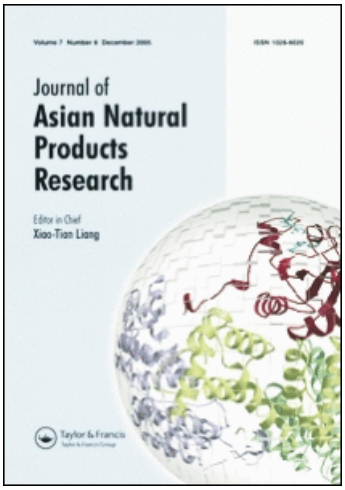

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Wang, Chang Zeng and Yu, De Quan(1998) 'Triterpenoid Saponins from *Aster auriculatus*', Journal of Asian Natural Products Research, 1: 1, 1 — 14
To link to this Article: DOI: 10.1080/10286029808039839
URL: http://dx.doi.org/10.1080/10286029808039839

JANPR, Vol. 1, pp. 1–14
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TRITERPENOID SAPONINS FROM *ASTER AURICULATUS*

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(Received 12 February 1998; Revised 23 February 1998; In final form 2 March 1998)

Three new triterpenoid saponins (**1**–**3**), together with one known saponin scaberoside B_6 methyl ester (**4**) were isolated from an ethanol extract of roots of *Aster auriculatus*. The structures of **1**–**3** have been determined on the basis of spectral and chemical evidence as 3-*O*-β-D-glucuronyl-16α-hydroxy-olean-12-en-28-oic acid 28-*O*-[α-L-arabinopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)]-[β-D-xylopyranosyl(1 → 3)-β-D-xylopyranosyl(1 → 3)]-α-L-arabinopyranoside (**1**), the methyl ester of 3-*O*-β-D-glucuronyl-16α-hydroxy-olean-12-en-28-oic acid 28-*O*-[β-D-xylopyranosyl(1 → 3)-α-L-arabinopyranosyl(1 → 4)]-[β-D-apiofuranosyl(1 → 3)]-α-L-rhamnopyranosyl(1 → 2)-β-D-xylopyranoside (**2**), and 3-*O*-β-D-glucuronyl-olean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl(1 → 3)-β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)-α-L-arabinopyranoside (**3**).

Keywords: Aster auriculatus, Compositae, triterpenoid saponins auriculatusaponin D(1), E(2), and F(3)

INTRODUCTION

Aster auriculatus Franch. (Compositae), commonly called "Yinxianju", is native to Yunnan, Guizhou and Sichuan Provinces of China [1]. The root is used in Chinese folk medicine for the treatment of cough and as an expectorant [2,3]. In a preliminary investigation an alcoholic extract of the roots exhibited anti-inflammatory and anti-ulcer activities, inhibitory effects on collagenase and mucinase, and 95% inhibition against ulcers (100 mg/kg) in

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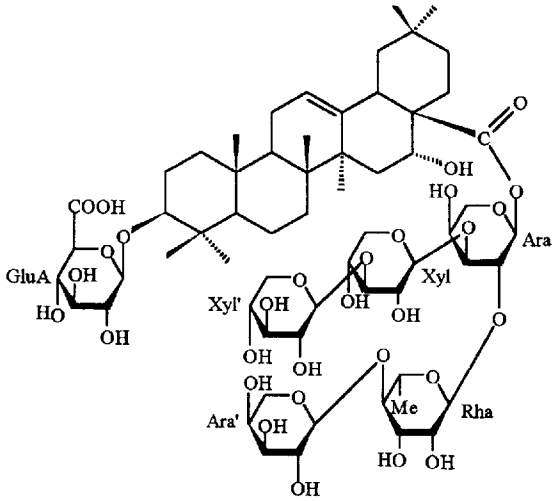
a rat pyloric ligature model [4]. As a result of our interest in chemical studies on the glycosidic constituents of higher plants, we wish to report the isolation and structural elucidation of three new triterpenoid saponins (**1**–**3**) from the roots of *A. auriculatus*, along with the identification of the known compound, scaberoside B_6 methyl ester (**4**).



