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TRITERPENOID GLYCOSIDES FROM *ANEMOCLEMA GLAUCIFOLIUM*

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Key Word Index—*Anemoclema glaucifolium*; Ranunculaceae; roots; triterpenoid glycosides; anemoclemosides A and B.

Abstract—Two novel triterpenoid glycosides, anemoclemosides A and B, were isolated from the roots of *Anemoclema glaucifolium*, along with four known compounds. The structures of anemoclemosides A and B were established mainly by NMR techniques. The two glycosides have the straight chain form of arabinose attached in acetal form to the aglycone. This is the first example of such glycosides from natural sources.

INTRODUCTION

Anemoclema glaucifolium (Fr.) W. T. Wang, monotype genus plant belonging to the Ranunculaceae, is distributed at an altitude of 1600–3000 m in the Yangtse River valley region between northwestern Yunnan and southwestern Sichuan, China. In the Lijiang area of Yunnan, native people use the roots of this plant to treat open wounds, fractures, contusions and strains. Owing to its unique taxonomic position, it was hoped that the chemical investigation of *A. glaucifolium* would provide some chemotaxonomic information. This paper describes the isolation and structural elucidation of two novel triterpenoid glycosides, anemoclemosides A (**5**) and B (**6**), along with four known compounds (**1**–**4**), from the roots of this plant.

RESULTS AND DISCUSSION

The methanol extract of the roots of *A. glaucifolium* was repeatedly chromatographed to yield **1**–**6**. By comparing their ^1H and ^{13}C NMR signals with reported data [1–3], compounds **1**–**4** were identified as hederagenin (**1**), widely distributed in Ranunculaceae, Lardizabalaceae, etc. [4–6], leontoside A (**2**), isolated from *Leontice eversmanii* [4], *Clematis chinensis* [1] and *Pulsatilla campanella* [2], and saponin CP_{3b} (**3**) and saponin CP_2 (**4**), found in *Clematis chinensis* [1], respectively.

Compound **5** (anemoclemoside A) showed a quasi-molecular ion peak at *m/z* 603 in its negative FAB-mass spectrum and 35 resonance signals in its ^{13}C NMR spectrum (DEPT). From these data, its molecular formula was determined to be $C_{35}H_{56}O_8$. Acid hydrolysis of **5** afforded hederagenin (**1**) and L-arabinose, indicating that **5** was composed of one mol of hederagenin and one mol of arabinose. However, some carbon signals of **5** were quite different from those of **2**, hederagenin 3-*O*-arabinopyranoside. ^1H–^1H COSY and ^1H–^{13}C COSY experiments determined the linkage of the arabinosyl unit and led to the assignment of its signals as well as most of those of the aglycone moiety (Tables 1 and 2). The set of carbon signals for the arabinosyl unit differed slightly from those of a normal terminal arabinopyranosyl unit such as in glycoside **2**. In addition, the typical carbon signals of C-3 and C-23 of the aglycone unit were δ85.6 (CH) and 78.3 (CH_2), respectively, suggesting that both of them might be substituted. In the HMBC [7] spectrum of **5**, cross-peaks appeared between H-1′ of arabinose and C-3 and C-23 of the aglycone. The H-23 signal of the aglycone δ3.97, (*d*, J = 10.5 Hz) correlated with C-3 of the aglycone and C-1′ of the arabinose unit. The H-23 signal of the aglycone δ3.35, (*d*, J = 10.5 Hz) also correlated with C-3 of the aglycone. The key long-range H–C correlations observed from the HMBC experiment are summarized in Table 3. These data revealed that the straight chain form of arabinose was linked to both C-3 and C-23 of the aglycone and thus formed a six-membered ring. The conformation of the six-membered ring was determined by using NOE correlations. In the NOESY spectrum of **5**, cross-peaks appeared between H-1′ and H-3, and between H-3 and H-5. H-23 (δ3.97) correlated with three protons, H-24, H-6α and H-23 (δ3.35) (Fig. 1). In a NOE differential spectrum, H-3 (δ3.30) and H-23 (δ3.35) were enhanced on irradiating the H-1′ signal δ5.30. Therefore, the six-membered ring had a chair form as depicted in

