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GLYCOSIDES FROM *ASTER YUNNANENSIS*

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Key Word Index—*Aster yunnanensis*; Compositae; triterpene saponins; acetylene glycoside; bayogenin; asteryunnanoside E and I.

Abstract—A new oleanane-type triterpene saponin named asteryunnanoside E and a new acetylene glycoside named asteryunnanoside I have been isolated from the roots of *Aster yunnanensis*, and their structures elucidated as 3-*O*-*β*-D-glucopyranosyl-bayogenin-28-*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranoside and 2*Z*,8*E*-decadiene-4,6-diyn-1-*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranoside by means of 1D and 2D NMR techniques (COSY, HETCOR, COLOC, HOHAHA and ROESY) and chemical transformations.

INTRODUCTION

In a previous paper [1], four new triterpene saponins, asteryunnanoside A–D have been reported from the roots of *Aster yunnanensis* collected from Li-Jiang County, Yunnan Province, China. Continuing the search for new bioactive glycosides from this plant, we have isolated a novel triterpenoid saponin named asteryunnanoside E (**1**) and one acetylene glycoside named asteryunnanoside I (**2**).

RESULTS AND DISCUSSION

The *n*-butanol-soluble part of the 70% ethanol extract from the roots of *A. yunnanensis* was chromatographed on a column of highly porous resin (SIP-1300) and rechromatographed on silica gel, Sephadex LH-20 and C-8 reverse-phase columns to afford **1** and **2**.

Asteryunnanoside E (**1**) was obtained as needles, mp 240–242°. The FAB-mass spectrum showed an $[M + Na]^+$ ion at *m/z* 997 and an $[M + Li]^+$ ion at *m/z* 981; thus, the molecular weight was 974. The result of elemental analysis (Found: C, 56.48; H, 8.53. Analyt. Calcd: C, 56.36; H, 8.16) suggested the molecular formula as $C_{48}H_{78}O_{20} \cdot 2.5H_2O$. The IR spectrum showed adsorptions at 3400 (OH), 1735 (C=O of ester group) and 1640 cm^{-1} (C=C). On mineral acid hydrolysis, **1** provided glucose as the sugar component.

The ^1H NMR spectrum gave the signals of six singlet methyl groups (*δ*0.87, 0.90, 0.91, 1.18, 1.37 and 1.62), one trisubstituted olefinic proton (*δ*5.40) and three anomeric protons [*δ*6.20 (*d*, *J* = 8.1 Hz), 5.72 (*d*, *J* = 7.7 Hz) and 5.18 (*d*, *J* = 7.6 Hz)]. The ^{13}C NMR spectrum revealed the signals of six C—C bonded saturated quaternary carbons (*δ*30.7, 36.9, 40.1, 42.8, 42.8, 47.0), a pair of olefinic carbons (*δ*122.8 and 144.5), one esteric carbonyl carbon (*δ*176.5) and three anomeric carbons (*δ*93.6, 104.6 and 105.7). The numbers and chemical shifts of the tertiary methyl functions and quaternary carbons suggested that **1** was an oleanane-type triterpene triglycoside. The ^1H signal at *δ*6.20 and the ^{13}C signal at *δ*93.6 indicated the presence of an ester-linked sugar moiety.

After selective cleavage of the ester-glycoside linkage with LiI in anhydrous methanol and 2,6-lutidine according to the method reported by Ohtanin *et al.* [2] and followed by treatment with diazomethane, **1** gave a prosapogenin methyl ester (**1a**). Compound **1a** showed an $[M + Na]^+$ ion at *m/z* 687 and an $[M + Li]^+$ ion at *m/z* 671 in its FAB-mass spectrum and it yielded the aglycone methyl ester (**1b**) upon methanolysis. The EI-mass spectrum of **1b** gave the molecular ion peak at *m/z* 502 together with the fragment ion peaks at *m/z* 262 and 203 deriving from the D/E ring and *m/z* 239 from the A/B ring, all of which were formed through the characteristic retro-Diels–Alder fragmentation at the C-ring in the olean-12-en-28-oic acid methyl ester skeleton. This indicated that there were no substitutions on the C, D and E-rings, and three hydroxyl groups on the A and B-rings [3]. The ^1H NMR spectrum of **1b** showed two signals corresponding to two methine protons on its carbons having hydroxyl groups at *δ*4.27 (*d*, *J* = 3.0 Hz, H-3*α*) and *δ*4.53 (*m*, H-2*α*), an AB type coupling at *δ*3.72 and 4.18 (each 1H, each *d*, *J* = 12 Hz) and the signals of seven tertiary methyls and one methoxy-carbonyl group. From these spectral data, and further comparison of its ^{13}C NMR chemical shifts (Table 1) with the data in the literature [4], **1b** was identified as the methyl ester of 2*β*,3*β*,23-trihydroxy-olean-12-en-28-oic acid (bayogenin methyl ester).

