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CHARACTERIZATION OF THREE OLEANE-TYPE SAPONINS FROM *Panax ginseng*

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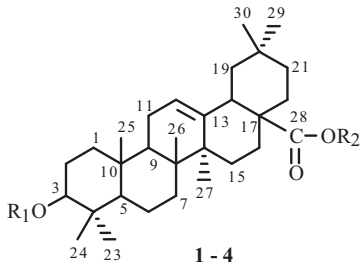
Ginseng, the root and rhizome of *Panax ginseng* C. A. Mey. (Araliaceae), has been used as a very old Chinese medicine by the people in the Eastern Asia regions for over 2000 years. *P. ginseng* has very extensive pharmacological activities, including antifatigue and antidiabetes, as well as activities in the prevention of cancer and slowing the aging process [1–3]. The pharmacological properties of ginseng are generally attributed to ginsenosides. Based on the structure of aglycone, the ginsenosides can be divided into protopanaxadiol-type saponins (PPD), protopanaxatriol-type saponins (PPT), and oleanane-type saponins. More than 150 ginsenosides have been isolated from various parts of ginseng and processed ginseng so far [4–11], which belong mainly to dammarane-type saponins. However, to date, only four oleanane-type saponins have been isolated from *P. ginseng*, including ginsenoside-Ro, ginsenoside-Ro methyl, polyacetylene ginsenoside-Ro [12], and ginsenoside-Ri [13]. In this study, three oleanane-type saponins, identified as chikusetsusaponin IVa (**1**), zingibroside R_1 (**2**), and oleanolic acid 28-*O*-β-D-glucopyranoside (**3**), were further isolated from the plant. Numerous activity studies have been performed on the dammarane-type ginsenosides, but little attention has been focused on oleanane-type ginsenosides. To further investigate their antitumor activities, cytotoxicity assays against HepG2, B16, and Lewis tumor cell lines *in vitro* have been performed using MTT methods.

Compound **1** was obtained as a white amorphous powder. Acid hydrolysis of compound **1** afforded glucose, glucuronic acid, and oleanolic acid, which were separately identified by direct comparison with authentic samples using TLC. The ^{1}H NMR spectrum of compound **1** showed seven singlet methyl signals (δ 0.81, 0.86, 0.92, 0.94, 0.96, 1.06, 1.17) together with an olefinic proton at δ 5.26 (1H, m) and two anomeric proton signals at δ 5.39 (1H, d, J = 8.2 Hz) and 4.39 (1H, d, J = 7.8 Hz). The presence of olefinic carbon signals (δ 122.4 and 143.4) suggested that compound **1** could be an oleanane-type saponin. The negative ion ESI-MS of compound **1** exhibited ions at *m/z* 793.7 $[M - H]^-$, 631.4 $[M - H - Glc]^-$, 613.4 $[M - H - Glc - H_2O]^-$, 569.3 $[M - H - Glc - H_2O - CO_2]^-$, 497.2 $[M - H - Glc - CO_2 - C_3H_6O_3]^-$, and 455.2 $[M - H - Glc - GluA]^-$. In comparison with the negative ion ESI-MS of ginsenoside-Ro [14], it had closely similar fragmentation pathways, except for a loss of 162 Da (one glucose unit). The physicochemical properties and spectral data (Table 1) of compound **1** were identical to chikusetsusaponin IVa [15].



1 - 4

1: R_1 = GlcUA, R_2 = Glc; **2:** R_1 = GlcUA^2→Glc, R_2 = H
3: R_1 = H, R_2 = Glc; **4:** R_1 = GlcUA^2→Glc, R_2 = Glc

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TABLE 1. ^{13}C NMR Data of Compounds **1–3** (150 MHz, TMS, δ, ppm)

C atom	**1***	**2****	**3****	C atom	**1***	**2****	**3****
					Glc UA		
1	38.4	38.4	40.6	3-*O*-Sugar			
2	25.6	26.4	29.7	1′	105.6	105.2	
3	89.7	88.9	78.6	2′	71.8	82.7	
4	39.3	39.3	41.0	3′	76.3	76.9	
5	55.6	55.5	56.3	4′	73.9	72.9	
6	17.9	18.2	20.4	5′	75.1	77.7	
7	31.7	33.0	34.8	6′	179.8	172.3	
8	38.8	39.5	41.5			Glc (1–2)	
9	47.6	47.7	49.8	1″		105.8	
10	36.5	36.7	39.0	2″		77.5	
11	23.1	23.5	25.0	3″		78.1	
12	122.4	122.3	123.4	4″		71.4	
13	143.4	144.6	144.6	5″		77.2	
14	41.5	41.9	43.8	6″		62.4	
15	27.5	28.1	29.9	28-*O*-Sugar	Glc		Glc
16	22.6	23.5	25.4	1‴	94.3		96.2
17	46.6	46.4	48.6	2‴	72.5		74.6
18	41.2	41.7	43.4	3‴	77.3		79.8
19	45.8	46.2	47.8	4‴	69.7		71.6
20	30.1	30.7	32.4	5‴	76.9		79.4
21	33.5	34.0	35.6	6‴	61.0		62.7
22	32.6	33.0	34.1				
23	27.1	27.9	30.4				
24	14.6	16.5	18.1				
25	15.5	15.2	17.2				
26	16.3	17.1	19.1				
27	24.9	25.9	27.7				
28	176.6	179.9	176.9				
29	32.1	33.0	34.7				
30	22.5	23.4	25.3				

*^{13}C NMR data were measured in CD_3OD; **^{13}C NMR data were measured in pyridine-d_5, Glc UA – β-D-glucuronic acid.