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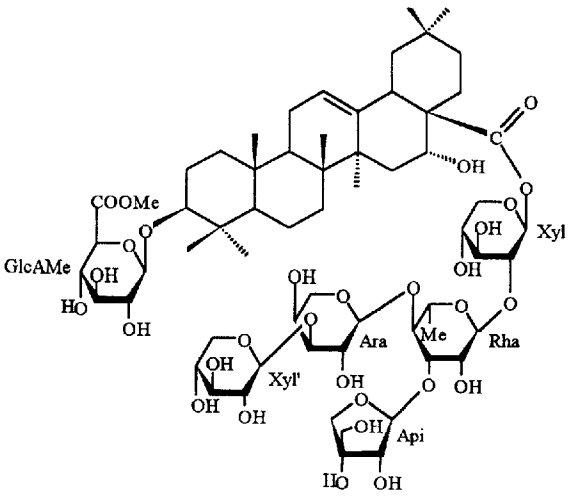

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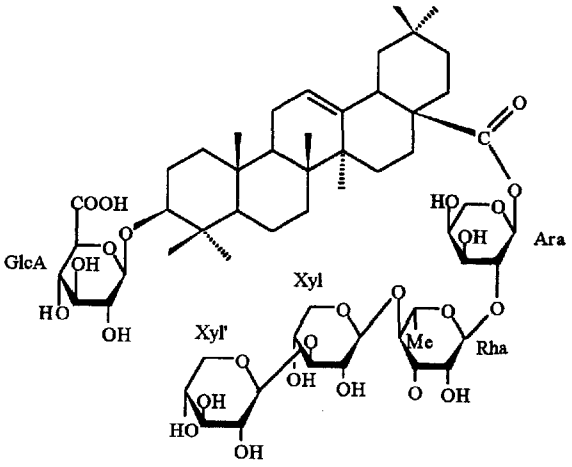

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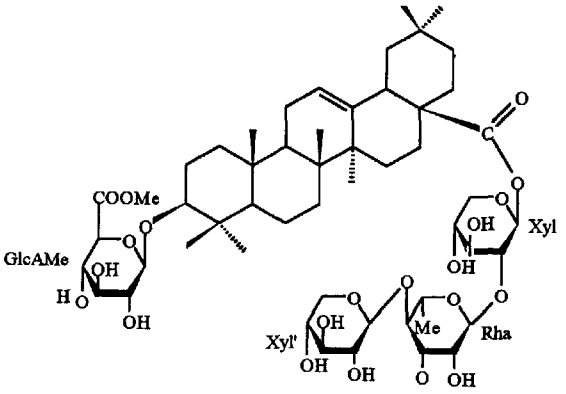

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RESULTS AND DISCUSSION

A 75% ethanolic extract from the roots of *Aster auriculatus* was fractionated over a highly porous polymer column, and the fractions were further purified by repeated column chromatography over silica gel and preparative thin-layer chromatography resulting in the isolation of three new saponins, (**1**–**3**), together with one known saponin (**4**).

Compound **1** showed a $[M+Na]^+$ ion at m/z 1345.6129 and a $[M+2Na-1]^+$ ion at m/z 1367.5881 in the HRFSIMS, corresponding to a molecular formula of $C_{62}H_{98}O_{30}$. The IR spectrum indicated the presence of hydroxyl (3430 cm^{-1}), ester group (1730 cm^{-1}), double bond (1634 cm^{-1}) and glycosidic linkage (1076–1043 cm^{-1}) absorptions. Its ^{1}H-NMR spectrum showed signals for seven tertiary methyl groups at δ 0.82, 0.98, 0.99, 1.70, 1.15, 1.27 and 1.82 (each 3H, s), one trisubstituted olefinic proton at δ 5.58 (1H, brs), and six anomeric protons at δ 4.98 (d, $J=7.5$ Hz), 5.03 (d, $J=7.5$ Hz), 5,15 (d, $J=7.2$ Hz), 5.46 (d, $J=7.8$ Hz), 5.64 (brs), and 6.55 (d, $J=1.6$ Hz). The ^{13}C-NMR spectrum of **1** revealed the presence of six sp^3 quaternary carbon atoms at δ 39.6, 40.1, 37.0, 42.1, 49.6, and 31.0, a pair of olefinic carbon atoms at δ 122.9 and 144.4, an ester carbonyl at δ 176.0, and six anomeric carbon atoms at δ 93.4, 100.9, 104.4, 106.0, 106.1 and 107.2. The NMR spectral data and molecular formula suggested that compound **1** was an oleanane-type triterpenoid saponin with six sugar units. The significant downfield shift of the anomeric proton at δ 6.55, and the upfield shift of anomeric carbon at δ 93.4 indicated that this sugar unit was attached to the aglycone through an ester linkage [5].

After acid hydrolysis, compound **1** yielded echinocystic acid, identified by ^{1}H-NMR, MS, and co-TLC with an authentic sample, and glucuronic acid, rhamnose, arabinose and xylose as sugar components, by comparison with authentic samples on co-TLC and paper chromatography. Upon alkaline hydrolysis, the ester-linked sugar chain was selectively cleaved and a prosapogenin was obtained, whose FABMS showed a $[M+Na]^+$ ion at m/z 671, corresponding to a molecular formula of $C_{36}H_{56}O_{10}$. Further acid hydrolysis furnished glucuronic acid and echinocystic acid. The NMR spectra of this prosaponin showed signals at δ 89.1 (C-3) and 3.35 (1H, dd, $J=10.5$, 3.0 Hz, H-3), suggesting that the glucuronic acid was linked at C-3 in a β-position. Accordingly, this prosapogenin was assigned as the known compound 3-*O*-β-D-glucuronyl-16α-hydroxy-olean-12-en-28-oic acid [6]. The above results indicated that compound **1** was a 3,28-bidesmoside and the remaining five sugar units must be bound to the C-28 of the aglycone through an ester linkage.