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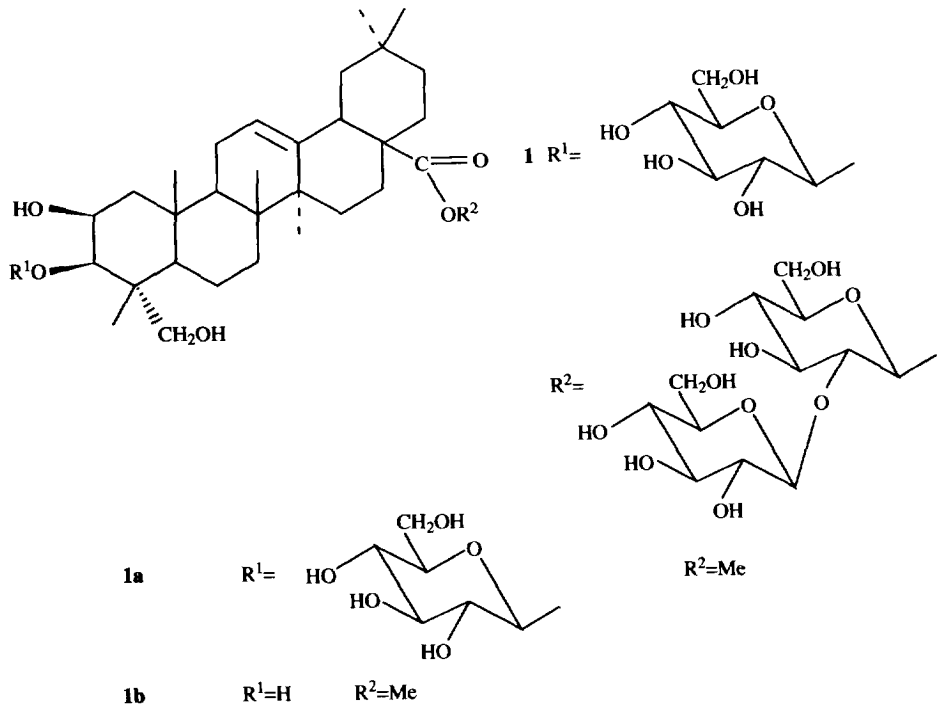

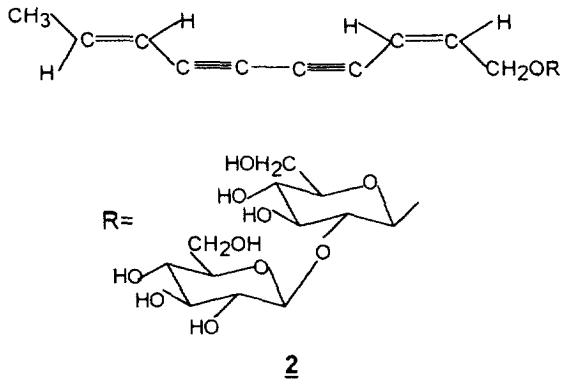

2

A comparison of the ^{13}C NMR signals of the aglycone moiety in **1a** with those of **1b** revealed that the C-3 signal in **1a** appeared at lower field (+ 9.94 ppm) than that of **1b**, which indicated that the glycosylation took place at the C-3 position. The glucose H-1′ signal at δ5.19 (*d*, *J* = 7.8 Hz) suggested that the glucosyl unit had the β-configuration. Thus, **1a** was a 3-*O*-β-D-glucopyranosyl-bayogenin methyl ester.

