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

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*The known compound cyclocantogenin (**1**) and a new cycloartane glycoside cyclounifolioside C (**2**), which has the structure 24R-cycloartan-3β,6α,16β,24,25-pentaol 3-O-β-D-glucopyranoside, were isolated from roots of* Astragalus unifoliolatus *Bunge. The structures of the isolated compounds are established using hydrolysis and spectral data.*

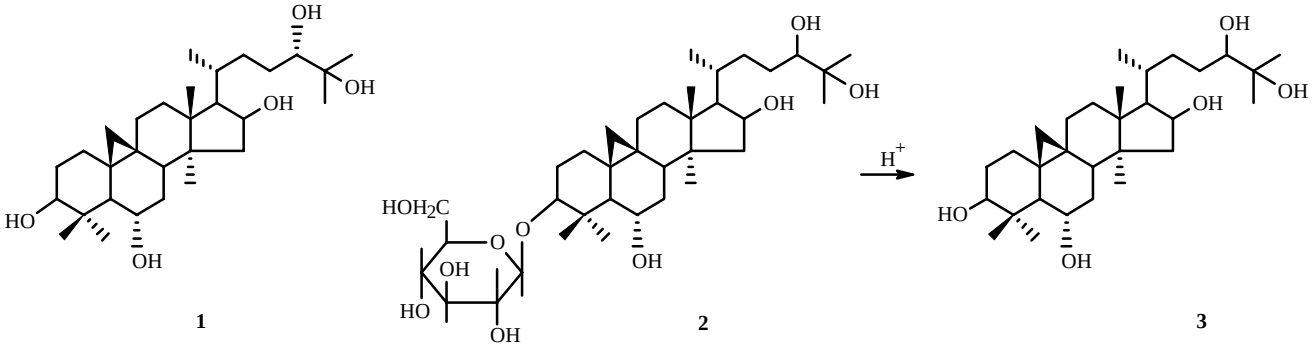
Key words: *Astragalus unifoliolatus*, triterpene glycoside, cycloartane, 24*R*-cycloartan-3*β*,6*α*,16*β*,24,25-pentaol 3-O-*β*-D-glucopyranoside, cycloasgenin C, cyclocantogenin.

In continuation of research on triterpene glycosides from the epigeal part of *Astragalus unifoliolatus* Bunge [1, 2], we have isolated from the roots the cycloartane triterpenoid cyclocantogenin (**1**) and a new glycoside cyclounifolioside C (**2**) and have determined their structures.

The PMR spectrum of compound **1** contains signals for seven methyls at 1.44, 1.47, 1.05, 1.38, 1.11, 1.90, and 1.49 ppm and for two 1H doublets of an AB system at 0.34 and 0.62 ppm, which were unequivocally assigned to methylene protons of a cyclopropane ring. This indicates that compound **1** is a cycloartane triterpenoid.

The ^{13}C NMR spectrum of **1** contains 30 signals, of which 7 belong to methyls; 9, to CH_2 groups; 8, to CH groups; and 6, to quaternary C atoms.

The structure of compound **1** was confirmed using PMR, ^{13}C NMR, and two-dimensional spectra (COSY, TOCSY). Comparison of the ^{13}C NMR spectra of compound **1** with the literature for cyclocantogenin [3] showed that they agree completely (Table 1). Thus, the structure of cyclocantogenin (**1**) is 24*S*-cycloart-3*β*,6*α*,16*β*,24,25-pentaol.



Cyclounifolioside C (2). The IR spectrum of compound **2** contains absorption bands at 3470 and 3035 cm^{-1}, which are characteristic of hydroxyls and cyclopropane CH_2 groups, respectively.

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

Atom	Chemical shifts					
	$^{13}C(2)$	$^{1}H(2)$	$^{13}C(3)$	$^{1}H(3)$	$^{13}C(1)$	$^{1}H(1)$
1	32.16	1.57; 1.17	32.96	1.54; 1.13	32.54	1.56; 1.14
2	32.91	2.49; 1.87	31.36	2.49; 1.79	31.17	2.40; 1.86
3	88.81	3.68	78.12	3.60	78.10	3.67
4	42.36	-	42.19	-	42.18	-
5	53.83	1.77	53.75	1.74	53.73	1.77
6	67.70	3.78	68.09	3.67	68.04	4.74
7	38.16	1.66; 1.59	38.37	1.69; 1.67	38.33	1.69; 1.67
8	46.76	1.85	47.02	1.84	46.94	1.85
9	21.02	-	21.05	-	21.02	-
10	29.91	-	30.16	-	29.71	-
11	26.06	1.83; 1.24	26.13	1.86; 1.27	26.17	1.86; 1.23
12	32.91	1.68; 1.47	32.55	1.67; 1.39	32.95	1.65; 1.41
13	45.45	-	45.48	-	45.47	-
14	46.64	-	46.69	-	46.67	-
15	48.43	2.139; 1.28	48.54	2.19; 1.27	48.16	2.16; 1.27
16	71.47	4.09	71.51	4.73	71.75	4.76
17	56.99	1.93	57.05	1.93	57.11	1.96
18	18.51	1.35	18.53	1.37	18.03	1.38
19	29.14	0.56; 0.23	29.36	0.61; 0.34	29.09	0.62; 0.34
20	31.35	1.98	31.86	1.98	28.44	1.98
21	18.69	1.12	18.85	1.12	18.77	1.11
22	34.58	2.16; 1.13	31.18	2.06; 1.13	32.81	2.06; 1.15
23	29.70	2.16; 1.55	34.62	2.07; 1.54	27.67	2.09; 1.53
24	80.29	3.69	80.31	3.81	76.99	3.94
25	72.46	-	72.49	-	72.88	-
26	25.62	2.01	25.65	1.89	25.48	1.90
27	25.86	1.52	25.88	1.52	26.07	1.49
28	19.95	1.04	20.03	1.04	19.93	1.05
29	28.66	1.49	29.18	1.49	29.34	1.47
30	16.44	1.40	15.89	1.40	15.87	1.44
			β-D-Glc*p*(1→3)Agl			
1	106.66	4.99	-	-	-	-
2	75.66	4.08	-	-	-	-
3	78.48	4.26	-	-	-	-
4	71.61	4.23	-	-	-	-
5	77.89	3.97	-	-	-	-
6	62.79	4.579; 4.40	-	-	-	-