Beginning from the anomeric protons and also from the well-resolved resonances of Rha H-6, Xyl and Ara H-5, the ^{1}H- and ^{3}C-NMR chemical shift assignments of the C-28 sugar chain of **1** were performed from its 2D NMR spectra including ^{1}H ^{1}H COSY, ^{13}C–^{1}H COSY and HMBC, which permitted unambiguous identification of the various sugar moieties, while the cross peaks in the HMBC and NOESY spectra allowed the determination of the sugar sequences and linkage sites. Comparison of the ^{13}C-NMR

spectral data of the sugar parts in **1** with those of the corresponding individual methyl glycosides [7,8] indicated that the C-28 sugar unit consisted of one rhamnose, two arabinose and two xylose residues, and that a xylose and an arabinose were in terminal locations. C-3 of the inner xylose residue in the C-28 sugar unit resonated at δ 87.9, C-4 of the inner rhamnose at δ 82.3, and C-2 and C-3 of the inner arabinose at δ 75.6 and 78.2, respectively, suggesting that the glycosylation positions were at Xyl C-3, Rha C-4, Ara C-2, and C-3. The HMBC spectrum of compound **1** showed strong cross peaks between Xyl′ H-1 and Xyl C-3, Xyl H-1 and Ara C-3, Ara′ H-1 and Rha C-4, Rha H-1 and Ara C-2, and Ara H-1 and the ester carbonyl at C-28. In addition, the NOESY spectrum of **1** showed correlations between the outer Xyl′ H-1 and the inner Xyl H-3, the inner Xyl H-1 and the inner Ara H-3, the outer Ara′ H-1 and the inner Rha H-4, the inner Rha H-1 and the inner Ara H-2. These results allowed the establishment of the linkage of the C-28 sugar chain of **1** as follows:

$$\begin{array}{l}\text{Xyl'-(1}\rightarrow\text{3)-Xyl-(1}\rightarrow\text{3)-Ara-(1}\rightarrow\text{28)- Aglycone}\\ \qquad\qquad\qquad\qquad\qquad\qquad\quad |\ \text{(1}\rightarrow\text{2)}\\ \qquad\qquad\qquad\qquad\qquad\qquad\text{Ara'-(1}\rightarrow\text{4)-Rha}\end{array}$$

Moreover, the presence of fragment ions at *m/z* 1213 $[M+Na-133+H]^+$, 1169 $[M+Na-177+H]^+$, 1081 $[M+Na-265+H]^+$, 1067 $[M+Na-279+H]^+$, 1037 $[M+Na-132-176]^+$ and 631 $[M-692+H]^+$ in the FABMS also supported the sugar sequence indicated above.

The $J_{1,2}$ couplings of the anomeric protons and the chemical shifts of the protons and carbons of the sugar residues suggested that the anomeric configurations of xylose and glucuronic acid units were β, and those of the rhamnose and arabinose units were α [9,10]. From all of the above results, compound **1** was established as 3-*O*-β-D-glucuronyl-16α-hydroxy-olean-12-en-28-oic acid 28-*O*-[α-L-arabinopyranosyl(1 → 4)-α-L-rhamnopyranosyl (1 → 2)]-[β-D-xylopyranosyl(1 → 3)-β-D-xylopyranosyl(1 → 3)]-α-L-arabinopyranoside.

The positive-ion FABMS of compound **2** provided a molecular ion at *m/z* 1336, corresponding to a molecular formula of $C_{63}H_{100}O_{30}$, which was consistent with the result of elemental analysis. The IR, ^{1}H- and ^{13}C-NMR spectra due to the aglycone part of **2** were almost identical with those of **1**. Acid hydrolysis of **2** afforded echinocystic acid, as well as glucuronic acid, arabinose, xylose, rhamnose and apiose, and on alkaline hydrolysis, the same prosapogenin as **1** was obtained. This evidence suggested that compound **2** was also an echinocystic acid 3-28-bidesmoside.