The above result indicated that the remaining 2 mol of glucosyl units must be bound to the genin by a glycosidic ester linkage at C-28. The ^1H and ^{13}C data (Table 2) of the sugar part were unambiguously assigned by ^1H–^1H COSY, HETCOR, ROESY [5–7] and HOHAHA [8, 9] techniques. The 2D NMR data are summarized in Table 3.

From the ROESY spectrum, two significant NOE correlation contours between glucose H-1′ with the H-3

Table 1. ^{13}C NMR chemical shifts of **1**, **1a**, and **1b** (pyridine-d_5, 100 MHz for δ_C, ppm)

C	1	1a	1b	DEPT
1	44.1	44.1	44.9	CH_2
2	70.6	70.5	71.6	CH
3	82.8	83.2	73.3	CH
4	42.8	42.3	42.2	C
5	48.6	48.5	48.5	CH
6	18.0	18.2	18.4	CH_2
7	33.0	33.0	32.8	CH_2
8	40.1	40.0	39.9	C
9	47.7	47.9	48.3	CH
10	36.9	37.0	37.2	C
11	24.0	24.1	24.0	CH_2
12	122.8	123.3	123.0	CH
13	144.5	144.2	144.2	C
14	42.8	42.8	42.4	C
15	29.1	28.2	28.1	CH_2
16	23.1	23.6	23.5	CH_2
17	47.0	47.1	47.0	C
18	41.8	42.0	41.9	CH
19	46.2	46.2	46.2	CH_2
20	30.7	30.9	30.8	C
21	34.0	34.1	34.2	CH_2
22	32.2	33.0	32.9	CH_2
23	65.3	65.8	67.9	CH_2
24	17.9	15.1	14.5	Me
25	17.5	17.3	17.3	Me
26	17.3	17.3	17.3	Me
27	26.2	26.3	26.2	Me
28	176.5	178.0	178.1	C
29	33.1	33.2	33.1	Me
30	23.7	23.8	23.7	Me
OMe		51.6	51.6	Me

Table 2. NMR data of sugar units of **1** and **1a** (pyridine-d_5, 100 MHz for δ_C, 400 MHz for δ_H, ppm, J = Hz)

	1		**1a**	
Position	δ_C	δ_H	δ_C	δ_H
3-*O*-Sugar				
glc-1′	105.7	5.18 (*d*, 7.6 Hz)	105.7	5.19 (*d*, 7.8 Hz)
2′	75.5	4.01	75.5	4.03
3′	78.3	4.25	78.6	4.34
4′	71.6	4.20	71.8	4.21
5′	78.3	3.91	78.3	3.95
6′a	62.6	4.35	62.8	4.44
6′b		4.44		4.47
28-*O*-Sugar				
Inner glc				
1″	93.6	6.20 (*d*, 8.1 Hz)		
2″	78.8	4.47		
3″	79.0	4.27		
4″	70.7	4.25		
5″	79.2	3.91		
6″	62.0	4.38		
Outer glc				
1‴	104.6	5.72 (*d*, 7.7 Hz)		
2‴	75.9	4.08		
3‴	78.6	4.22		
4‴	72.9	4.08		
5‴	78.9	4.01		
6‴a	63.9	4.38		
6‴b		4.67		

Table 3. Summary of the 2D NMR correlations of **1**

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′, 6a	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	6′b, 5′
6′b	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″	2″
5″	4″, 6″	5″	2″, 3″, 4″, 6″	1″, 3″, 6″
6″	5″	6″	4″, 5″	5″
Outer glc				
1‴	2‴	1‴	2‴, 3‴	3‴, 5‴, 2″
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

signal of aglycone, and glucose H-1‴ with the 28-*O*-linked inner glucose H-2″, were observed. Glycosylation shifts by + 4.77 ppm for C-2″ of the 28-*O*-inner glucosyl unit by comparison with that of methyl β-D-glucopyranoside [10] favoured that the outer glycosyl unit was attached to the C-2″ position of the inner glucosyl unit. Three glucose units were determined to have β-configurations based on the coupling constants of their anomeric protons (7.6, 7.7 and 8.1 Hz, respectively). Consequently, the structure of **1** was concluded to be 3-*O*-β-D-glucopyranosyl-bayogenin-28-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside.

