Asteryunnanosides F and G: Two New Triterpenoid Saponins from *Aster yunnanensis*

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Abstract

Two new triterpenoid saponins, asteryunnanosides F and G, were isolated from the roots of *Aster yunnanensis*. Their structures were determined as oleanolic acid-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside and 3-*O*-β-glucopyranosyloleanolic acid-28-*O*-β-D-glucopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside by spectral data, especially 2D NMR analysis including COSY, HETCOR, HOHAHA, and ROESY techniques, and chemical transformation.

Key words

Aster yunnanensis, Compositae, asteryunnanosides F and G, triterpenoid saponins, oleanolic acid, 2D NMR.

Introduction

Some plants in the genus *Aster* (Compositae) have been used as drugs for the treatment of fever, cold, tonsillitis, snake bite, and bee stings in Chinese traditional medicine (1). During our studies on the bioactive compounds from medicinal plants, the potent analgesic and sedative activities of the *n*-butanol extract of the roots of *A. yunnanensis* encouraged us to study the glycosides of this plant. The present paper deals with the isolation and structure elucidation of two new triterpenoid saponins, asteryunnanoside F (**1**) and G (**2**), from its roots.

Materials and Methods

General experimental procedures

Melting points were determined on a Kofler apparatus and are uncorrected; $[\alpha]_D$ values were measured at 28 °C on a JASCO DIP-181 polarimeter. IR spectra were obtained on a Perkin-Elmer 599B infrared spectrometer. FAB-MS were recorded by the direct-inlet method on a VG ZAB-HS mass spectrometer using glycerol as matrix. EI-MS were obtained on a MAT-95 mass spectrometer. ^{1}H and ^{13}C-NMR, ^{1}H-^{1}H COSY, HETCOR spectra were obtained on a Bruker AM400 spectrometer operating at 400 MHz for δ_H and 100 MHz for δ_C. HOHAHA and ROESY spectra were obtained on a GE OMEGA-500 spectrometer operating at 500 MHz for δ_H. PC of sugars were run on Whatman No. 1 using the solvent systems *n*-BuOH-pyridine-H_2O (6 : 4 : 3) and *n*-BuOH-AcOH-H_2O (4 : 1 : 5), respectively, and detected with aniline phthalate reagent. MPLC was carried out on a LiChroprep RP-8 (40–63μm) Labor column (31 cm × 25 mm i.d.) with MeOH-H_2O (5.5–6 : 4) as eluent (flow rate 2 ml/min). SiO_2, 200–300 mesh (Qingdao Marine Chemical Factory, Qingdao, China) was used for column chromatography and SiO_2 GF_{254} for TLC.

Plant material

The roots of *A. yunnanensis* were collected in August, 1992 in Li-Jiang County, Yunnan Province, southwest China. A voucher specimen was identified by Prof. Z. W. Lu and is deposited in the Herbarium of Kunming Institute of Botany, Academia Sinica, Kunming, China.

Extraction and isolation

The dried roots (15 kg) of *A. yunnanensis* were extracted with 70 % ethanol five times at room temperature. After concentration under vacuum, the residue (3.8 kg) was suspended in H_2O and then extracted successively with petroleum ether, EtOAc, and *n*-BuOH. The residue (698 g) from the *n*-BuOH extract was chromatographed on highly porous resin (SIP-1300, 1.5 kg) eluting initially with H_2O, followed by EtOH. The EtOH eluate (350 g) was subjected to gradient CC on silica gel (200–300 mesh, 1.5 kg) eluting with a $CHCl_3$-MeOH-H_2O (8 : 1 : 0.1 to 1 : 1 : 0.1) mixtures, (500 ml each eluent) and separated into 8 crude fractions (fractions 1–8) monitoring by TLC. Fraction 3 was separated by repeated CC over silica gel (200–300 mesh, 40 g) with $CHCl_3$-MeOH-H_2O (4 : 1 : 0.1) as eluent (each 25 ml) and finally purified by LiChroprep (40–63μm) column (31 × 2.5 cm) with MeOH-H_2O (5 : 5) (each 50 ml) to yield asteryunnanoside F (**1**, 85 mg, 0.00057 %). Fraction 6 was further subjected to CC on silica gel (100 g) eluting with $CHCl_3$-MeOH-H_2O (9 : 3 : 0.5) to give 3 fractions, and the second fraction was repeatedly chromatographed on LiChroprep RP-8 with MeOH-H_2O (6 : 4) to afford asteryunnanoside G (**2**, 480 mg, 0.0032 %).