The NMR spectral data of **2** showed the presence of six sugar moieties from the six anomeric proton signals at δ 5.17 (d, $J=7.5$ Hz), 5.24 (d, $J=7.6$ Hz), 5.27 (brs), 5.62 (brs), 5.99 (d, $J=4.2$ Hz) and 6.56 (d, $J=6.3$ Hz) and the six anomeric carbon signals at δ 112.0, 107.2, 105.9, 104.8, 101.2, and 93.4, together with a methoxyl group at δ_H: 3.59 (3H, s) and δ_C: 49.6 (CH_3). One of these sugar residues was rhamnose (δ 101.2), suggested by the distinct methyl proton doublet (Rha H-6: δ 1.69, d, $J=6.0$ Hz), and the significant downfield signal of the anomeric carbon at δ 112.0 was assigned for apiose, suggested by the quaternary carbon at δ 79.9. The above results indicated that the C-3-sugar unit was glucuronic acid methyl ester, and that the C-28-sugar moiety consisted of one unit

TABLE I NMR chemical shift assignments of aglycone moieties of compounds **1** and **2**. (500 MHz for ^1H and 125 MHz for ^{13}C in C_5D_5N)

Position	**1**		**2**		*DEPT*
	δ_H	δ_C	δ_H	δ_C	
1	0.88, 1.40	38.8	0.89, 1.39	38.9	CH_2
2	1.83	26.9	1.84	27.0	CH_2
3	3.38 dd, (10.5, 3.0)	89.1	3.32 dd (10.2, 3.5)	89.1	CH
4		39.6		39.5	C
5	0.80 brd (11.6)	55.9	0.76 brd (11.2)	56.0	CH
6	1.31–1.35	18.5	1.31–1.35	18.5	CH_2
7	1.33–1.40	33.6	1.31–1.44	33.6	CH_2
8		40.1		40.1	C
9	1.69	47.2	1.69	47.2	CH
10		37.0		37.0	C
11	1.89	23.9	1.89	23.8	CH_2
12	5.58	122.9	5.58	123.1	CH
13		144.4		144.5	C
14		42.1		42.1	C
15	2.15–2.45	36.2	2.15–2.47	36.2	CH_2
16	5.25 brs	74.1	5.25 brs	74.1	CH
17		49.6		49.6	C
18	3.58 dd (11.3, 3.0)	41.3	3.57 dd (11.3, 3.0)	41.3	CH
19	1.34, 2.77	47.1	1.35, 2.78	47.1	CH_2
20		31.0		31.0	C
21	1.28	36.0	1.26	36.0	CH_2
22		32.2		32.1	CH_2
23	1.27	28.3	1.250	28.3	CH_3
24	0.98	17.1	0.972	17.1	CH_3
25	0.82	15.7	0.819	15.7	CH_3
26	1.07	17.7	1.060	17.7	CH_3
27	1.82	27.3	1.806	27.3	CH_3
28		176.0		176.0	C
29	1.01	33.3	1.016	33.3	CH_3
30	1.15	24.9	1.170	24.9	CH_3

TABLE II NMR chemical shift assignments of the sugar moieties of compounds **1** and **2** (500 MHz for ^1H and 125 MHz for ^{13}C in C_5D_5N)

Position	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
	GlcA		GlcAme	
1	107.2	4.98 d 7.5	107.2	5.17 d 7.5
2	75.5	4.12	74.7	
3	77.8	4.49	77.8	
4	70.9	4.14	71.0	
5	78.2	4.50	78.5	
6	176.7		176.0	
OMe			49.6	3.59 s
	Ara		Xyl	
1	93.4	6.55 d 1.6	93.4	6.56 d 6.3
2	75.6	4.52 brd 5.8	79.8	4.56
3	78.2	4.34	75.3	4.15
4	65.5	4.45	70.9	4.12
5	62.4	3.94 dd 8.3, 12.0 4.56	65.5	3.49 dd 11.0, 11.0 4.10
	Rha		Rha	
1	100.9	5.64 brs	101.2	5.62 brs
2	71.6	4.79 dd 8.7, 2.8	71.5	4.84 brd 4.2
3	72.9	4.73	82.4	4.60 dd 4.2, 8.0
4	82.3	4.60 dd 3.1, 9.6	78.3	4.52
5	68.7	4.43 dd 9.6, 6.1	68.7	4.46
6	18.6	1.76 d 6.1	18.6	1.69 d 6.0
	Xyl		Ara	
1	104.4	5.46 d 7.8	104.8	5.27 brs
2	74.7	3.97 dd 7.8, 9.0	71.0	4.63
3	87.9	4.05 dd 9.0, 9.0	86.6	4.01
4	69.4	4.00	66.5	4.50
5	66.4	3.31 dd 11.0, 11.0 4.10	67.1	3.99, 4.60
	Xyl′		Xyl′	
1	106.1	5.15 d 7.2	105.9	5.24 d 7.6
2	75.4	4.01	75.4	4.04
3	77.9	4.08	77.9	4.09
4	69.6	4.17	69.4	4.18
5	67.1	4.09 dd 11.3, 11.3 4.11	67.2	3.42 dd 11.0, 11.0 4.10
	Ara′		Api	
1	106.0	5.03 d 7.5	112.0	5.99 d 4.2
2	72.9	4.47	77.7	4.76 d 4.2
3	74.5	4.02	79.9	—
4	68.9	4.57	74.6	4.22 d 9, 4.52
5	67.2	3.43 dd 11.0, 11.0 4.10	64.5	4.02