Asteryunnanoside I (**2**) was obtained as a powder, mp 170–172°. Its FAB-mass spectrum gave three quasimolecular ions at *m/z* 471 $[M + H]^+$, 493 $[M + Na]^+$ and 477 $[M + Li]^+$ to confirm the molecular weight of 470 for $C_{22}H_{30}O_{11}$. The ^1H NMR spectrum (Table 4) showed one methyl group at δ1.55 (*dd*, *J* = 7.0, 1.6 Hz), two *trans*-coupled olefinic proton resonances at δ5.59 (*dd*, *J* = 15.8 Hz) and 6.22 (*dd*, *J* = 15.8, 7.0 Hz), two *cis*-coupled olefinic proton resonances at δ6.44 (2H, *br*, *s*), 15 proton resonances in the region δ3.82–5.31 including two anomeric proton signals at δ4.85 (*d*) and 5.31 (*d*). The ^{13}C NMR spectrum (Table 4) revealed the presence of methyl at δ18.6, four olefinic carbons at δ143.8, 109.6, 110.0 and 144.2, four quaternary carbons at δ75.0, 80.3, 81.0 and 73.2, 10 methines including two anomeric carbon signals at δ102.1 and 106.5 and three methylene at δ62.3, 62.5 and 68.3. These data suggested that **2** was a C_{10}-acetylene diglycoside.

Upon acid hydrolysis of **2**, the sugar constituent was identified by PC as glucose in direct comparison with an authentic sample and the aglycone could not be obtained. Two glucose units were deduced as β-configurations based on their coupling constants (7.7 and 7.8 Hz, respectively).

The ^1H–^1H COSY spectrum showed two separate spin systems, the first one was a Me–CH=CH– unit related to three protons at δ1.55, 5.59 and 6.22, and the second fragment was a –CH=CH–CH_2– which corresponded to the four protons at δ6.44 (2H, *br s*) and 4.55, 4.30 (each 1H, overlap). The chemical shifts of the last two protons showed that this methylene must be bound to a oxygen atom. The two fragments were linked through two acetylenic bonds. Therefore, the aglycone part was determined to be Me–CH=CH–C≡C–C≡C–CH=CH–CH_2O–.

Using the HETCOR spectrum, the chemical shifts of the carbons attached to the hydrogens were assigned. The remaining four quaternary carbon resonances were assigned by the COLOC (8 Hz) map [11] which displayed correlations between H-3 and carbon signals at δ75.0 and 80.3, the coupling of the former was stronger than that of the latter. Thus, the δ75.0 signal was assigned as the C-4 chemical shift and the δ80.3 signal was assigned as C-5. In

Table 4. NMR data of **2** (pyridine-d_5, 100 MHz for δ_C, 400 MHz for δ_H, ppm, *J* = Hz)

Position	δ_C	δ_H	COLOC
Aglycone			
1a	68.3	4.30 (overlap)	H-1a/C-2
1b		4.55 (overlap)	H-1b/C-2
2	143.8	6.44 (*br s*)	
3	109.6	6.44 (*br s*)	H-2/C-4 (strong), H-2/C-5
4	75.0		
5	80.3		
6	81.1		
7	73.2		
8	110.0	5.59 (*dd*, 15.8, 1.6)	H-8/C-7, H-8/C-9
9	114.2	6.22 (*dq*, 15.8, 7.0)	H-9/C-8, H-9/C-10
10	18.6	1.55 (*dd*, 7.0, 1.6)	H-10/C-7
Sugar			
Inner glc			
1′	102.1	4.85 (*d*, 7.7)	H-1′/C-6
2′	84.1	4.13 (*dd*, 7.7, 9.0)	H-2′/C-1″
3′	77.9	4.27 (*dd*, 9.0, 9.0)	
4′	71.4	4.24 (*dd*, 9.0, 8.5)	
5′	78.5	3.92 (*ddd*, 8.5, 4.8, 2.0)	
6a′	62.5	4.40 (*dd*, 11.6, 4.8)	
6′b		4.53 (*dd*, 11.6, 2.0)	
Outer glc			
1″	106.5	5.31 (*d*, 7.8)	H-1″/C-2′
2″	76.5	4.09 (*dd*, 7.8, 8.5)	
3″	78.0	4.21 (*dd*, 8.5, 9.5)	
4″	71.0	4.17 (*dd*, 9.5, 8.0)	
5″	78.3	3.82 (*ddd*, 8.0, 5.0, 2.0)	
6″a	62.3	4.26 (*dd*, 11.7, 5.0)	
6″b		4.46 (*dd*, 11.7, 2.0)	

