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ECHINOCYSTIC ACID SAPONINS FROM *ASTER YUNNANENSIS*

YU SHAO, BING-NAN ZHOU,*

Shanghai Institute of Materia Medica, Academia Sinica, Shanghai 200031, People's Republic of China

KAN MA, and HOU-MING WU

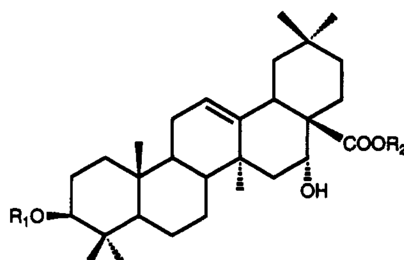
National Key Laboratory of Bio-Organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Academia Sinica, Shanghai 200032, People's Republic of China

ABSTRACT.—A novel echinocystic acid saponin, asteryunnanoside H [**1**], along with four known saponins, has been isolated from the roots of *Aster yunnanensis*. The structure of **1** was determined unambiguously by nmr and chemical transformations.

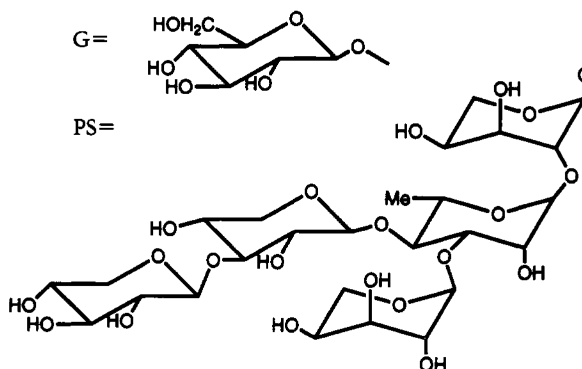
Plants of the genus *Aster* (Compositae) have been used as drugs for the treatment of fever, colds, tonsillitis, snake bite, and bee stings in Chinese folk medicine (1). During a search for novel bioactive compounds from medicinal plants, we found that *n*-BuOH extracts of *Aster yunnanensis* Franch showed potent analgesic and sedative activities. This observation encouraged us to study the glycosides of this plant that have not been chemically investigated before. In this paper we report the isolation and structural elucidation of a new echinocystic acid saponin named asteryunnanoside H [**1**], as well as four known saponins, aster saponins Ha, Hb, and Hc, and foetidissimoside A.

RESULTS AND DISCUSSION

The *n*-BuOH-soluble fraction of a 70% EtOH extract from the roots of *A. yunnanensis* was chromatographed on a column of SIP-1300 polymeric adsorbent, followed by cc on SiO_2, Sephadex LH-20, and C_8 reversed-phase columns to afford five compounds.



	R_1	R_2
1	G	PS
2	G	CH_3
3	H	CH_3



Asteryunnanoside H [1] was obtained as colorless needles from MeOH, mp 254–255°, [α]D −41.66° (*c*=0.36, pyridine). It showed in the fabms a $[M+Na]^+$ ion at *m/z* 1331, which indicated the molecular formula to be $C_{62}H_{100}O_{29}\cdot 2H_2O$ in accordance with the eims (*anal.*, calcd C, 55.36, H, 7.74; found C, 55.31, H, 7.77). The ir spectrum indicated the presence of hydroxyl groups (3400 cm^{-1}), an ester group (1730 cm^{-1}), a double bond (1630 cm^{-1}), and glycosidic linkages (1000–1100 cm^{-1}). Acid hydrolysis of compound **1** gave arabinose, rhamnose, xylose, and glucose, which were identified by tlc and paper chromatography in comparison with authentic samples. Its ^{1}H-nmr spectrum showed signals of seven tertiary methyl groups at δ 0.84, 0.97, 0.99, 1.06, 1.13, 1.26, and 1.79, one secondary methyl group at δ 1.69 (d, *J*=6.0 Hz), one trisubstituted olefinic proton at δ 5.62 (br, s) and six anomeric protons at δ 4.90 (d), 5.05 (d), 5.13 (d), 5.45 (d), 5.59 (br, s), and 6.53 (d). The ^{13}C-nmr spectrum showed signals of six C-C bonded saturated quaternary carbons at δ 39.60, 40.25, 37.19, 42.22, 49.80, 30.97, an ester carbonyl carbon at δ 175.91, a pair of olefinic carbons at δ 123.25 and 144.47, and six anomeric carbons at δ 93.46, 100.90, 105.82, 105.85, 106.00, and 106.77 (Table 1). The number and chemical shifts of the tertiary methyl groups and quaternary carbons suggested that the aglycone was an oleanane-type triterpene and the

