P. ginseng*, we describe the structural determination of a new dammarane-type triterpene monoglucoside named as isoginsenoside-Rh_3 (**1**).

RESULTS AND DISCUSSION

1 was isolated as white powder. It gave positive Liebermann–Burchard and Molish reactions, and electro-spray ionization mass spectrometry (ESI-MS) showed quasi-molecular ion peaks

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at *m/z* 627 $[M + Na]^+$ and 443 $[M - glucosyl + H]^+$. Its molecular formula $C_{36}H_{60}O_7$ was determined by high-resolution secondary-ion mass spectrometry (HR-SIMS), *m/z* 627.4225 $[M + Na]^+$. The IR spectrum displayed strong absorption bands at 3407 and 1075 cm^{-1}, suggestive of the glycosidic structure. Characteristic signals owing to a glucosyl group were observed in the NMR spectra of **1** [^{1}H NMR δ (ppm): 4.01 (1H, dd, $J = 6.0$, 8.5 Hz, glc-H-5), 4.03 (1H, t, $J = 8.5$ Hz, glc-H-2), 4.21 (1H, t, $J = 8.5$ Hz, glc-H-4), 4.25 (1H, t, $J = 8.5$ Hz, glc-H-3), 4.40 (1H, dd, $J = 6.0$, 11.5 Hz, glc-Ha-6), 4.59 (1H, d, $J = 11.5$ Hz, glc-Hb-6), 4.95 (1H, d, $J = 8.0$ Hz, glc-H-1); ^{13}C NMR δ (ppm): 106.9 (C-1), 75.8 (C-2), 78.7 (C-3), 71.8 (C-4), 78.4 (C-5), 63.1 (C-6)]. Acid hydrolysis of **1** with 9% methanolic HCl yielded an aglycone and one sugar which were identified as D-glucose by PC comparison with authentic sample. The large J (8.0 Hz) indicated β-glucosidic linkages.

Eight methyl groups [^{1}H NMR δ (ppm): 0.79 (s), 0.96 (s), 0.98 (s), 1.00 (s), 1.31 (s), 1.56 (s), 1.60 (s), 1.80 (s); ^{13}C NMR δ: 13.1, 15.8, 16.5, 16.8, 17.0, 17.7, 25.7, 28.1] and two olefinic bonds signals [^{1}H NMR δ: 5.49 (1H, t, $J = 7.0$ Hz, H-22), 5.21 (1H, t, $J = 6.8$ Hz, H-24) ^{13}C NMR δ: 123.8 d, 124.5 d, 131.2 s, 140.1 s] were observed. All these data suggested that **1** is a dammarane-type triterpenoid glycoside with double bonds [4,5]. Compared with ginsenoside Rg_6 (**2**) [4] and ginsenoside Rh_3 (**3**) [5], **1** was similar to **2**, except for the signals assigned to cycles A, B, C, D and sugar moieties (Fig. 1), and similar to **3** except for