TABLE III ^{13}C-NMR data of aglycone moieties of compounds **3** and **4** (125 MHz for **3** in C_5D_5N; for **4** in CD_3OD)

Position	δ_C		*DEPT*	*Position*	δ_C		*DEPT*
	3	**4**			**3**	**4**	
1	38.8	40.2	CH_2	16	23.5	23.8	CH_2
2	26.5	27.0	CH_2	17	47.4	49.9	C
3	89.1	91.1	CH	18	41.7	42.7	CH
4	39.5	39.9	C	19	46.4	47.2	CH_2
5	56.0	57.0	CH	20	30.9	31.8	C
6	18.6	18.4	CH_2	21	34.2	35.0	CH_2
7	33.0	34.2	CH_2	22	32.6	33.9	CH_2
8	39.9	40.8	C	23	28.2	28.5	CH_3
9	48.0	48.3	CH	24	17.1	16.2	CH_3
10	37.0	38.0	C	25	15.6	14.9	CH_3
11	23.8	24.6	CH_2	26	17.6	17.3	CH_3
12	123.1	124.9	CH	27	26.0	26.4	CH_3
13	144.4	144.8	C	28	176.2	177.8	C
14	42.2	43.0	C	29	33.3	33.6	CH_3
15	28.3	28.9	CH_2	30	23.9	24.1	CH_3

of rhamnose, one unit of apiose, two units of xylose, and one unit of arabinose. The unambiguous assignments of the ^{1}H- and ^{13}C-NMR resonances of each monosaccharide were made (Table II) from 2D NMR spectra. The ^{13}C-NMR spectral data of the C-28 sugar moiety in **2** showed that one apiose and one xylose were at terminal positions, and that the linkage sites of glycosylation were at C-3 of the inner arabinose (+12.2 ppm), C-2 of the inner xylose (+5.8 ppm), and C-3 and C-4 of the inner rhamnose (+11.3 and +5.0 ppm, respectively). In the HMBC spectrum of compound **2**, the cross peaks between Api H-1 with Rha C-3, Xyl′ H-1 with Ara C-3, Ara H-1 with Rha C-4, Rha H-1 with Xyl C-2 and Xyl H-1 with the aglycone C-28 were observed, suggesting that the 28-*O*-oligosaccharide chain had the structure:

$$\begin{array}{c} \text{Xyl}'\text{-}(1\rightarrow3)\text{-Ara-}(1\rightarrow4)\text{-Rha-}(1\rightarrow2)\text{-Xyl-}\ (1\rightarrow28)\text{-Aglycone} \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad |\ (1\rightarrow3) \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad \text{Api} \end{array}$$

The HMBC spectrum also displayed cross peaks between GlcAMe H-1 with the aglycone C-3, showing that the glucuronic acid methyl ester moiety was affixed to C-3 of the aglycone.

The ^{1}H-NMR coupling constants of H-1 and H-2 and the ^{13}C-NMR chemical shifts of each monosaccharide also showed β-configuration for GluAMe. Xyl and Api and α-configuration for Rha and Ara [9,10].

TABLE IV NMR data of sugar moieties of compounds **3** and **4** (125 MHz for ^{13}C and 500 MHz for ^{1}H; for **3** in C_5D_5N; for **4** in CD_3OD)