TABLE 1. ^{1}H- and ^{13}C-Nmr Data of Compound **1** and ^{13}C-Nmr Data of Compounds **2** and **3**.

Position	Compound 1		Compound 2	Compound 3	DEPT
	δ_H	δ_C	δ_C	δ_C	
1	0.94, 1.45	39.00	38.63	39.01	CH_2
2	1.79, 2.20	26.67	26.44	28.07	CH_2
3	3.37	89.01	88.68	78.05	CH
4	—	39.60	39.41	39.27	C
5	0.80	56.15	55.73	56.00	CH
6	1.69	18.62	18.33	18.79	CH_2
7	1.35, 1.56	33.50	33.19	33.38	CH_2
8	—	40.25	40.10	40.05	C
9	1.70	47.34	46.90	47.18	CH
10	—	37.19	36.98	37.35	C
11	1.90	23.95	23.59	23.80	CH_2
12	5.62	123.25	122.59	122.75	CH
13	—	144.47	143.97	144.43	C
14	—	42.22	42.00	41.78	C
15	1.77, 2.30	36.18	35.75	36.01'	CH_2
16	5.24	74.15	74.22	74.14	CH
17	—	49.80	49.48	49.17	C
18	3.60	41.15	41.11	41.42	CH
19	1.33, 2.76	47.24	46.83	46.98	CH_2
20	—	30.97	30.77	30.78	C
21	1.26, 2.40	36.08	35.75	35.96	CH_2
22	2.20, 2.30	31.99	32.35	32.33	CH_2
23	1.26 s	28.40	28.08	28.65	CH_3
24	1.06 s	17.15	16.89	16.77	CH_3
25	0.84 s	15.79	15.43	15.61	CH_3
26	0.97 s	17.78	17.06	17.27	CH_3
27	1.79 s	27.07	27.07	27.04	CH_3
28	—	175.91	176.49	177.69	C
29	0.99 s	33.29	33.04	33.08	CH_3
30	1.13 s	25.03	24.42	24.54	CH_3
OMe			51.61	51.70	CH_3

^{1}H-nmr signals at δ 6.53 and ^{13}C-nmr signals at δ 93.46 indicated the presence of an ester-linked sugar moiety.

Upon alkaline hydrolysis, compound **1** gave a prosapogenin and an oligosaccharide, which was subsequently hydrolyzed by acid to give rhamnose, arabinose, and xylose, identified by tlc and paper chromatography in direct comparison with authentic sugar samples. The methyl ester [**2**] of the prosapogenin showed an $[M+Na]^+$ ion at *m/z* 671 and an $[M+Li]^+$ ion at *m/z* 655 in its fabms. Acid hydrolysis of compound **2** afforded glucose, identified by tlc and paper chromatography with an authentic sample, and an aglycone. After treatment with CH_2N_2, the aglycone was converted to its methyl ester [**3**], which showed an $[M]^+$ ion at *m/z* 486 ($C_{31}H_{50}O_4$) in the eims, and fragment ions at *m/z* 468, 278, 260, 219, and 201. The ^{1}H-nmr spectrum of **3** showed the signals of two hydroxymethine groups at δ 3.45 (dd, J=10 and 4.5 Hz) and δ 5.04 (br s), a carbomethoxyl group at δ 3.69, and a trisubstituted olefinic proton at δ 5.59 (br s). The fragment ions at *m/z* 278 and 260 (*m/z* 278-H_2O) suggested that **3** was an olean-12-ene derivative with one hydroxyl group at ring A or ring B, and one hydroxyl group and a carbomethoxyl group in either ring C, D, or E. Compound **3** was identified as the methyl ester of 3β,16α-dihydroxolean-12-en-28-oic acid (the methyl ester of echinocystic acid) by comparison of the ^{13}C-nmr data with literature values (2).