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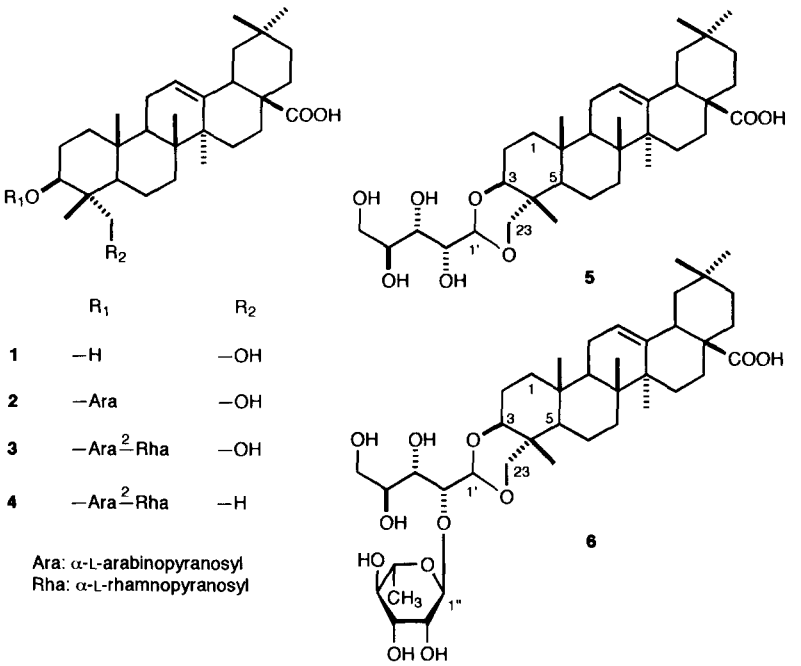

Fig. 1. The signals at δ3.35 and 3.97 were assigned to the axial H-23 and equatorial H-23, respectively. A comprehensive analysis of the NMR spectra assigned the carbon and proton signals of **5** as shown in Tables 1 and 2. Thus, the structure of **5** is (1*R*)-L-arabinose hederagenin 1′,3;1′,23-acetal. Alternatively, it could be named 3,23-*O*-(2*R*),(3*S*),(4*S*),5-tetrahydroxypentylidene olean-12-en-28-oic acid.

Compound **6** (anemoclemoside B) was hydrolysed with acid to give hederagenin (**1**) as aglycone and L-arabinose and L-rhamnose as the sugar components. Its negative FAB-mass spectrum exhibited a quasimolecular ion peak at *m/z* 749 [M ($C_{41}H_{66}O_{12}$) − H]$^-$ and a fragment ion at *m/z* 603 [M − Rha]$^-$. The ^{13}C NMR spectrum of **6** displayed signals similar to **5** with the exception of a set of additional α-L-rhamnopyranosyl signals. This suggested that **6** was formed from **5** by glycosylation with a rhamnopyranosyl unit. The nature of the interglycosidic linkage of **6** was established in a similar manner to that used for **5**. Tables 1 and 2 show the signals of **6** assigned by ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, HMBC and NOESY experiments. Table 3 summarizes the key long-range H–C correlations observed from the HMBC experiment and Fig. 2 shows the key NOE correlations observed from the NOESY experiment. The HMBC and NOESY spectra demonstrated that the rhamnosyl unit was linked to the hydroxy group of C-2′ of the arabinosyl unit. The NOESY spectrum of **6** showed that H-1′ of arabinose correlated not only with H-3 of the aglycone but also with the axial H-23 of the aglycone, although the latter cross-peak could not be observed in the NOESY spectrum of **5**. Thus, **6** is α-L-rhamnopyranosyl (1 → 2)-(1*R*)-L-arabinose hederagenin 1′,3;1′,23-acetal. Alternatively, it could be named 3,23-*O*-[2-(*O*-α-L-rhamnopyranosyl) (2*R*),(3*S*),(4*S*), 5-tetrahydroxypentylidene] olean-12-en-28-oic acid.