Compound **2** was obtained as a white amorphous powder. Acid hydrolysis of compound **2** afforded glucose, glucuronic acid, and oleanolic acid, which were separately identified by direct comparison with authentic samples using TLC. The ^{1}H NMR spectrum of compound **2** showed seven singlet methyls (δ 0.77, 0.92, 0.95, 0.97, 1.07, 1.25, 1.27) together with an olefinic proton at δ 5.42 (1H, m) and two anomeric proton signals at δ 5.39 (1H, d, J = 7.2 Hz), 5.00 (1H, d, J = 7.2 Hz). The presence of olefinic carbon signals (δ_C 122.3 and 144.6) suggested that compound **2** could be an oleanane-type saponin. The negative ion ESI-MS of compound **2** exhibited ions at *m/z* 793.6 $[M - H]^-$, 731.5 $[M - H - CO_2 - H_2O]^-$, 631.4 $[M - H - Glc]^-$, 613.4 $[M - H - Glc - H_2O]^-$, 569.3 $[M - H - Glc - H_2O - CO_2]^-$, 551.5 $[M - H - Glc - 2H_2O - CO_2]^-$, and 455.2 $[M - H - Glc - GluA]^-$. In comparison with compound **1**, it had closely similar fragmentation pathways, except for the characteristic ion moiety *m/z* 731.5 (CO_2 and H_2O unit) [12]. The physicochemical properties and spectral data (Table 1) of compound **2** were identical to zingibroside R_1 [16].

Compound **3** was obtained as a white amorphous powder. Acid hydrolysis of compound **3** afforded glucose and oleanolic acid, which were separately identified by direct comparison with authentic samples using TLC. The ^{1}H NMR spectrum of compound **3** showed seven singlet methyls (δ 0.90, 0.92, 0.94, 1.04, 1.15, 1.24, 1.26) and an olefinic proton at δ 5.47 (1H, m) and 6.36 (1H, d, J = 8.4 Hz) for the anomeric proton signal. The presence of olefinic carbon signals (δ 123.4 and 144.6) suggested that compound **3** could be an oleanane-type saponin. The configuration of the anomeric position was determined to be β on the basis of the coupling constant of the anomeric proton signal in the ^{1}H NMR spectrum of compound **3** (1H, d, J = 8.4 Hz). The signals due to the β-D-glucopyranosyl moiety were observed in the ^{13}C NMR spectrum at δ 96.2, 74.6, 79.8, 71.6, 79.4, and 62.7. The physicochemical properties and spectral data (Table 1) of compound **3** were identical to oleanolic acid 28-*O*-β-D-glucopyranoside [16].

The isolated oleanane-type saponins were all tested for their cytotoxicities against HepG2, B16, and Lewis tumor cell lines using the MTT method with compound K as positive control. Among them, compounds **1**, **3**, and **4** showed no cytotoxicity effect, while compound **2** showed potent cytotoxic activities against HepG2, B16, and Lewis cell lines with IC_{50} values of 35.39, 35.44, and 47.30 μM, respectively.

Plant Material. The roots of *P. ginseng* C. A. Mey. were collected from Jilin Province, China, in August 2010. Red ginseng, obtained by steaming fresh *P. ginseng* roots at 120°C for 8 h, is manufactured by the National R & D Center for Ginseng and Pilose Antler Product Processing. The botanical origin of the material was identified by Dr. Jing-hui Zhao, Institute of Special Wild Economic Animals and Plants, CAAS, Jilin Province. A voucher specimen (No. 2010127) was deposited at the Institute of Special Wild Economic Animals and Plants, CAAS, Jilin, China.

Extraction and Isolation. The air-dried roots of red ginseng (12.8 kg) was extracted with methanol, sonified (4 × 60 L; each 1 h), and concentrated *in vacuo* to give the MeOH extract. The MeOH extract was suspended in H_2O and extracted with *n*-BuOH. The concentrated butanol fraction was suspended with excess water and then subjected to D_{101} macroporous absorption resin eluting with H_2O, 20% EtOH, 70% EtOH, and 95% EtOH in sequence. The 70% EtOH fraction (294.0 g) was subjected to silica gel column chromatography eluted with CH_2Cl_2–MeOH (100:1→0:1) to yield seven fractions (Fr.1–7). Fractions 1–7 were subjected to silica gel and RP-18 column chromatography and preparative HPLC, affording 19 saponins (**1**–**19**). On the basis of spectroscopic and chemical methods, the structures of compounds **1**–**19** were elucidated as chikusetsusaponin IVa (**1**), zingibroside R_1 (**2**), oleanolic acid 28-*O*-*β*-D-glucopyranoside (**3**), and ginsenoside – Ro (**4**), 20 (*S*)-Rh_1 (**5**), 20 (*R*)-Rh_1 (**6**), 20 (*S*)-Rg_2 (**7**), 20 (*R*)-Rg_2 (**8**), 20 (*S*)-Rg_3 (**9**), 20 (*R*)-Rg_3 (**10**), Rg_5 (**11**), Rk_1 (**12**), Rs_3 (**13**), Re (**14**), Rg_1 (**15**), Rb_1 (**16**), Rb_2 (**17**), Rc (**18**), and Rd (**19**) [12]. Compounds **1**–**3** were oleanane-type saponins isolated for the first time from *P. ginseng*.

Cytotoxicity Assays. The cytotoxic activities of compounds **1**–**4** against HepG2, B16, and Lewis tumor cell lines were evaluated using the MTT assay. The assay was performed in the Cell Culture Laboratory, Pharmaceutical College, Jilin University. Compound K was used as the positive control with IC_{50} of 8.1, 6.1, and 8.9 μM for HepG2, B16, and Lewis tumor cells, respectively.

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