The position of the sugar linkage in **2** was determined as being affixed to the C-3-hydroxy group, based on the glycosylation shift of the C-3 ^{13}C-nmr signal which appeared at lower field by +10.52 ppm in comparison with that of **3**. The nmr data showed an anomeric proton signal at δ 4.87 (d,J=7.7 Hz) and an anomeric carbon signal at δ 106.70, which indicated the β-configuration of the glucosyl moiety (3). Therefore, **2** was identified as 3-*O*-β-D-glucopyranosyl-echinocystic acid methyl ester.

The above result indicated that the remaining five sugar units in **1** must be bound to the C-28 position of its genin by a glycosidic ester linkage. The complete structure of the 28-*O*-oligosaccharide chain was determined by DQ COSY (4), HMQC (5), TOCSY (6), ROESY (7–9), and HMBC (10) nmr spectra. The individual spin-systems in the saccharide unit were assigned by COSY and TOCSY experiments starting from the six anomeric proton signals at δ 6.53 (d,J=3 Hz), 5.59 (br s), 5.45 (d,J=7.7 Hz), 5.13 (d,J=7.0 Hz), 5.05 (d,J=7.4 Hz), and 4.90 (d,J=7.6 Hz). Their ^{13}C-nmr chemical shifts were assigned using DEPT and HMQC spectra. The ^{13}C- and ^{1}H-nmr chemical shifts are listed in Table 2. The nmr data and J values shown in Table 2 indicated that the 28-*O*-sugar moiety consisted of two arabinopyranosyl units, two xylopyranosyl units, and one rhamnopyranosyl unit by comparison with individual methyl glycopyranosides (3). Furthermore, one xylosyl unit (X′) and one arabinosyl unit (A′) were located at terminal positions, because their ^{13}C-nmr data were identical with those of the corresponding methyl β-xylopyranoside and methyl α-L-arabinopyranosides. In addition, ^{13}C-nmr glycosylation shifts were observed for the C-2 signal of the inner arabinosyl group (A) by +2.24 ppm, for the C-3 signal of the rhamnosyl group by +10.06 ppm, for the C-4 signal of the rhamnosyl unit (R) by +5.33 ppm, and for the C-3 signals of the inner xylosyl group (X) by +9.84 ppm. The HMBC spectrum of **1** showed contours between the C-2 signal of the inner arabinosyl unit (A) and the H-1 signal of rhamnosyl unit (R), the C-3 of the rhamnosyl unit and the H-1 of outer arabinosyl unit (A′), the H-4 of rhamnosyl unit (R) and the C-1 of the inner xylosyl unit (X), and the C-3 of the inner xylosyl unit (X) and H-1 of the outer xylosyl unit (X′). Therefore, the sequence of the saccharide moiety in the 28-*O*-oligosaccharide as deduced from ^{13}C-nmr and HMBC spectral data can be summarized as: X′(1→3)-X-(1→4)-[A′-(1→3)]-R-(1→2)-A-(1→28)-aglycone. This conclusion was further confirmed by the nOes observed between X′-H-1 and X-H-3, X-H-1 and R-H-4, A′-H-1 and R-H-3, and R-H-1 and A-H-2 in the ROESY spectrum.