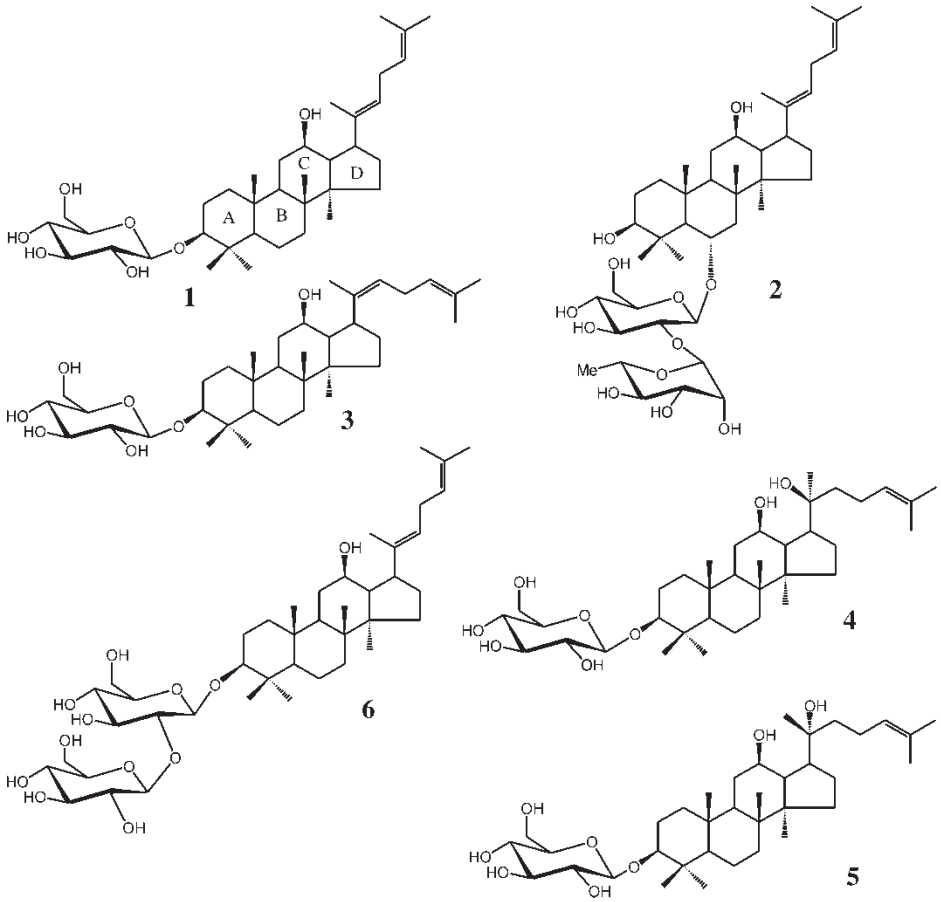

FIGURE 1 Structures of compounds **1**–**6**.

the signals assigned to the C_{17} side-chain in the ^{13}C NMR spectral data. These results indicated that **1** is closely related to **2** and **3**. Further comparison of the ^{1}H and ^{13}C NMR data of **1** with those of **2** [4], **3** [5], 20(*S*)-ginsenoside Rh_2 (**4**) [6], 20(*R*)-ginsenoside Rh_2 (**5**) [5], and ginsenoside-Rg_5 (**6**) [7] (Fig. 1, and Table I) showed that the signals of the C_{17} side-chain are similar to those of **2** and **6**. The stereochemistry of the double bond at C-20(22) was proposed to be *E* from the C_{21}-methyl signal at δ 13.0 [4,7] in the ^{13}C NMR spectrum. These results suggest that **1** is 3-*O*-β-D-glucopyranosyl-dammarane-(*E*)-20(22),24-diene-3β,12β-diol (Fig. 1), a novel compound, named isoginsenoside-Rh_3.

Meanwhile, the ^{1}H and ^{13}C NMR signals of **1** were assigned by DEPT and 2D NMR with pulse-field gradient (PFG) techniques (gCOSY, gHMQC and gHMBC).

TABLE I ^{13}C NMR spectral data (δ in C_5D_5N) of **1**–**6**

	*Compounds**					
Carbon no.	***1***	***2*** [4]	***3*** [5]	***4*** [6]	***5*** [5]	***6*** [7]
1	39.2 t	39.6 t	39.3	39.4	39.1	39.2
2	26.7 t	27.6 t	27.0	27.3	26.7	28.0
3	88.7 d	78.3 d	88.3	88.9	88.8	88.8
4	39.7 s	39.6 s	40.3	40.3	40.1	40.1
5	56.4 d	60.7 d	56.4	56.7	56.4	56.3
6	18.4 t	74.1 d	18.5	18.7	18.5	18.3
7	35.3 t	46.1 t	35.3	36.0	35.2	35.2
8	40.2 s	41.3 s	37.1	37.2	37.0	39.6
9	50.7 d	50.2 d	50.9	50.7	50.4	50.7
10	37.0 s	39.4 s	39.7	39.8	39.7	36.9
11	32.2 t	32.1 t	32.2	32.2	32.2	32.1
12	72.4 d	72.5 d	71.9	71.1	70.9	72.5
13	50.8 d	50.6 d	50.4	48.8	49.2	50.3
14	50.9 s	50.8 s	51.2	51.9	51.8	50.9
15	32.6 t	32.5 t	32.6	31.5	31.4	32.5
16	26.7 t	27.6 t	26.8	26.8	26.7	26.6
17	50.4 d	50.0 d	51.2	54.8	50.6	50.8
18	15.8 q	17.6 q	16.8	16.8	16.8	16.4
19	16.8 q	17.6 q	16.5	16.4	16.4	16.5
20	140.1 s	140.0 s	140.2	73.2	73.0	140.1
21	13.1 q	13.0 q	27.4	27.0	22.8	13.1
22	123.8 d	123.0 d	123.8	35.4	43.3	123.2
23	27.4 t	27.4 t	30.0	23.1	22.6	27.4
24	124.5 d	123.7 d	125.4	126.4	126.1	123.5
25	131.2 s	131.2 s	131.5	130.7	130.8	131.2
26	25.7 q	25.6 q	25.7	25.7	25.8	25.6
27	17.7 q	16.8 q	17.7	17.7	17.7	17.7
28	28.1 q	32.1 q	28.2	28.3	28.2	28.7
29	16.5 q	17.5 q	15.8	16.0	15.8	15.7
30	17.0 q	17.1 q	17.0	17.3	17.4	16.9
1′	106.9 d	101.7 d	106.9	106.7	106.9	105.0
2′	75.8 d	79.3 d	75.7	75.8	75.8	83.3
3′	78.7 d	78.4 d	78.7	78.7	78.8	78.1
4′	71.8 d	72.5 d	71.9	72.2	71.9	71.5
5′	78.4 d	78.4 d	78.3	78.0	78.3	77.8
6′	63.1 t	63.0 t	63.1	63.3	63.1	62.6
1″		101.8 d				105.9
2″		72.2 d				77.0
3″		72.3 d				78.2
4″		74.3 d				71.5
5″		69.4 d				78.0
6″		18.7 q				62.7

* **1**, isoginsenoside Rh_3; **2**, ginsenoside Rg_6; **3**, ginsenoside Rh_3; **4**, 20(*S*)-ginsenoside Rh_2; **5**, 20(*R*)-ginsenoside Rh_2; **6**, ginsenoside Rg_5.