Position	**3**		**4**	
	δ_C	δ_H	δ_C	δ_H
	GlcA		GlcAme	
1	107.2	4.96 d (8.0)	107.2	4.36 d (7.8)
2	75.2	4.06 d (8.9)	75.3	3.61 dd (8.0, 9.0)
3	78.7	4.24 dd (9.0, 9.0)	77.8	3.79 dd (9.0, 9.0)
4	71.0	4.45 dd (9.0, 9.0)	73.5	3.82 dd (9.0, 9.0)
5	79.9	4.58 d (9.0)	76.9	3.87 d (9.0)
6	176.5		172.3	
OMe			52.9	3.75 s
	Ara		Xyl	
1	93.6	6.45 d (3.0)	93.8	5.66 d (3.5)
2	75.4	4.55 dd (3.0, 4.0)	76.3	3.49 dd (3.5, 9.0)
3	70.1	4.50	77.8	3.45
4	66.4	4.40	71.4	
5	63.3	3.94 dd (12.0, 4.0), 4.50	67.1	3.82, dd (11.0, 9.0), 3.44 dd (11.0, 5.0)
	Rha		Rha	
1	101.2	5.77 brs	101.6	5.01 brs
2	71.7	4.86 brs	72.5	4.56 brs
3	72.8	4.53	73.5	3.87
4	84.4	4.37	83.7	3.70
5	68.5	4.40	69.2	3.80
6	18.5	1.77 d (6.0)	18.4	1.26 d (6.3)
	Xyl		Xyl′	
1	106.1	5.42 d (7.8)	107.1	4.48 d 7.7
2	74.2	4.00	76.1	3.21 dd (7.7, 9.0)
3	87.3	4.04	78.4	3.34 dd (9.0, 9.0)
4	69.2	4.00	71.3	*ca.* 3.50
5	67.5	3.45, 4.20 dd (5.0, 11.0)	67.5	3.78 dd (11.0, 9.0), 3.30 dd (11.0, 5.0)
	Xyl′			
1	106.9	5.16 d (8.0)		
2	76.2	4.02		
3	78.3	4.12		
4	70.1	4.18		
5	67.7	3.94 dd (11.0, 11.0), 4.10		

Therefore, the structure of compound **2** was elucidated as methyl ester of 3-*O*-β-D-glucuronyl-16α-hydroxy-olean-12-en-28-oic acid 28-*O*-[β-D-xylopyranosyl(1 → 3)-α-L-arabinopyranosyl(1 → 4)]-[β-D-apiofuranosyl(1 → 3)]-α-L-rhamnopyranosyl(1 → 2)-β-D-xylopyranoside.

Compound **3** showed a $[M+Na]^+$ ion at *m/z* 1197 in the FABMS, corresponding to a molecular formula of $Cl_{57}H_{90}O_{25}$. On acid hydrolysis, compound **3** gave xylose, arabinose, rhamnose and glucuronic acid as sugar components which were identified in the same manner as described for **1** and **2**. Its IR spectrum showed the presence of hydroxy, ester group, double bond, and C–O–C bonds. The ^{1}H-NMR spectrum showed signals of seven

tertiary methyl groups (δ 0.80, 0.93, 0.98, 1.02, 1.14, 1.27, and 1.77), one trisubstituted olefinic proton (δ 5.67, dd, $J = 3.0$, 3.0 Hz) and five anomeric protons (δ 4.96, d, $J = 8.0$ Hz; 6.45, d, $J = 3.0$ Hz; 5.77, brs; 5.42, d, $J = 7.8$ Hz; 5.16, d, $J = 8.0$ Hz). The ^{13}C-NMR and DEPT spectra showed signals for six sp^3 quaternary carbons (δ 30.9, 37.0, 39.5, 39.9, 42.2, and 47.4), two carbonyl carbons (δ 176.2 and 176.5), a pair of olefinic carbons (δ 123.1 and 144.4), ten methylene signals (δ 18.6, 23.5, 23.8, 26.5, 28.3, 32.6, 33.0, 34.2, 38.8, and 46.4) and four methine signals (δ 89.1, 56.0, 48.0, and 41.7) in the aglycone moiety, together with five anomeric carbons (δ 93.6, 101.2, 106.1, 106.9, and 107.2). Overall features of the NMR spectra suggested compound **3** to be an oleanolic acid 3-28-bisdemoside having five sugar units [one unit of rhamnose from signals at δ 101.2 and 18.5 (CH_3), one unit of glucuronic acid from signals at δ 107.2 and 176.5, and three pentose units from signals at δ 63.3, 67.5, and 67.7 ($3 \times CH_2$)]. The signals at δ 93.6 and 63.3 were due to C-1 and C-5 of arabinose, respectively, which was connected to C-28 through an ester linkage [6,11].

Alkaline hydrolysis of **3** afforded a prosapogenin identified as 3-*O*-β-D-glucuronylolean-12-en-28-oic acid, which furnished oleanolic acid and glucuronic acid by further acid hydrolysis. Thus, the C-28 sugar unit contained four sugar units (one unit of Rha, two units of Xyl, and one unit of Ara).

From the chemical shifts of the ^{13}C-NMR signals due to the sugar moieties (Table IV), glycosidation shifts were observed at C-2 of Ara (+3 ppm), C-4 of Rha (+11.1 ppm) and C-3 of Xyl (+10.4 ppm) in comparison with methyl-α-L-arabinopyranoside, methyl-α-L-rhamnopyranoside and methyl-β-D-xylopyranoside, respectively [7,8]. The cross peaks between Rha H-1 and Ara C-2, Xyl H-1 and Rha C-4, Xyl′ H-1 and Xyl C-3 in the HMBC spectrum showed the sequences of the sugars at C-28 as: Xyl′-(1 → 3)-Xyl-(1 → 4)-Rha(1 → 2)-Ara-. This was further confirmed by the fragment ions at *m/z* 1065 $[M + Na - 132]^+$, 1021 $[M + Na - 177 + H]^+$, 933 $[M + Na - 2Xyl]^+$, 787 $[M + Na - 2Xyl\text{-}Rha]^+$ in the FABMS. From all this information, compound **3** was established as 3-*O*-β-D-glucuronyl-olean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl(1 → 3)-β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)-α-L-arabinopyranoside.