TABLE 2. Nmr Data of Sugar Moieties of Compounds **1** and **2** in Pyridine-d_6.[a]

Position	Compound 1 δ_C	Compound 1 δ_H	Compound 2 δ_C	Compound 2 δ_H
3-*O*-sugar[b]				
G-1	106.77	4.90 (d, 7.6)	106.70	4.87 (d, 7.7)
G-2	75.87	4.05	75.64	3.94
G-3	78.80	4.22	78.59	4.25
G-4	71.51	4.22	71.71	4.23
G-5	78.16	3.98	78.10	3.90
G-6a	62.47	3.93	62.88	4.31 (dd, 11.8, 5.0)
G-6b		4.54		4.46 (dd, 11.8, 2.0)
28-*O*-sugar[b]				
A-1	93.46	6.53 (d, 3)		
A-2	75.32	4.54		
A-3	69.52	4.59		
A-4	65.52	4.39		
A-5	63.24	4.37, 4.57		
R-1	100.90	5.59 (br, s)		
R-2	72.09	4.77 (br, s)		
R-3	82.36	4.63		
R-4	77.83	4.53		
R-5	68.76	4.44		
R-6	18.62	1.69 (d, 6.0)		
X-1	105.82	5.45 (d, 7.7)		
X-2	74.64	3.94		
X-3	87.85	4.09		
X-4	69.13	4.03		
X-5	66.44	3.29, 4.10		
X'-1	106.00	5.13 (d, 7.0)		
X'-2	75.32	3.97		
X'-3	78.01	4.02		
X'-4	70.89	4.08		
X'-5	67.15	3.46, 4.13		
A'-1	105.84	5.05 (d, 7.4)		
A'-2	72.95	4.45		
A'-3	74.45	4.04		
A'-4	69.52	4.12		
A'-5	67.15	3.93, 4.54		

[a]Measured at 100 MHz for ^{13}C-nmr and at 400 MHz for ^{1}H nmr; J values (ppm) are shown in parentheses.

[b]G=β-D-glucopyranosyl-, A=inner α-L-arabinopyranosyl-, R=α-L-rhamnopyranosyl, X=inner β-D-xylopyranosyl-, X'=outer β-D-xylopyranosyl-, A'=outer α-L-arabinopyranosyl.

The β-configuration at the anomeric centers of the two xylopyranosyl moieties was suggested by the ^{1}H-nmr chemical shifts and the large $J_{1,2}$ couplings (7.7 and 7.0 Hz, respectively) of the anomeric protons in the ^{1}H-nmr spectrum. The configuration of the L-rhamnosyl group was determined as α on the basis of its C-5 ^{13}C-nmr signal at δ 68.76 (3). The outer arabinosyl group (A') was concluded to be in the α configuration by the observed $J_{1,2}$=7.4 Hz coupling. The ester-linked inner arabinopyranosyl group was presumed to be in the α-configuration and to possess $^{1}C_4$ conformation based on the $J_{1,2}$=3 Hz coupling (11,12) and its ^{1}H- and ^{13}C-nmr chemical shifts, which were quite similar to those of desacyl-lobatoside B (3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-bayogenin-28-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (13).

Thus, asteryunnanoside H [**1**] was established as 3-*O*-β-D-glucopyranosyl-echinocystic acid-28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyra-nosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, on the basis of the above results.