It is worth emphasizing that glycosides **5** and **6** have the straight chain form of arabinose attached in an acetal form to the aglycone. To the best of our knowledge, this is the first example of such glycosides isolated from natural sources. The glycosides isolated from *A. glaucifolium* show some similarities as well as distinctive aspects with those isolated from *Anemone*, *Clemitis* and *Pulsatilla* of the same family [2, 4–6]. This is of significance regarding their chemotaxonomy.

EXPERIMENTAL

Mps: uncorr; ^{1}H NMR: 400 MHz for **1**–**4** and 600 MHz for **5** and **6**; ^{13}C NMR: 100 MHz for **1**–**4** and 150 MHz for **5** and **6**. 2D NMR spectra were performed with standard pulse sequences. All NMR spectra were recorded in pyridine-d_5 using TMS as int. standard.

Plant material. The roots of *Anemoclema glaucifolium* (Fr.) W. T. Wang were collected in Lijiang, Yunnan, China and identified by Mr Hui-Jin Guo. A voucher specimen is deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation. The dried roots (5.0 kg) were extracted with hot MeOH. Removal of the solvent by evapn afforded a MeOH extract (1.2 kg). One half of the extract (600 g) was dissolved in H_2O and then subjected to CC on macroporous absorption resin D-101 with aq. MeOH. The 80% MeOH eluate was concd to dryness resulting in a pale yellow residue (27 g). This residue was chromatographed on silica gel with a $CHCl_3$–MeOH gradient (20 : 1 to 5 : 1) to afford frs 1–5. Fr. 1 (11.7 g) was

Table 1. ^{13}C NMR spectral data of compounds **1**–**6** in pyridine-d_5*

C	1	2	3	4	5	6
1	39.0	38.9	39.1	39.0	38.8	38.8
2	27.7	26.1	26.3	26.6	23.6[a]	23.7[a]
3	73.8	82.1	81.2	89.0	85.6	86.0
4	43.0	43.5	43.6	39.6	37.1	36.9
5	48.9	47.8	47.9	56.1	51.5	51.5
6	18.8	18.3	18.3	18.7	17.6	17.8
7	33.1[a]	33.0	33.0[a]	33.4	32.6	32.6
8	40.0	40.0	39.9	39.9	39.8	39.8
9	48.3	48.2	48.3	48.2	47.9	47.9
10	37.4	37.1	37.0	37.2	37.3	37.3
11	23.9[b]	23.8[a]	23.9	23.9	23.5[a]	23.6[a]
12	122.7	122.6	122.5	122.6	122.3	122.2
13	145.0	144.9	145.1	145.0	144.8	144.8
14	42.4	42.3	42.3	42.3	42.1	42.1
15	28.5	28.4	28.5	28.5	28.2	28.3
16	24.0[b]	23.9[a]	23.9	23.9	23.6[a]	23.6[a]
17	46.7	46.5	46.7	46.7	46.5	46.5
18	42.2	42.1	42.1	42.1	42.0	42.0
19	46.8	46.7	46.8	46.9	46.6	46.6
20	31.1	31.0	31.1	31.1	30.9	30.9
21	34.4	34.3	34.4	34.4	34.2	34.2
22	33.36[a]	33.0	33.4[a]	33.36	33.1	33.1
23	68.3	64.7	64.2	28.2	78.3	78.3
24	13.2	13.6	14.0	17.1	13.7	13.6
25	16.1	16.2	16.2	15.7	16.4	16.4
26	17.7	17.5	17.6	17.5	17.2	17.3
27	26.3	26.2	26.3	26.3	26.1	26.1
28	180.4	180.2	180.3	180.5	180.1	180.1
29	33.4	33.3	33.4	33.4	33.2	33.2
30	24.0	23.8	23.9	23.9	23.7	23.7
1′		106.6	104.2	104.7	103.7	103.0
2′		73.2	76.0	76.2	71.6	78.2
3′		74.7	74.4	73.7	71.8	72.1
4′		69.5	69.1	68.5	72.6	72.2
5′		66.7	65.4	64.7	65.4	65.1
1″			101.7	101.9		103.3
2″			72.4	72.5		72.3
3″			72.6	72.7		72.9
4″			74.2	74.1		74.1
5″			69.8	70.0		69.9
6″			18.6	18.7		18.5