Asteryunnanoside F (**1**): Amorphous powder; m.p. 192–194 °C; $[\alpha]_D$: +16.7° (pyridine, *c* 0.22); IR ν_{KBr}: 3400, 1730, 1630 and 1000–1100 cm^{-1}; FAB-MS *m/z*: 803 $[M + Na]^+$ and 787 $[M + Li]^+$; ^{1}H-NMR: aglycone moiety; δ 0.86, 0.87, 0.92, 1.02, 1.13, 1.25, 1.26 (each 3H, s, tert-Me x 7), 3.19 (1H, dd, J = 14.3, 4.1 Hz, 18-H), 3.43 (1H, dd, J = 10.3, 5.3 Hz, 3-H) and 5.43 (1H, br. s, 12-H); sugar moiety: see Table **2**; ^{13}C-NMR: see Tables **1** and **2**.

Acid hydrolysis of **1**: A solution of compound **1** (20 mg) was hydrolyzed in 2 N HCl-MeOH (5 ml) at 100 °C for 4 h. From the ether extract, oleanolic acid (7 mg) was obtained by recrystallization from MeOH and identified by direct comparison with an authentic sample by co-TLC, co-m.p. and ^{1}H-NMR data.

After repeated evaporation of the H_2O layer at 40 °C until the solution no longer showed an acidic reaction, glucose was detected by PC and TLC in direct comparison with an authentic sample.

Asteryunnanoside G (**2**): Colorless crystals; m.p. 230–232 °C; $[\alpha]_D$: −21.0° (pyridine, *c* 0.43); IR ν_{KBr}: 3400, 1735, 1640, 1000–1100 cm^{-1}; Elem. Anal.: Calcd for $C_{54}H_{88}O_{22} \cdot H_2O$: C, 58.59; H, 8.14; Found: C, 58.57; H, 8.17; FAB-MS *m/z*: 1111 $[M + Na]^+$ and 1095 $[M + Li]^+$; ^{1}H-NMR (pyridine-d_5): aglycone moiety; δ 0.83, 0.84, 0.85, 0.95, 1.05, 1.22, 1.25 (each 3H, s, tert-Me x 7), 3.15 (1H, dd, *J* = 14.5, 4.0 Hz, 18-H), 3.34 (1H, dd, *J* = 10.5, 5.0 Hz, 3-H), 5.43 (1H, br. s, 12-H); sugar moiety: see Table **2**; ^{13}C-NMR: see Tables **1** and **2**.

Alkaline hydrolysis of **2**: A solution of **2** (50 mg) in 5% KOH-MeOH (5 ml) was heated at 100 °C for 2 h. After neutralization with dilute HCl, and removal of MeOH, the remaining mixture was passed through a column of highly porous resin (SIP-1300, 50 g) eluted H_2O and then MeOH. From the MeOH eluent after treatment with CH_2N_2 and evaporation to dryness, the methyl ester of prosapogenin (**2a**, 28 mg) was isolated by silica gel column chromatography (10 g) eluting with $CHCl_3$-MeOH (10 : 1). **2a**: colorless needles; m.p. 158–160 °C; FAB-MS *m/z*: 655 $[M + Na]^+$ and 639 $[M + Li]^+$; ^{1}H-NMR (pyridine-d_5): aglycone: δ 0.79, 0.83, 0.84, 0.90, 0.99, 1.21 and 1.30 (each 3H, s, tert-Me x 7), 3.69 (3H, s, OMe), 5.36 (1H, br. s, 12-H), sugar moiety: see Table **2**. ^{13}C-NMR: see Tables **1** and **2**.

Acid hydrolysis of **2** *and* **2a**: Solutions of **2** and **2a** (each 5 mg) in 2 N HCl-MeOH (2 ml) were heated at 100 °C for 4 h. The precipitates formed in both cases were collected and identified as oleanolic acid. The filtrate was repeatedly evaporated at 40 °C until the solution showed a neutral reaction. The residue was examined by PC and TLC in direct comparison with an authentic sample. Compound **2** gave glucose and rhamnose, and **2a** gave glucose, respectively.

Table 1 ^{13}C-NMR data of the aglycones of **1**, **2**, and **2a***.