Compound **4** gave xylose, rhamnose, glucuronic acid and oleanolic acid on acid hydrolysis and the same prosapogenin of **3** on alkaline hydrolysis. The NMR spectra showed the presence of four sugar units in **4**, and the ^{13}C-NMR spectral data were almost identical with those of a known compound scaberoside B_6 methyl ester which is the methyl ester of 3-*O*-β-D-glucuronyl-olean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)-β-D-xylopyranoside.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on a XT4-100X micro-melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 automatic digital polarimeter. The IR spectra were recorded on a Perkin-Elmer 683 IR spectrometer. NMR spectra were run on a Bruker AM-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer. FAB-MS were taken on a ZAB SpecE instrument, ESIMS on an IonSpec HiRes-MALDI instrument and EIMS on a VG ZAB-2f instrument. TLC was performed on silica gel GF and HRTLC on silica gel H (5–7 μm). Separation and purification were performed by column chromatography on silica gel (300–400 mesh and 180–200 mesh).

Plant Material

The roots of *Aster auriculatus* were collected in August 1988 from Yun Long County, Sichuan Province, People's Republic of China. A voucher specimen (880801) was identified by Prof. W. Z. Song and deposited in the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, People's Republic of China.

Extraction and Isolation

Air-dried roots of plants (2.5 kg) were extracted with 70% ethanol (5 × 5 l) under reflux. The combined extracts were evaporated under reduced pressure to obtain a crude syrup (500 g), which was chromatographed over a highly porous polymer (RA, Seventh Chemical and Industrial Factory, Beijing) column (9.5 × 50 cm, 2 × 750 g) eluted successively with H_2O, 30% EtOH, 60% EtOH, and 95% EtOH.

The 60% EtOH part (250 g) was subjected to column chromatography over silica gel (8.0 × 64 cm, 180–200 mesh, 2 kg) eluted with a solvent system of $CHCl_3$–MeOH–H_2O gradient (500 ml each eluent) yielding eight crude fractions monitored by HRTLC (silica gel 5–7 μm). Fraction 8 eluted with $CHCl_3$–MeOH–H_2O, (1 : 1 : 0.2) was purified by column chromatography over silica gel with $CHCl_3$–MeOH–H_2O (26 : 14 : 3) as solvent to afford compound **1** (5 g).

The 95% EtOH part (54 g) was subjected to column chromatography over silica gel (5.5 × 85 cm, 180–200 mesh, 1 kg) eluted with $CHCl_3$–MeOH

(10 : 1), followed by $CHCl_3$–MeOH mixture of increasing polarity yielding seven fractions. Fraction 5 eluted with $CHCl_3$–MeOH (2 : 1) was further purified by column chromatography over silica gel using $CHCl_3$–MeOH–H_2O as solvent (2 : 1 : 0.2) to give compound **4** (45 mg) and **3** (120 mg), elution with $CHCl_3$–MeOH–H_2O (1 : 1 : 0.1) yielding compound **2** (80 mg).

Compound 1 White needles; m.p. 232–235°C, $[\alpha]_D^{21}$ – 56.1 (*c* 0.027, MeOH); IR (KBr) ν_{max} 3431, 2930, 1730, 1634, 1387, 1076–1043 cm^{-1}; ^1H- and ^{13}C-NMR, see Tables I and II; FABMS *m/z* 1345 $[M+Na]^+$, 1213, 1169, 1081, 1067, 1037, 631, 585, 543, 455, 133, 115; ESIMS (HR) *m/z* 1367.5881 (5) $[M+2Na-H]^+$ (calcd. 1367.5860), 1345.6129 (5) $[M+Na]^+$ (calcd. 1345.6041), 1171.5769 (**4**), 1170.5753 (8), 1169.5788 (16), 1037.5260 (5), 727.2260 (19), 698.2193 (34), 697.2150 (100), 493.3577 (16).