*Compounds **1**–**4**: recorded at 100 MHz; compounds **5** and **6**: recorded at 150 MHz.

[a,b]Signals may be interchangable within each column.

Table 2. ^1H NMR spectral data of **5** and **6** in pyrideine-d_5 (600 MHz)*

H	5	6
1ax.	1.48	1.48
1eq.	0.95	0.94
2ax.	1.67	1.62
2eq.	1.45	1.45
3	3.30 *br d* (12.0)	3.32 *dd* (12.0, 3.7)
5	0.79 *d* (10.9)	0.78
6ax.	1.28	1.30
6eq.	1.09	1.07
7ax.	1.39 *dd* (8.9, 3.6)	1.40
7eq.	1.24	1.23
9	1.67	1.67
11ax.	1.89 *dd* (8.9, 3.6)	1.87 *dd* (8.9, 3.3)
11eq.	1.65	1.64
12	5.48 *t* (3.6)	5.47 *t* (3.3)
15ax.	2.13	2.13
15eq.	1.17	1.20
16ax.	1.96	1.96
16eq.	1.80	1.82
18	3.30 *br d* (12.0)	3.31 *br d* (12.0)
19ax.	1.82	1.81
19eq.	1.34	1.33
21ax.	1.45	1.42
21eq.	1.24	1.23
22ax.	2.04 *dt* (14, 4)	2.04
22eq.	1.82	1.83
23ax.	3.35 *d* (10.5)	3.24 *d* (10.5)
23eq.	3.97 *d* (10.5)	3.88 *d* (10.5)
24	1.14 *s*	1.15 *s*
25	0.83 *s*	0.80 *s*
26	0.96 *s*	0.96 *s*
27	1.26 *s*	1.28 *s*
29	0.97 *s*	0.97 *s*
30	1.03 *s*	1.02 *s*
1′	5.30 *d* (6.1)	5.41 *d* (4.8)
2′	4.77 *dd* (6.3, 1.2)	4.84 *dd* (4.8, 1.8)
3′	4.67 *dd* (8.5, 1.2)	4.73 *m*
4′	4.61 *m*	4.41 *m*
5′†	4.37 *dd* (11.0, 5.9)	4.33 *m*
5′‡	4.53 *dd* (11.0, 3.6)	4.48 *dd* (10.9, 3.2)
1″		5.98 *d* (1.0)
2″		4.73 *m*
3″		4.59 *dd* (9.3, 3.4)
4″		4.31 *m*
5″		4.73 *m*
6″		1.72 *d* (6.2)

*Assignments were made on the basis of ^1H–^1H COSY, ^1H–^{13}C COSY, HMBC and NOESY spectra. Coupling constants which were well resolved are expressed with multiplicities and coupling constants in Hz in parentheses.

†Upfield proton.