addition, a correlation, showing the H-10 signal with the carbon resonance at δ73.2, confirmed the assignment of C-7 at δ73.2. The remaining acetylenic carbon signal at δ81.0 was assigned to C-6.

The NMR parameters of two sugar units were assigned by COSY and HETCOR spectra. The COLOC spectrum showed the correlations between the inner glucose H-1′ (δ4.85) and aglycone C-1 (δ68.3), the outer glucose H-1″ (δ5.31) and the inner glucose C-2′ (δ84.1), which suggested the direct linkage between the inner glucose unit with the aglycone and a (1 → 2) linkage between the inner glucose with the outer glucose unit. The downfield shift of the inner glucose C-2′ by + 9.8 ppm in comparison with that of methyl β-D-glycopyranoside further confirmed the presence of a (1 → 2) linkage between two glucose units. The observation of two prominent fragment ion peaks at *m/z* 331 $[glc(OAc)_4]^+$ and 619 $[glc(OAc)_4glc(OAc)_3]^+$ in the EI mass spectrum of peracetylated **2** provided additional evidence for the sequence of the sugar part.

From the above-mentioned evidence, **2** proved to be 2*Z*,8*E*-decadiene-4,6-diyn-1-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside.

EXPERIMENTAL

Mps were determined on a Kofler apparatus and uncorr.; $[\alpha]_D$ were measured at 28° 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 on a VG ZAB-HS mass spectrometer using glycerin as matrix. EIMS were obtained on a MAT-95 mass spectrometer. ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY and HETCOR spectra were obtained on a Bruker AM-400 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, high layer), respectively, and detected with aniline phthalate.

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

Extraction and separation. The dried roots (15 kg) of *A. yunnanensis* were extracted 5 × with 70% EtOH at room temp. After concn *in vacuo*, the residue (3.8 kg) was suspended in H_2O and then extracted with petrol, EtOAc and *n*-BuOH, successively. The *n*-BuOH layer was evapd under red. pres. to give a residue (698 g). This residue was chromatographed on a column of highly porous resin (SIP-1300) eluting initially with H_2O and followed by EtOH. The EtOH eluate (350 g) was subjected to CC on silica gel (1.5 kg) eluting with $CHCl_3$–MeOH–H_2O (8:1:0.1–1:1:0.1) gradient to separate into 8 crude frs (frs 1–8). Fr. 2 was further rechromatographed on silica gel with $CHCl_3$–MeOH (6:1) and then purified by Sephadex LH-20 eluting with MeOH to afford 160 mg of **2**. Fr. 6 was further subjected to CC on silica gel eluting with $CHCl_3$–MeOH–H_2O (9:3:0.5) to give 3 frs, and the less polar fr. was repeatedly chromatographed on Lichroprep RP-8 with MeOH–H_2O (6:4) to give 550 mg of **1**.