C	1	2	2a	DEPT
1	39.04	39.01	39.23	CH_2
2	28.20	26.71	27.05	CH_2
3	78.48	88.98	89.38	CH
4	39.46	39.59	39.99	C
5	55.99	56.09	56.32	CH
6	18.90	18.77	18.98	CH_2
7	33.36	33.50	33.53	CH_2
8	40.08	40.09	40.18	C
9	48.30	48.21	48.42	CH
10	37.53	37.17	37.48	C
11	23.91	23.96	24.21	CH_2
12	122.98	122.68	123.34	CH
13	144.24	144.33	144.67	C
14	42.30	42.49	42.47	C
15	28.45	28.92	28.57	CH_2
16	23.56	23.58	23.90	CH_2
17	47.20	47.36	47.46	C
18	41.86	42.13	42.31	CH
19	46.44	46.64	46.59	CH_2
20	31.00	30.82	31.29	C
21	34.13	34.26	34.46	CH_2
22	32.62	32.45	33.29	CH_2
23	28.87	28.41	28.75	CH_3
24	16.65	17.60	17.65	CH_3
25	15.78	15.77	15.96	CH_3
26	17.64	17.10	17.50	CH_3
27	26.17	25.99	26.63	CH_3
28	176.54	176.54	176.49	C
29	33.19	33.24	33.61	CH_3
30	23.81	23.99	24.14	CH_3
OCH_3			52.07	CH_3

* In pyridine-d_5, 100 MHz for δ_c, ppm.

Results and Discussion

Two new triterpenoid saponins, asteryunnanosides F (**1**) and G (**2**) have been isolated from the *n*-butanol soluble fraction of the roots of *A. yunnanensis*.

Asteryunnanoside F (**1**) was obtained as a white amorphous powder, m.p. 192–194 °C, $[\alpha]_D$: +16.7° (pyridine, *c* 0.22). Its IR spectrum indicated the presence of hydroxy (3400 cm^{-1}) and ester (1730 cm^{-1}) groups, a double bond (1630 cm^{-1}) and glycosidic linkages (1000 – 1100 cm^{-1}). Its FAB-MS gave two quasimolecular ion peaks at *m/z* 803 $[M + Na]^+$ and 787 $[M + Li]^+$ indicating a molecular weight of 780 amu. Interpretation of the ^{1}H-, ^{13}C- and DEPT spectra of **1** led to a molecular formula of $C_{42}H_{68}O_{13}$. The ^{1}H-NMR spectrum showed the signals of seven singlet methyl groups at δ 0.86, 0.87, 0.92, 1.02, 1.13, 1.25, and 1.26, a trisubstituted olefinic proton at δ 5.43, and two anomeric protons at δ 6.29 (d, *J* = 8.1 Hz) and 5.05 (d, *J* = 7.8 Hz). The ^{13}C-NMR data revealed the signals of six aliphatic quaternary carbons at δ 31.00, 37.53, 39.46, 40.08, 42.30, and 47.20, a pair of olefinic carbons at δ 122.98 and 144.24, an esterified carbonyl carbon at δ 176.54, and two anomeric carbons at δ 95.76 and 105.33. These data indicated that **1** was an esterified olean-12-ene type saponin with two sugar units.

Acid hydrolysis of **1** gave glucose and oleanolic acid as its aglycone by direct comparison of co-TLC, co-m.p., and EI-MS with an authentic sample. The C-28 signals of **1** in the ^{13}C-NMR at δ 176.54 ppm instead of 180.1 ppm in oleanolic acid caused by glycosylation shifts (2) suggested that the glycosylation had taken place at the C-28 position.

The downfield shift of C-6 of the inner glucosyl unit by +7.2 ppm to 69.65 ppm showed that the outer glucosyl unit must be attached at the C-6′ position of the inner glucose unit. The anomeric centers of both glucosyl units were confirmed as having β-configurations based on the coupling constants of their anomeric protons (8.1 Hz and 7.8 Hz, respectively). Therefore, the structure of asteryunnanoside F (**1**) was elucidated as oleanolic acid-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Asteryunnanoside G (**2**) was obtained as a crystalline products m.p. 230–232 °C, $[\alpha]_D$: −20.98° (pyridine, *c* 0.43). The FAB-MS showed two quasimolecular ion peaks at *m/z* 1111 $[M + Na]^+$ and 1095 $[M + Li]^+$ indicating a molecular mass of 1088. In addition, from the elemental analysis (Calcd: C, 58.59; H, 8.14; Found: C, 58.57; H, 8.17) the molecular formula $C_{54}H_{88}O_{22} \cdot H_2O$ was deduced. The IR spectrum showed absorptions at 3400 cm^{-1} (OH groups), 1640 cm^{-1} (double bond), and 1735 cm^{-1} (ester group). The data of chemical shifts in the ^{1}H- and ^{13}C-NMR corresponding to the aglycone moiety were identical with those of asteryunnanoside F (**1**), but the C-3 signal showed a significant downfield shift of +10.5 to 88.98 ppm and the C-2 signal a slight upfield of −1.5 to 26.71 ppm due to the glycosylation effect. It was suggested that **2** was an oleanolic acid 3, 28-bisdesmoside.