‡Downfield proton.

crystallized with MeOH to yield **1** (9.2 g). Fr. 2 (3.5 g) was chromatographed on silica gel with $CHCl_3$–MeOH (9 : 1) to afford **5** (128 mg) and a mixture containing mainly **2**. The mixture was further purified on a silica gel column using $CHCl_3$–MeOH–H_2O (50 : 10 : 1) to furnish **2** (35 mg). Fr. 4 was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (50:10:1 or 45:10:1) and reversed phase silica gel (RP-8) with 80% MeOH to yield **3** (90 mg), **4** (18 mg) and **6** (80 mg).

Hederagenin (**1**). Needles from MeOH, mp 306–308°, $[\alpha]_D^{16}$ +78° (MeOH; *c* 0.69). ^1H NMR: δ0.93, 0.95, 0.99, 1.03, 1.04, 1.23 (3H each, *s*, Me × 6), 3.28 (1H, *dd*, J = 13.6, 3.8 Hz, H-18), 3.71, 4.16 (2H, AB*q*, J = 10.4 Hz, H-23), 4.20 (1H, *m*, H-3), 5.49 (1H, *br s*, H-12); ^{13}C NMR: Table 1.

Leontoside A (**2**). Powder from MeOH, mp 221–223° (dec.), $[\alpha]_D^{16}$ +63° (MeOH; *c* 0.45). FAB-MS (neg.) *m/z* 603 $[M(C_{35}H_{56}O_8) - H]^-$, 471 $[M - Ara]^-$, ^1H NMR:

Table 3. Key long-range H–C correlations of **5** and **6** observed from HMBC experiments

H	Correlated C	
	5	**6**
3	2	
12	9, 14	9, 14
18	13, 14, 17, 19	14, 19
23ax.	3, 24	3, 24
23eq.	3, 4, 24, 1′	3, 24, 1′
24	3, 5, 7, 23	3, 5, 7, 23
25	1, 5, 7, 9	1, 5, 7, 9
26	8, 9, 14, 15	8, 9, 14, 15
27	8, 13, 14	8, 13, 14
29	19, 20, 30	19, 20, 30
30	19, 20	19, 20
1′	3, 23, 2′	3, 23, 2′
2′	1′, 3′	1′, 3′, 1″
3′	2′, 4′, 5′	
4′	3′	
5′*	3′, 4′	
5′†	3′, 4′	
1″		2′, 3″, 5″
4″		3″, 5″, 6″
6″		4″, 5″

*Upfield proton.
†Downfield proton.

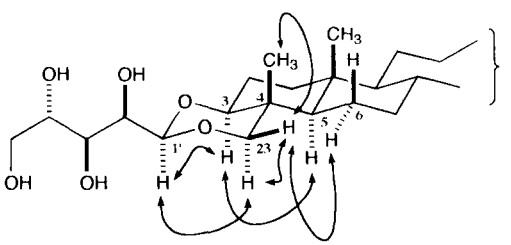

Fig. 1. Key NOE correlations of **5** observed in NOESY and NOEDS experiments.

δ0.90 × 2, 0.92, 0.98, 1.00, 123 (3H each, *s*, Me × 6), 3.28 (1H, *br d*, J = 10.4 Hz, H-18), 3.69 (1H, *d*, J = 10.6 Hz, H-23), 4.98 (1H, *d*, J = 7.1 Hz, Ara H-1′), 5.46 (1H, *br s*, H-12); ^{13}C NMR: Table 1.

Saponin CP$_{3b}$ (**3**). Powder from MeOH, mp 229–231° (dec.), $[\alpha]_D^{15}$ + 22° (MeOH; *c* 0.81). FAB-MS (neg.) *m/z*: 749 [M ($C_{41}H_{66}O_{12}$) − H]$^-$, 603 [M − Rha]$^-$, 471 [M − Rha − Ara]$^-$. ^{1}H NMR: δ0.92 × 2, 0.98 × 2, 1.05, 1.21 (3H each, *s*, Me × 6), 1.62 (3H, *d*, J = 6.0 Hz, Rha Me), 5.09 (1H, *d*, J = 6.1 Hz, Ara H-1′), 5.44 (1H, *br s*, H-12), 6.24 (1H, *s*, Rha H-1″); ^{13}C NMR: Table 1.