EXPERIMENTAL

General Experimental Procedures

IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR Spectrometer with KBr pellets. Optical rotations were determined on a Perkin-Elmer 243 Polarimeter. NMR spectra were obtained on a Varian INOVA-500 spectrometer in pyridine-d_5 at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts (δ ppm) are given relative to TMS as internal standard. ESI-TOF-MS and HR-SIMS spectra were recorded on MDS SCIEX API QSTAR and APEX II FT-ICR (Bruker Daltonics) mass spectrometers, respectively. Macroporous resin Diaion-101 was produced by Nankai University of China.

Plant Material

The fresh fruits of *Panax ginseng* C. A. Mey. were collected in Changbai Country of Jilin Province, and were identified by Profesor Xiang-gao Li. The sarcocarp was taken from the fresh fruits and stored at 0°C before extraction.

Extraction and Isolation

The fresh fruits (10 kg) of *Panax ginseng* were mixed with water (50 L) to yield a syrup solution and then the seeds were removed. The syrup solution was centrifuged to get a supernatant. After evaporation of water solution *in vacuo*, the residue (480 g) was chromatographed over the Diaion-101 resin, eluting with H_2O (30 L) and 70% EtOH (30 L), respectively. Removal of the EtOH from the 70% EtOH fraction under reduced pressure gave a water solution that was extracted with n-BuOH saturated with H_2O to afford n-BuOH extracts (80 g). The latter extracts were further purified by silica gel column chromatography using $CHCl_3$–MeOH (10 : 2 → 10 : 2.5 → 10 : 3 → 10 : 4 → 10 : 5) as solvent and then repeatedly chromatographed on silica gel column under low pressure to yield isoginsenoside-Rh_3 (**1**) (31 mg), ginsenosides-Rh_2 (10 mg), -Rh_1 (12 mg), -Rg_1 (40 mg), -Re (45 mg), -Rd (11 mg), -Rc (8 mg), -Rb_2 (16 mg), -Rb_1 (32 mg), respectively.

Isoginsenoside-Rh$_3$ (1)

A white powder that gave positive Liebermann–Burchard and Molish reactions; $[\alpha]_D$ + 25.6 (*c*, 1.0, MeOH); IR ν_{max} (KBr) (cm^{-1}): 3407 (OH), 1636 (C=C), 1453, 1385 (–CH_3), 1075 (O-gly), 1029; ESI-TOF-MS: *m/z* 627 [M + Na]$^+$, 443 [M − glucosyl + H]$^+$; HR-SIMS: *m/z* 627.4225 [M + Na]$^+$ (calcd for $C_{36}H_{60}O_7Na$ 627.4231); ^{1}H NMR (500 MHz, py-d_5) δ (ppm): 0.74 (1H, d, J = 12.0 Hz, H-5), 0.79 (3H, s, Me-19), 0.96 (3H, s, Me-30), 0.98 (3H, s, Me-29), 1.00 (3H, s, Me-18), 1.31 (3H, s, Me-28), 1.56 (3H, s, Me-27), 1.60 (3H, s, Me-26), 1.80 (3H, s, Me-21), 2.77 (2H, dd, J = 7.0, 12.0 Hz, H-23), 2.81 (1H, m, H-17), 3.37 (1H, d.t., J = 3.5, 8.0 Hz, H-3), 3.90 (1H, m, H-12), 4.01 (1H, dd, J = 6.0, 8.5 Hz, glc-H-5), 4.03 (1H, t, J = 8.5 Hz, glc-H-2), 4.21 (1H, t, J = 8.5 Hz, glc-H-4), 4.25 (1H, t, J = 8.5 Hz, glc-H-3), 4.40 (1H, dd, J = 6.0, 11.5 Hz, glc-Ha-6), 4.59 (1H, d, J = 11.5 Hz, glc-Hb-6), 4.95 (1H, d, J = 8.0 Hz, glc-H-1), 5.21 (1H, t, J = 7.0 Hz, H-24), 5.49 (1H, t, J = 7.0 Hz, H-22); ^{13}C NMR data see in Table I.

Acid Hydrolysis of 1

Compound **1** (10 mg) was dissolved in MeOH (10 ml) and refluxed with 9% HCl (3 ml) on a water bath for 5 h. The reaction mixture was concentrated and then dissolved in MeOH (1 ml)

for PC (n-BuOH–EtOH–H_2O–conc. NH_4OH, 45:5:49:1) together with authentic D-glucose ($R_f = 0.19$).

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