Chemistry of Natural Compounds, Vol. 38, No. 2, 2002

TRITERPENE GLYCOSIDES FROM PLANTS OF THE *Astragalus* GENUS. STRUCTURE OF CYCLOUNIFOLIOSIDE A FROM *Astragalus unifoliolatus*

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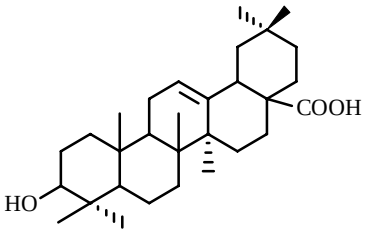
UDC 547.918:547.926

*The known compound oleanolic acid (**1**) and a new cycloartane glycoside cyclounifolioside A (**2**), which has the structure 6,16-di-O-acetyl-24R-cycloartan-3β,6α,16β,24,25-pentaol 3-O-β-D-glucopyranoside, were isolated from* Astragalus unifoliolatus *Bunge. The structures of the isolated compounds were established using chemical transformations and two-dimensional spectra (TOCSY, ROESY, HMBC, HSQC, COSY).*

Key words: *Astragalus unifoliolatus*, triterpene glycoside, cycloartane, 6-16-di-O-acetyl-24R-cycloartan-3*β*,6*α*,16*β*,24,25-pentaol 3-O-*β*-D-glucopyranoside.

Seven triterpenes are detected by TLC in the methanol extract of the epigeal part of *Astragalus unifoliolatus* Bunge [1]. Column chromatography of the ethylacetate fraction affords oleanolic acid (**1**) [2] and the new cycloartane glycoside cyclounifolioside A (**2**) [3]. The present article describes the structure determination of these glycosides.



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Oleanolic Acid (1). The IR spectrum of **1** contains absorption bands characteristic of hydroxyl and free carboxyl at 3447 and 1699 cm^{-1}, respectively.

The PMR spectrum of **1** has signals at strong field for seven methyls that resonate at 0.91, 0.97, 1.03, 1.04, 1.05, 1.26, and 1.31 ppm; at weak field, protons of a double bond at 5.52 ppm.

The structure of **1** was confirmed using ^{1}H and ^{13}C NMR and two-dimensional spectra (COSY, TOCSY). C-12 and C-13 of the double bond resonate at weak field at 122.48 and 144.90 ppm, respectively. The ^{13}C NMR of **1** and data for oleanolic acid in the literature [2] are in agreement.

Cyclounifolioside A (2). The IR spectrum of **2** contains absorption bands characteristic of hydroxyl, methylene, and ester at 3435, 3050, and 1734 and 1251 cm^{-1}, respectively.

The PMR and ^{13}C NMR contain two 3H singlets at 2.10 and 2.26 ppm and signals for C atoms at 21.45, 21.80, and 170.70 and 171.10 ppm that are consistent with the presence in **2** of two acetyls.

The PMR spectrum of **2** has 1H doublets of an AB system at strong field at 0.23 and 0.52 ppm. This is unambiguously assigned to methylene H of the cyclopropane ring. It also has seven methyl peaks that resonate at 0.93, 1.04, 1.12, 1.21, 1.42, 1.52, and 1.55 ppm. Thus, we propose that **2** is a cycloartane triterpene glycoside.

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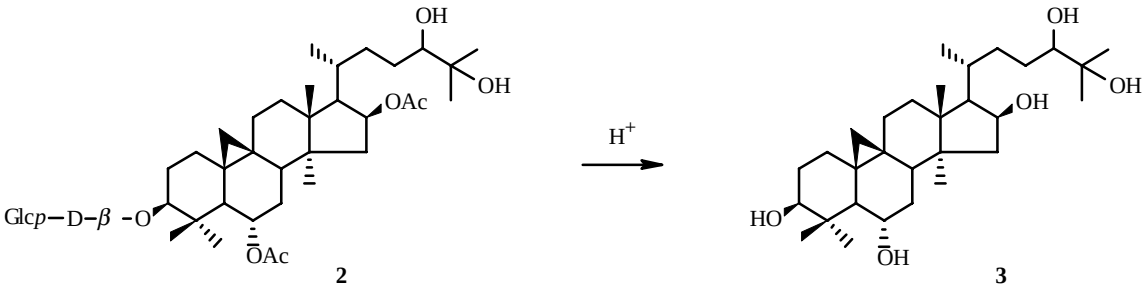
0009-3130/02/3802-0175$27.00

TABLE 1. Chemical Shifts in ^{1}H and ^{13}C NMR of Cyclounifolioside A (**2**) and Cycloasgenin C (**3**) (δ, ppm, 0 = TMS, C_5D_5N)

Atom	Chemical shifts		
	^{13}C (2)	^{1}H (2)	^{13}C(3)
1	32.00	1.57; 1.14	32.81
2	29.80	2.49; 1.89	31.48
3	87.95	3.56	78.84
4	42.10	-	42.43
5	50.00	1.76	53.88
6	70.70	4.98	68.25
7	32.60	1.61; 1.59	38.56
8	45.30	1.83	47.02
9	20.80	-	21.17
10	28.40	-	29.55
11	25.90	1.82; 1.25	26.26
12	33.25	1.62; 1.40	34.37
13	46.90	-	45.55
14	45.95	-	46.90
15	45.60	2.13; 1.25	47.82
16	75.50	5.51	71.77
17	55.30	1.92	57.19
18	18.10	1.12	18.08
19	28.80	0.52; 0.23	30.30
20	31.70	1.98	31.96
21	18.50	1.04	19.18
22	34.10	2.08; 1.11	30.63
23	30.10	2.13; 1.52	34.37
24	79.30	3.68	79.96
25	72.10	-	72.70
26	25.90	1.55	25.42
27	25.70	1.52	26.39
28	19.80	0.93	20.25
29	27.00	1.42	29.44
30	16.60	1.21	16.23
Ac (6)	21.8; 171.1	2.10	
Ac (16)	21.45; 170.70	2.26	
	β-D-Glc*p*-(1→3)Agl		
1	106.70	4.94	
2	75.50	4.06	
3	78.40	4.29	
4	71.70	4.18	
5	78.10	4.02	
6	62.80	4.57; 4.37	

Acid hydrolysis of **2** gives the genin, which was identified as cycloasgenin C (**3**) [4] and the glycoside.