Table 2 NMR data of the sugar moieties of **1**, **2**, and **2a***.

Position	1 δ_C	1 δ_H	2 δ_C	2 δ_H	3 δ_C	3 δ_H
3-*O*-Sugar						
Glc						
1′			106.66	4.93 (d,7.7)	107.35	4.92 (d, 7.8)
2′			75.88	4.05	76.26	4.01 (dd, 7.8, 8.5)
3′			78.83	4.25	79.21	4.22 (dd, 8.5, 8.1)
4′			72.05	4.27	72.36	4.22 (dd, 8.1, 8.5)
5′			78.24	4.03	78.72	4.00 (ddd, 8.5, 5.2)
6′a			63.21	4.41	63.54	4.38 (dd, 11.1, 5.2)
6′b				4.60		4.56 (dd, 11.1, 2.0)
28-*O*-Sugar						
Inner Glc						
1″	95.75	6.29 (d, 8.1)	94.69	6.12 (d, 8.1)		
2″	74.00	4.14	75.26	4.42		
3″	78.83	4.25	79.60	4.29		
4″	71.22	4.36	71.52	4.33		
5″	78.06	4.13	77.78	4.07		
6″a	69.65	4.38	69.79	4.30		
6″b		4.73		4.67		
Outer Glc						
1‴	105.33	5.05 (d, 7.8)	105.37	4.98		
2‴	75.25	4.03	75.54	3.99		
3‴	78.57	4.21	78.40	4.18		
4‴	71.74	4.23	71.74	4.22		
5‴	78.48	3.90	78.24	3.86		
6‴a	62.67	4.39	62.85	4.35		
6‴b		4.49		4.45		
rham						
1⁗			101.39	6.63		
2⁗			72.27	4.78		
3⁗			72.64	4.57		
4⁗			73.97	4.35		
5⁗			69.79	4.59		
6⁗			18.77	1.79 (d, 6.2)		

* In pyridine-d_5, 100 MHz for δ_C and 400 MHz for δ_H, ppm, J = Hz.

Acid hydrolysis of **2** afforded glucose and rhamnose which were determined by TLC and PC with authentic samples. Four anomeric proton signals were observed in the ^{1}H-NMR spectrum at δ 6.12 (d, J = 8.1 Hz), 6.63 (br. s), 4.98 (d, J = 7.7 Hz), and 4.93 (d, J = 7.7 Hz), and one doublet methyl signal at δ 1.79 (d, J = 6.2 Hz) and the ^{13}C-NMR spectrum, with signals at δ 94.69, 106.66 and 105.37, suggested the presence of three β-D-glucose units and one mol of α-L-rhamnose unit in compound **2**. The anomeric configuration of the rhamnosyl unit was confirmed as α by the ^{13}C-NMR data which showed its C-5⁗ signal at δ 69.79 ppm (3).

On alkaline hydrolysis with 5% KOH-methanol, followed by treatment with CH_2N_2, compound **2** was converted to a prosapogenin methyl ester **2a**, which showed ions at *m/z* 655 $[M + Na]^+$ and 639 $[M + Li]^+$ in the FAB-mass spectrum. Acid hydrolysis of **2a** generated glucose which was identified by co-TLC and PC in direct comparison with authentic sample. The ^{1}H-NMR spectrum showed an anomeric proton signal at δ 4.92 (d, J = 7.8 Hz) which was consistent with a β-D-glucosyl unit. A comparison of its ^{13}C-NMR data with those of oleanolic acid revealed that C-3 resonated at lower field by +11.18 ppm, i.e. that the glucose unit was linked to the C-3 position. Therefore, the structure of **2a** was 3-*O*-β-D-glucopyranosyloleanolic acid methyl ester, androseptoside A methyl ester, which has been isolated from *Androsace septentrionolis* (4). This conclusion suggested that the 28-*O*-sugar chain consisted of two glucosyl and one rhamnosyl units.

In order to determine the structure of the esterifying sugar chain, the ^{1}H- and ^{13}C-NMR parameters were unambiguously assigned by ^{1}H-^{1}H COSY, HETCOR, HOHAHA (5, 6), and ROESY (7, 8) spectra. The 2D-NMR data were summarized in Table **3**.

Starting from the anomeric signals, the connectivities of the protons in the individual monosaccharide were established from the COSY and HOHAHA spectra and all one-bond carbon-proton connectivities were elucidated by HETCOR. The ^{1}H- and ^{13}C-NMR data of the sugar moiety are listed in Table **2**.