Compound 2 Amorphous powder; m.p. 280–284°C, $[\alpha]_D^{31}$ – 23.7 (*c* 0.046, MeOH); IR (KBr) ν_{max} 3400, 1735, 1640, 1100–1000 cm^{-1}; ^1H- and ^{13}C-NMR, see Tables I and II; FABMS *m/z* 1359 $[M+Na]^+$, 1227 $[M+Na-132]^+$, 1169 $[M+Na-GluAMe]^+$, 1095 $[M+Na-Xyl'-Ara]^+$, 631, 585, 483, 455, 437, 391, 330, 303, 207, 133, 115; *Anal* found: C 53.33%, H 7.72%; calcd. For $C_{63}H_{100}O_{30} \cdot 9/2H_2O$; C 53.35%; H 7.75%

Compound 3 Amorphous powder; $[\alpha]_D^{21}$ – 23.2° (*c* 0.056, MeOH); IR (KBr) ν_{max} 3406, 2924, 1711, 1605, 1386, 1077–1044 cm^{-1}; ^1H-NMR (C_5D_5N, 500 MHz) δ 0.80, 0.93, 0.98, 1.02, 1.14, 1.27 and 1.77, each 3H, s), 5.67 (1H, dd, J = 3.0, 3.0 Hz, H-12); Sugar moieties, see Table IV; ^{13}C-NMR, see Table III and IV; FABMS *m/z* 1197 $[M+Na]^+$, 1065 $[M+Na-132]^+$, 021 $[M+Na-177+H]^+$, 933 $[M+Na-2Xyl.]^+$, 787 $[M+Na-2Xyl-Rha]^+$.

Scaberoside B_6 methyl ester (**4**). Amorphous powder; ^1H-NMR (CD_3OD, 500 MHz) δ 0.76, 0.83, 0.89, 0.92, 0.93, 1.03 and 1.14 (each 3H, s, 7 × Me), 2.90 (1H, dd, J = 4.3, 11 Hz, H-18), 3.14 (1H, dd, J = 4.1, 11.3 Hz, H-3), 5.27 (1H, t, J = 3.0 Hz, H-12); Sugar moieties, see Table IV. ^{13}C-NMR, see Tables III and IV. Its ^{13}C-NMR data in CD_3OD was closely comparable with those of scaberoside B_6 methyl ester in C_5D_5N [11]. FABMS *m/z* 1079 $[M+Na]^+$, 947 $[M+Na-Xyl]^+$, 889 $[M+Na-GluAMe]^+$.

Acid hydrolysis of 1–4 A solution of each sample (20 mg) in 4 ml of 2 N HCl–MeOH (1 : 1) was refluxed for 2 h. After cooling to room temperature, the reaction mixture was neutralized with 0.05 N NaOH, and then extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated to dryness and chromatographed over silica gel eluted with petroleum ether–acetone (4 : 1) to obtain the aglycones echinocystic acid for compounds **1** and **2**, and oleanolic acid for **3** and **4**.

The aqueous layer was evaporated *in vacuo* and subjected to TLC analysis on Kieselgel 60 F_{254} [using $CHCl_3$–MeOH–H_2O (30 : 12 : 4) and HOAc, 9 : 1 ml] and paper chromatography [using *n*-BuOH–benzene–C_5H_5N–H_2O (5 : 1 : 3 : 3) and *n*-BuOH–HOAc–H_2O (4 : 1 : 5)] by comparison with authentic samples, indicating the presence of glucuronic acid, xylose, arabinose, rhamnose, and apiose in **1** and **3**, glucuronic acid, xylose, arabinose, rhamnose, and apiose in **2**, and glucuronic acid, xylose, and rhamnose in **4**.

Alkaline hydrolysis of 1–4 A solution of each sample (20 mg) in 2% NaOH–EtOH (1 : 1, 10 ml) was refluxed for 6 h. The reaction mixture was cooled to room temperature, and carefully neutralized with 0.1 N HCl, then extracted with *n*-BuOH three times. The *n*-BuOH solution was evaporated under reduced pressure, and the residue was chromatographed over silica gel, eluted with $CHCl_3$–MeOH–H_2O (3 : 1 : 0.1), recrystallization in 95% EtOH yielded the prosapogenin 3-*O*-β-D-glucuronyl-16α-hydroxy-olean-12-en-28-oic acid for both **1** and **2**. The prosapogenin, amorphous powder, m.p. 182–186°C, $[\alpha]_D^{31}$ + 23.6 (*c* 0.051, MeOH); FABMS *m/z* 671 $[M+Na]^+$, 455, 391, 207, 115. ^{1}H- and ^{13}C-NMR data were consistent with the literature values [6]. Similarly, the prosapogenin from the akaline hydrolysis of compounds **3** and **4** was identified as 3-*O*-β-D-glucuronyl-olean-12-en-28-oic acid by comparison of its physical properties and spectral data with those reported in the literature.

Acknowledgement

This work was partially supported by the Chinese Postdoctoral Scientific Foundation. The authors are grateful to Mr. W. Y. He and Ms. M. Kong, Department of Instrumental Analysis, Institute of Materia Medica, Chinese Academy of Medical Sciences for NMR measurements.

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