In addition to **1**, four other echinocystic acid glucuronide saponins, namely, aster saponins Ha, Hb, and Hc, and foetidissimoside A, were isolated as their methyl esters. Their structures were identified by comparison of their spectral data (fabms, ^{1}H- and ^{13}C-nmr) with those reported in the literature (14,15). These saponins have a common prosaponin, echinocystic acid-3-*O*-β-D-glucopyranosiduronic acid monomethyl ester, and differ in the structures of the 28-*O*-linked sugar moiety, i.e., aster saponin Ha is the 28-*O*-α-L-arabinopyranoside, Hb the 28-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, Hc the 28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, and festissimoside A, the 28-*O*-β-D-xylopyranosyl-(1→4)-α-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside of the prosaponin. These compounds were first isolated from *Aster tataricus* by Tanaka *et al.* (13) and from *Cucurbita foetidissima* by Dubois *et al.* (15).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler apparatus and are uncorrected and optical rotations were measured at 28° on a Jasco DIP-181 polarimeter. Ir spectra were obtained on a Perkin-Elmer 599B infrared spectrometer. Fabms were recorded in the positive-ion mode by direct-inlet on a VG ZAB-HS mass spectrometer using glycerol as maxtrix. Eims were obtained on a MAT-95 mass spectrometer. ^{1}H- and ^{13}C-nmr and DEPT spectra were obtained on a Bruker AM-400 spectrometer operating at 400 MHz for δ_H and 100 MHz for δ_C. HMQC, HMBC, HOHAHA, and ROESY nmr spectra were obtained on a Bruker AMX-600 spectrometer operating at 600 MHz for δ_H and 150 MHz for δ_C. All 1D and 2D nmr spectra were recorded using the standard Bruker software package, and data manipulations of the 2D spectra were performed on a Bruker Aspect×32 station. Paper chromatography of sugars was run on Whatman No. 1 paper using the solvent systems *n*-BuOH-pyridine-H_2O (6:4:3) and *n*-BuOH-AcOH-H_2O (4:1:5, upper layer), respectively, and detected with aniline phthalate. Mplc was carried out on a LiChroprep RP-8 (40–60 μm) Lobar column (31 cm×25 mm i.d.) with MeOH-H_2O (5:5→6:4) as eluent (flow rate 2 ml/min). Si gel, 200–300 mesh (Qingdao Marine Chemical Factory) was used for cc and SiO_2 GF_{254} for tlc.

PLANT MATERIAL.—The roots of *Aster yunnanensis* were collected in August 1992, from Li-Jiang County, Yunnan Province, People's Republic of China. A voucher specimen was identified by Prof. Z.W. Lu and is deposited in the Herbarium of the Kunming Institute of Botany, Academia Sinica, People's Republic of China.

EXTRACTION AND ISOLATION.—The dried roots (15 kg) of *Aster yunnanensis* were percolated with 70% EtOH five times (6 days each) at room temperature. After concentration *in vacuo*, the residue (3.8 kg) was suspended in H_2O, and then was successively extracted with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH layer was evaporated *in vacuo* to give a residue (698 g). This residue was chromatographed on a column of highly porous resin (SIP-1300, 1.5 kg) eluting initially with H_2O followed by EtOH. From the EtOH fraction, aster saponin Hb (2.37 g) was crystallized by treatment of MeOH. A portion of the crude saponin (350 g) from the mother liquor of Hb after treatment with CH_2N_2 was subjected to cc on SiO_2 (1.5 kg) eluting with a $CHCl_3$-MeOH-H_2O gradient (8:1:0.1→1:1:0.1). Fractions 7 and 8 containing **1** (tlc) were repeatedly chromatographed on a SiO_2 column using H_2O-saturated *n*-BuOH as the eluent and the same components were combined to give two fractions. The more polar fraction was further purified by LiChroprep RP-8 cc with MeOH-H_2O (7:3) as eluent to yield 84 mg of asteryunnanoside H [**1**] and 2.4 g of aster saponin Hc. Aster saponin Ha methyl ester (250 mg) was isolated from fraction 4 by sequential cc on LH-20 (MeOH), and SiO_2 with $CHCl_3$-MeOH-H_2O (3:1:0.1), and LiChroprep RP-8 with MeOH-H_2O (6:4). Hb methyl ester (55 g) was purified from fraction 5 by cc on SiO_2 with $CHCl_3$-MeOH-H_2O (3:1:0.1), Sephadex LH-20 (MeOH), and LiChroprep RP-8 with MeOH-H_2O (6:4). Foetidissimoside A methyl ester (1.5 g) was obtained from fraction 6 by cc on SiO_2 with $CHCl_3$-MeOH-H_2O (9:3:0.5) and LiChroprep RP-8 with MeOH-H_2O (6:4).