The ^{13}C-NMR data of the two glucosyl units and the rhamnosyl unit were closely identical with those of the corresponding methyl β-D-glycopyranoside (3) and indicated that they located at terminal positions. Furthermore, in the EI-MS of peracetylated **2**, the characteristic peaks at *m/z* 331 $[glc(OAc)_4]^+$, 519 $[rham(OAc)_3\text{-}glc(OAc)_2]^+$ and 577 $[glc(OAc)_4\text{-}glc(OAc)_2]^+$ provided additional evidence for the presence of terminal glucose and rhamnose units in the 28-*O*-sugar chain. The spin systems of two terminal glucose units were finally discriminated by analysis of the ROESY spectrum of **2**. A significant nOe correlation contour between the anomeric proton signal at δ 4.93 and the H-3 signal in its aglycone at δ 3.34 was observed. Thereby the proton signals of the spin system with the δ 4.93 signal were assigned to be those of the 3-*O*-glucose unit. The proton spin system corresponding to the anomeric proton at δ 4.98 were due to those of the 28-*O*-terminal glucosyl unit.

Table 3 Summary of the two-dimensional NMR correlations of **2**.

Proton	COSY (^{1}H)	HETCOR (^{13}C)	HOHAHA (^{1}H)	ROESY (^{1}H)
3-*O*-glc				
1′	2′	1′	2′,3′	3,3′,5′
2′	1′,3′	2′	1′,3′,4′	4′
3′	2′,4′	3′	1′,2′,4′,5′	1′,5′
4′	3′,5′	4′	2′,3′,5′,6′a,6′b	2′
5′	4′,6′a,6′b	5′	2′,3′,4′,6′a,6′b	1′,3′,6′a
6′	5′,6′b	6′	4′,5′,6′b	6′b,5′
6′	5′,6′a	6′	5′,6′a	6′a
28-sugar inner-glc				
1″	2″	1″	2″,3″	3″,5″
2″	1″,3″	2″	1″,3″,4″	4″,1‴
3″	2″,4″	3″	1″,2″,4″,5″	1″,5″
4″	3″,5″	4″	2″,3″,5″,6″a	2″
5″	4″,6″	5″	2″,3″,4″,6″a,6″b	1″,3″,6″
6″	5″	6″	4″,5″	5″,1‴
outer glc				
1‴	2‴	1‴	2‴,3‴	3‴,5‴,6″
2‴	1‴,3‴	2‴	1‴,3‴,4‴	4‴
3‴	2‴,4‴	3‴	1‴,2‴,4‴,5‴	1‴,5‴
4‴	3‴,5‴	4‴	2‴,3‴,5‴,6‴a	2‴
5‴	4‴,6‴a,6‴b	5‴	2‴,3‴,4‴,6‴a,6‴b	1‴,3‴6,‴a
6‴a	5‴,6‴b	6‴	4‴,5‴,6‴b	5‴,6‴b
6‴b	5‴,6‴a	6‴	5‴,6‴a	6‴a
rham				
1⁗	2⁗	1⁗	2⁗	2⁗,4⁗,2″
2⁗	1⁗,3⁗	2⁗	1⁗,3‴,4⁗	1⁗,4⁗
3⁗	2⁗,4⁗	3⁗		2⁗,4⁗,5⁗
4⁗	3⁗,5⁗	4⁗	2⁗,3⁗,5⁗,6⁗	1⁗,2⁗
5⁗	4⁗,6⁗	5⁗	3⁗,4⁗,6⁗	3⁗,6⁗
6⁗	5⁗	6⁗	4⁗,6⁗	5⁗

Examination of the ^{13}C chemical shifts in the sugar region indicated that the C-2″ and C-6″ signals of the 28-*O*-inner glucose unit were shifted downfield by 1.26 ppm to 75.26 ppm and by 7.3 ppm to 69.79 ppm, respectively. These data suggested that glycosylation had occurred at the C-2″ and C-6″ positions of the inner glucosyl unit. In the ^{1}H-NMR spectrum, the observations of H-2″ and H-6″ signals deshielded at δ 4.42 and 4.67, 4.30, respectively, further supported this conclusion. In the ROESY spectrum nOe correlations between glucose the H-1‴ and H-6″, as well as the rhamnose H-1 and glucose H-2″ were observed, which suggested that the sequences of the sugars on C-28, i.e. the (1→6) linkage was between two glucose units and the (1→2) linkage between the rhamnose and the inner glucose unit.

Finally, the structure of asteryunnanoside G (**2**) was determined to be 3-*O*-β-D-glucopyranosyl-oleanolic acid-28-*O*-β-D-glucopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside.

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