Saponin CP$_2$ (**4**). Powder from MeOH, mp 224–226° (dec.). FAB-MS (neg.) *m/z*: 733 [M($C_{41}H_{66}O_{11}$) − H]$^-$ 587 [M − Rha]$^-$, 455 [M − Rha − Ara]$^-$. ^{1}H NMR: δ0.85, 0.96, 0.99, 1.02, 1.05, 1.17, 1.31 (3H each, *s*, Me × 7), 1.62 (3H, *d*, J = 6.0 Hz, Rha Me), 3.27 (1H, *m*, H-18), 4.91 (1H, *d*, J = 4.8 Hz, Ara H-1′), 5.48 (1H, *br s*, H-12), 6.07 (1H, *s*, Rha H-1″); ^{13}C NMR: Table 1.

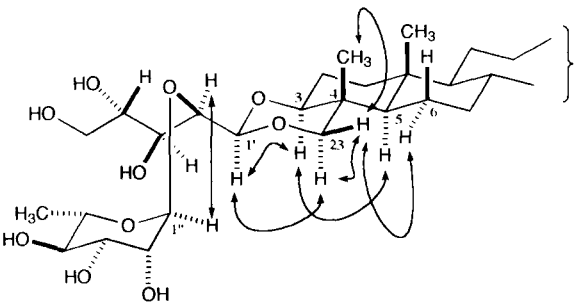

Fig. 2. Key NOE correlations of **6** observed in NOESY experiment.

Anemoclemoside A (**5**). Powder from MeOH, mp 194–198° (dec.), $[\alpha]_D^{16}$ +55° (MeOH; *c* 0.89). FAB-MS (neg.) *m/z*: 603 [M($C_{35}H_{56}O_8$)]$^-$; ^{1}H and ^{13}C NMR: Tables 1 and 2.

Anemoclemoside B (**6**). Powder from MeOH, mp 220–223° (dec.), $[\alpha]_D^{15}$ +22° (MeOH; *c* 0.90). FAB-MS (neg.) *m/z*: 749 [M($C_{41}H_{66}O_{12}$) − H]$^-$, 603 [M − Rha]$^-$; ^{1}H and ^{13}C NMR: Tables 1 and 2.

Acid hydrolysis of glycosides **5** *and* **6**. A soln of either **5** or **6** (25 mg each) and 5% HCl–dioxane (1:1; 3 ml) was heated at 95° for 4 hr. After cooling, the reaction mixt. was diluted with H_2O and then extracted with EtOAc. The EtOAc layer was washed with H_2O. After removal of the solvent, both **5** and **6** afforded hederagenin (**1**) as aglycone. Identifications were made by comparing TLC behaviour and the ^{1}H NMR spectra with an authentic sample.

The H_2O layer was neutralized with MB-3 (H^+, OH^- form) and concd to dryness. The residue resulting from **5** was dissolved in H_2O and then passed through a Sep-Pak C-18 cartridge to give L-arabinose [5.5 mg; $[\alpha]_D^{16}$ +112° (H_2O; *c* 0.34)]. The residue from **6** was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (14:6:1) to give L-arabinose [2.5 mg: $[\alpha]_D^{18}$ +116° (H_2O; *c* 0.70)] and L-rhamnose [1.4 mg; $[\alpha]_D^{18}$ +7° (H_2O; *c* 1.26)]. Qualitative identification of sugars was made by comparing their TLC behaviour on a silica gel plate with authentic samples. In this case, $CHCl_3$–MeOH–HOAc–H_2O (14:6:2:2:1) was used as solvent system and aniline/phthalate as colour reagent. Arabinose and rhamnose had R_f values of 0.25 and 0.36, respectively.

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