The PMR of cyclounifolioside C **(2)** has strong-field signals at 0.23 and 0.56 ppm that are 1H doublets split into an AB system and are assigned to methylene protons of a cyclopropane ring and signals of seven methyls. This suggested to us that compound **2** is a cycloartane triterpenoid.

The PMR and ^{13}C NMR spectra of **2** have signals for one anomeric proton at 4.98 ppm and one carbon atom at 106.66 ppm. Therefore, compound **2** contains one carbohydrate unit.

Acid hydrolysis of **2** produces the genin, which was identified as cycloasgenin C (**3**) [2]. The hydrolysate contains D-

glucose according to paper chromatography with authentic samples. A comparison of the chemical shifts for the C atoms in the ^{13}C NMR spectra of cycloasgenin C **(3)** and cyclounifolioside C **(2)** showed that the C-3 hydroxyl was glycosylated (Table 1).

The location of the carbohydrate unit was found using COSY, TOCSY, ROESY, HSQC, and HMBC spectra.

According to COSY and TOCSY spectra, the single carbohydrate unit in the glycoside is a β-D-glucopyranose (Table 1). The location on C-3 of the aglycon was confirmed by the presence in the ROESY spectrum of correlation peaks for H-1 of the glucopyranose with H-3 and H-29 of the aglycon and in the HMBC spectrum of a correlation peak for H-1 of the glucopyranose with C-3 of the aglycon.

The COSY and TOCSY spectra also contain several closed spin systems for protons on rings A, B, and C of the aglycon. Partial assignment of these protons was attempted.

The ROESY spectrum permitted the assignment of the aglycon protons to be completed and the types of fusion of all rings to be determined.

Thus, the fusions of rings A, B, and C and the cyclopropane ring C-9, C-10, and C-19 were found as follows. Through-space contacts of H-3 and H-5 and each of these with H-29 were observed. According to cross-peaks in the ROESY spectrum, proton H-30 couples with H-29, H-2_{ax}, H-6, and one of the protons on C-19 of the aforementioned AB system that resonates at weaker field (0.23-0.56 ppm, H-19_{endo}). Therefore, the cyclopropane is situated in the β,β-position on C-9 and C-10.

The type of fusion for rings C and D was determined from the through-space contacts of methyl protons C-18 and C-28 according to cross-peaks in the ROESY spectrum. H-18 couples with H-11_{ax}, H-12_{eq}, H-20, and H-21; H-28, with H-12_{ax}, H-7, H-15, H-17, and H-16. These data indicate that not only does C-18 have the β-position and C-28 the α-position but also the OH on C-16 has the β-position. This corresponds to *trans*-fusion of rings C and D.

Thus, the structure 24*R*-cycloart-3β,6α,16β,24,25-pentaol 3-O-β-D-glucopyranoside is established for cyclounifoliside C (**2**).

EXPERIMENTAL

General comments have been published [1].

Separation of Ethylacetate Fraction. The ethylacetate fraction was repeatedly chromatographed over a silica-gel column with elution by $CHCl_3$:CH_3OH (9:1) to afford **1**, 25 mg (0.0008%), $C_{30}H_{52}O_5$, mp 190-193°C (MeOH).

Table 1 lists the PMR and ^{13}C NMR spectra.

Separation of Butanol Fraction. Repeated chromatography of the total more polar compounds using $CHCl_3$:CH_3OH:H_2O (70:23:3) afforded **2**, 47 mg (0.0021%), $C_{36}H_{62}O_{10}$, mp 192-195°C (MeOH).

IR spectrum (KBr, ν, cm^{-1}): 3470 (OH), 3035 (cyclopropane ring). Table 1 lists the PMR and ^{13}C NMR spectra.

Acid Hydrolysis. Glycoside **2** (20 mg) was hydrolyzed in methanolic H_2SO_4 (10 mL, 0.5%) at 70°C for 1 h. The reaction mixture was cooled and treated with water (10 mL). The MeOH was distilled off. The solid was filtered off to afford the genin, which was identified using TLC as cycloasgenin (**3**).

Paper chromatography of the hydrolysate detected D-glucose by comparison with authentic samples.

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