The aforementioned acetyls replace hydroxyls on C-6 and C-16 of the aglycone. This is evident in the ROESY spectrum from correlation peaks for couplings of CH_3 with chemical shift of 2.10 ppm to H-29 and CH_3 with chemical shift of 2.26 ppm to H-23. The presence of acetyls on C-6 and C-16 also explains the weak-field shifts of the corresponding protons H-6 (4.98 ppm) and H-16 (5.51 ppm) (Table 1).



2 **3**

The ^{1}H and ^{13}C NMR spectra of **2** contain signals for one anomeric proton at 4.94 ppm and one anomeric C atom at 106.70 ppm.

These data indicate that **2** is a monoside.

Table 1 shows that C-3 in **2** was glycosylated and resonates at 87.95 ppm.

Thus, glucose is bonded to the genin through the hydroxyl on C-3.

Cyclounifolioside A (**2**) is 6,16-di-O-acetyl-24R-cycloartan-3β,6α,16β,24,25-pentaol 3-O-β-D-glucopyranoside.

EXPERIMENTAL

Silica gel containing 10% gypsum and Silufol plates were used for TLC; silica gel (KSK) of particle size 0.1-0.08 and 0.16-0.1 mm; for column chromatography. Cycloartanes and their derivatives were visualized on TLC using methanolic phosphotungstic acid (20%) with subsequent heating to 120°C for 5-10 min. IR spectra were recorded on a Perkin—Elmer System 2000 FT-IR in KBr pellets; NMR spectra, on Unity 400 plus (Varian) and Bruker DRX-500 spectrometers using deuteropyridine solutions of glycosides at 30°C with TMS internal standard. Two-dimensional spectra were recorded using standard Bruker methods. The delay time for recording TOCSY and ROESY spectra was 0.2 sec.

Paper chromatography was performed on FN-11 paper. Sugars were detected using anilinium phthalate.

The following solvent systems were used: $CHCl_3$—CH_3OH—H_2O (70:23:2, 1; 9:1:0.05, 2) and *n*-butanol—pyridine—water (6:4:3, 3).

Isolation of Cycloartanes. The air-dried and ground epigeal part of *Astragalus unifoliolatus* Bunge (3.0 kg) that was collected in June 1998 on the collective farm Berdakh of Amudar′ya Region of the Republic of Karakalpakstan was extracted with methanol (6×10 L). The extract was concentrated and diluted with water. The precipitate was removed. The aqueous solution was extracted first with ethylacetate and then with isoamyl alcohol. The solvents were evaporated in vacuum to afford ethylacetate (84.95 g) and isoamyl (42.02 g) fractions.

Separation of Ethylacetate Fraction. The ethylacetate fraction was chromatographed over a silica-gel column with elution by system 1 to give fractions consisting of 2-3 compounds. Fractions containing **1** and **2** were rechromatographed using system 2 to give **1** [5 g, 16% (calculated here and hereafter based on air-dried weight)] and **2** (200 mg, 0.006%).

Oleanolic Acid (1). $C_{40}H_{66}O_{13}$, mp 206-207°C (methanol).

IR spectrum (KBr, ν, cm^{-1}): 3447 (OH), 1699 (COOH).

PMR spectrum (C_5D_5N, TMS, δ, ppm): 0.91, 0.97, 1.03, 1.04, 1.05, 1.26, 1.31 (3H each, tertiary methyls), 3.35 (H-3), 5.52 (H-12).

^{13}C NMR spectrum (δ, ppm, C_5D_5N, TMS): 38.95 (C-1), 28.34 (C-2), 78.09 (C-3), 39.37 (C-4), 55.82 (C-5), 18.81 (C-6), 32.22 (C-7), 39.76 (C-8), 48.04 (C-9), 37.39 (C-10), 23.82 (C-11), 122.48 (C-12), 144.90 (C-13), 42.18 (C-14), 28.09 (C-15), 23.74 (C-16), 46.70 (C-17), 42.04 (C-18), 46.54 (C-19), 30.96 (C-20), 34.26 (C-21), 32.22 (C-22), 28.77 (C-23), 16.52 (C-24), 15.55 (C-25), 17.46 (C-26), 26.16 (C-27), 180.38 (C-28), 33.28 (C-29), 23.78 (C-30).

Cyclounifolioside A (2). $C_{40}H_{66}O_{14}$, mp 208-210°C (methanol).

IR spectrum (KBr, ν, cm^{-1}): 3435 (OH), 3050 (CH_2–), 1734, 1251 (ester).

PMR and ^{13}C NMR spectra are listed in Table 1.

Acid Hydrolysis. Compound **2** (50 mg) was hydrolyzed in methanolic H_2SO_4 (15 mL, 0.5%) at 70°C for 4 h. The reaction mixture was cooled and treated with water (15 mL). The methanol was distilled off. The precipitate was filtered off to give the genin, which was identified by TLC as cycloasgenin C (**3**).

Paper chromatography using system 3 of the hydrolysate after neutralization with $BaCO_3$ and evaporation detected D-glucose by comparision with an authentic sample.

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