Asteryunnanoside H [**1**].—Colorless needles; mp 254–255°. $[\alpha]_D$ −41.66° (c=0.36, pyridine); ir (KBr) ν max 3400 (OH), 1730 (C=O), 1630, 1000–1100 cm^{-1}; fabms *m/z* 1331 $[M+Na]^+$; *anal.*, calcd for $C_{62}H_{100}O_{29}\cdot 2H_2O$; C, 55.36, H, 7.74; found C, 55.31, H, 7.77; ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2.

ACID HYDROLYSIS OF **1**.—A solution of **1** (5 mg) in 2 N HCl/MeOH (3 ml) was heated at 100° for 4 h. After cooling to room temperature, the reaction mixture was evaporated to dryness at 40°, and additional H_2O was repeatedly added and evaporated until neutrality was achieved. The residue was examined by paper chromatography and tlc, and showed the presence of glucose, rhamnose, xylose, and arabinose by direct comparison with the authentic samples.

ALKALINE HYDROLYSIS OF **1**.—A solution of **1** (40 mg) in 5% KOH/MeOH (8 ml) was heated at 100° for 4 h. The reaction mixture was cooled to room temperature and neutralized with dilute HCl. After removal of MeOH, the remaining mixture was passed through a column of highly porous resin eluted with H_2O and then MeOH. The H_2O eluent was evaporated and followed by acidic hydrolysis to give the residue which showed the presence of glucose and rhamnose identified by paper chromatography and tlc in direct comparison with authentic samples. The MeOH eluent was treated with CH_2N_2 and evaporated to dryness. The residue was subjected to cc over SiO_2 using $CHCl_3$-MeOH (10:1) as the solvent to afford **2** (17 mg): colorless needles; mp 207–209°; ir ν max (KBr) 3400, 1740, 1645, 1000–1100 cm^{-1}; ^1H nmr (pyridine-d_5) aglycone moiety: δ 0.77, 0.79, 0.91, 0.99, 1.20, 1.69 (3H each, s, *tert*-Me×7), 3.59 (3H, s, OMe), 5.45 (1H, br s, H-12); sugar moiety, see Table 2; ^{13}C-nmr data, see Tables 1 and 2; fabms *m/z* 671 $[M+Na]^+$ and 655 $[M+Li]^+$.

ACID HYDROLYSIS OF **2**.—A solution of compound **2** (15 mg) in 2N HCl/MeOH (4 ml) was heated at 100° for 4 h. After cooling to room temperature, the reaction mixture was extracted with Et_2O. After evaporation of Et_2O, the residue was treated with CH_2N_2 and recrystallized from MeOH to give the methyl ester of the aglycone, echinocystic acid methyl ester (**3**, 9 mg). After repeated evaporation and treatment with addition of H_2O, the neutral residue was examined by paper chromatography and tlc in direct comparison with an authentic sample to show the presence of glucose. Compound **3** exhibited colorless needles; $[\alpha]_D$ +25.3° (c=0.34, MeOH); $C_{31}H_{50}O_4$; eims *m/z* 486 $[M]^+$, 468, 278, 260, 219, 201; ^1H nmr (pyridine-d_5) aglycone moiety: δ 0.88, 0.90, 1.02, 1.04, 1.10, 1.23, 1.70 (3H each, s, *tert*-Me×7), 3.41 (1H, dd, J=4.0 and 13.8 Hz, H-18), 3.45 (1H, dd, J=10.0 and 4.5 Hz, H-3), 3.69 (3H, s, OMe), 5.04 (1H, br s, H-16), 5.59 (1H, br s, H-12); ^{13}C-nmr data, see Table 2.

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