Asteryunnanoside E (**1**). Needles, mp 240–242°. IR ν_{max}^{KBr} cm^{-1}; 3400, 1735, 1640, 1000–1100. FAB-MS *m/z* 997 $[M + Na]^+$ and 981 $[M + Li]^+$. Analyt. calcd for $C_{48}H_{78}O_{20} \cdot 2.5H_2O$: C, 56.63; H, 8.16. Found: C, 56.48; H, 8.53. ^{1}H NMR (pryidine-d_5): aglycone moiety: δ0.82, 0.82, 1.04, 1.21, 1.25, 1.47 (each 3H, each *s*, tert-Mex × 6), 3.11 (1H, *dd*, *J* = 12.5, 4.0 Hz, H-18), 3.59 and 4.35 (each 1H, each *d*, *J* = 10.0 Hz, H-23), 5.41 (*br s*,H-12), sugar moiety: Table 2. ^{13}C NMR data: Tables 1 and 2.

Selective cleavage of the ester–glycoside linkage of **1**. Compound **1** (100 mg) was dissolved in 2,6-lutidine (4 ml) containing anhydrous MeOH (2 ml) and LiI (150 mg). After heating at 160° for 16 hr, the reaction mixt. was diluted with H_2O (20 ml) and passed through a column of highly porous resin (SIP-1300) eluted with H_2O and then MeOH. The MeOH eluate was methylated with etheral CH_2N_2 and evapd to dryness. The residue was chromatographed on a silica gel column eluting with $CHCl_3$–MeOH (10:1) to afford a prosapogenin methyl ester (**1a**, 35 mg). Compound **1a**: needles from MeOH, mp 249–251°. $[\alpha]_D$ + 49.51° (MeOH; *c* 0.48). IR ν_{max}^{KBr} cm^{-1}: 3400, 1740, 1640, 1000. FAB-MS *m/z*: 687 $[M + Na]^+$ and 671 $[M + Li]^+$. ^{1}H NMR (pyridine-d_5): aglycone moiety; δ0.87, 0.88, 0.90, 1.18, 1.36, 1.57 (each 3H each *s*, *tert*-Me × 6), 3.08 (1H, *dd*, *J* = 13.7, 4.0 Hz, H-18), 3.69 (3H, *s*, OMe) 3.69 and 4.15 (each 1H, each *d*, *J* = 10.0 Hz, H-23), 4.34 (1H, overlap, H-3α), 4.82 (1H, *m*, H-2α), 5.37 (1H, *br s*, H-12), sugar moiety: Table 1. ^{13}C NMR: Tables 1 and 2.

Acid hydrolysis of **1**. Compound **1** (50 mg) was hydrolysed in 2 N HCl–MeOH (5 ml) at 100° for 4 hr. The aglycone was obtained. By methylation with etheral CH_2N_2, the aglycone was converted to the methyl ester (20 mg **1b**). The sugar part was identified as glucose by PC and TLC in direct comparison with a standard sugar. Compound **1b**: needles, mp 198–200°. $[\alpha]_D$ + 85° ($CHCl_3$; *c* 0.51). IR ν_{max}^{KBr} cm^{-1}: 3450, 1740, 1660. EI-MS *m/z*: 502 $[M]^+$, 487, 484, 469, 466, 292, 239, 203, 189. $C_{31}H_{50}O_5$. ^{1}H NMR (pyridine-d_5): δ0.87, 0.90, 0.91, 1.18, 1.37, 1.62 (each 3H, each *s*, tert-Me × 6), 3.09 (1H, *dd*, *J* = 13.7, 4.0 Hz, H-18), 3.69 (3H, *s*, OMe), 3.72 and 4.18 (each 1H, *J* = 10.1 Hz, H-23), 4.27 (1H, *d*, *J* = 3.0 Hz, H-3), 4.53 (1H, *m*, H-2), 5.40 (1H, *br s*, H-12). ^{13}C NMR: Table 1.

Asteryunnanoside I (**2**). Mp 170–172°. FAB-MS *m/z*: 493 $[M + Na]^+$, 471 $[M + Li]^+$ and 471 $[M + H]^+$. $C_{22}H_{30}O_{11}$. ^{1}H and ^{13}C NMR: Table 4.

Acid hydrolysis of **2**. A soln of **2** (5 mg) in 2 N HCl–MeOH (4 ml) was heated at 100° for 4 hr. The reaction mixt. was evapd repeatedly at 40° until the soln showed a neutral reaction. The residue was examined by PC and TLC in direct comparison with an authentic sample to show the presence of glucose.

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