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TRITERPENE GLYCOSIDES FROM *Astragalus* AND THEIR GENINS. LXXXVIII. CYCLOASCIDOSIDE A, A NEW BISDESMOSIDE OF CYCLOASGENIN C

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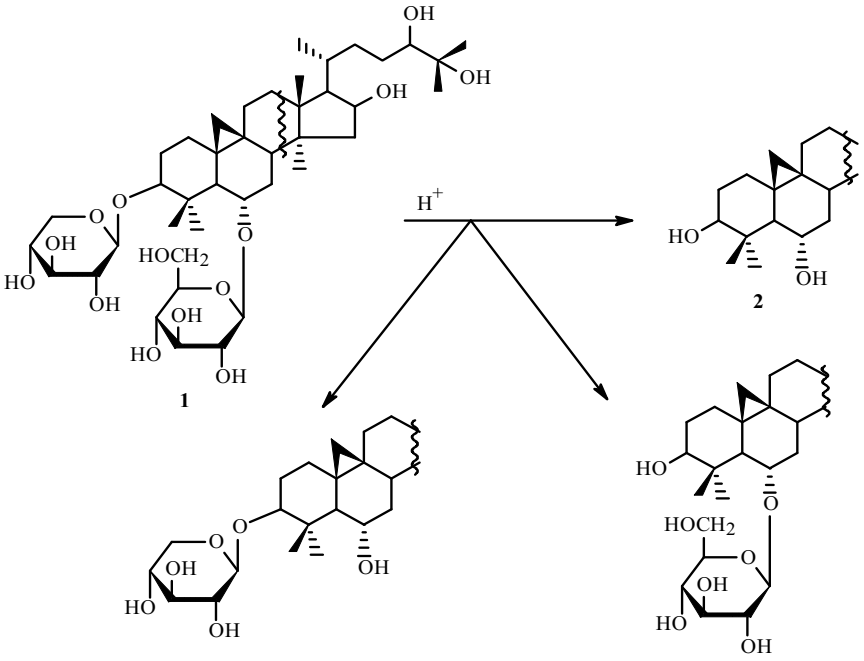
The aerial part of Astragalus mucidus *Bunge (Leguminosae) contains several isoprenoid compounds, two of which were isolated pure. The structure of a new triterpene glycoside of the cycloartane series, cycloascidoside A, was determined as 24*R*-cycloartan-3*β*,6*α*,16*β*,24,25-pentaol-3-*O*-*β*-D-xylopyranoside-6-*O*-*β*-D-glucopyranoside. D-3-*O*-Methyl-chiro-inositol was also identified.*

Keywords: *Astragalus mucidus* Bunge; Leguminosae; cycloartane triterpenoids; cycloascidoside A; cycloasgenin C; PMR, ^{13}C NMR, DEPT, COSY, Hetcor, NOE spectra.

In continuation of our research on the chemistry of plant isoprenoids [1], we initiated a study of *Astragalus mucidus* Bunge (Leguminosae). TLC of the MeOH extract of the aerial part of the plant showed at least 18 spots, which were called compounds 1-18 according to increasing polarity. Column chromatography over silica gel isolated compounds 9 and 11. Compound 9 was identified as D-3-*O*-methyl-*chiro*-inositol. The structure of 11, called by us cycloascidoside A (**1**), was determined. Herein the structure of this glycoside is proved.



1 **2** **3** **4**

The PMR spectrum of **1** (Table 1) contained at strong field δ 0.06 and 0.45 two 1H doublets of an AX system with characteristic spin–spin coupling constant (SSCC) ^{2}J = 4.3 Hz and resonances for seven methyls, which enabled the examined compound to be assigned as a cycloartane-type triterpenoid [2–5]. In fact, the genin, which was identified as cycloasgenin C (**2**) [6], was isolated from the products of partial acid hydrolysis of **1**.

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TABLE 1. Chemical Shifts of C and H Atoms and Parameters of DEPT, ^1H–^1H COSY, and Hetcor Spectra of **1**; Chemical Shifts of C Atoms of **2**–**4** (C_5D_5N, δ, ppm, J/Hz, 0 = TMS)*

C atom	DEPT	**1**		**2**	**3**	**4**
		δ_C	δ_H	δ_C	δ_C	δ_C
1	CH_2	32.20		32.83	32.97	32.61
2	CH_2	30.18	2.25 m, 2.25 m	31.45	30.75	31.29
3	CH	88.55	3.40 dd (11.6, 4.3)	78.41	89.20	78.38
4	C	42.64	–	42.45	43.14	42.44
5	CH	52.49	1.80 d (8.6)	54.05	54.58	52.58
6	CH	79.16	3.68 td (8.4, 8.4, 4.3)	68.35	68.44	79.79
7	CH_2	34.24	2.12 dt (13.2, 4, 4)	38.62	38.88	34.68
8	CH	45.59	1.85 dd (9.7, 5.1)	47.27	47.51	46.21
9	C	21.41	–	21.34	21.83	21.44
10	C	28.73	–	29.67	29.74	29.31
11	CH_2	26.29		26.43	26.79	26.42
12	CH_2	33.14		33.28	33.66	33.26
13	C	45.75	–	45.78	46.19	45.81
14	C	46.99	–	47.00	47.37	47.09
15	CH_2	48.19		48.83	49.15	48.44
16	CH	71.69	4.57 td (7.5, 7.5, 4.9)	71.83	72.24	71.98
17	CH	57.02	1.71 m	57.31	57.74	57.16
18	CH_3	18.50	1.26 s	18.80	19.20	18.77
19	CH_2	28.16	0.06 and 0.45 d (4.3)	30.40	30.51	28.99
20	CH	31.63	2.25 m	31.66	32.07	31.66
21	CH_3	18.82	0.97 d (6.4)	19.10	19.42	18.85
22	CH_2	34.82	1.27 m, 2.35 m	34.86	35.29	34.84
23	CH_2	29.32	1.60 m, 2.03 m	29.43	29.89	29.39
24	CH	80.58	3.67 dd (10.5, 2.4)	80.58	81.00	80.60
25	C	72.67	–	72.71	73.16	72.71
26	CH_3	25.95	1.39 s	25.86	26.28	25.89
27	CH_3	26.10	1.36 s	26.22	26.60	26.20
28	CH_3	19.86	0.85 s	20.31	20.66	20.02
29	CH_3	28.53	1.90 s	29.34	29.31	29.07
30	CH_3	16.64	1.24 s	16.12	17.13	16.14
			β-D-Xyl*p*			
1	CH	107.63	4.71 d (7.5)		107.96	
2	CH	75.59	3.92 dd (8.6, 7.5)		76.01	
3	CH	78.51	4.02 t (8.6)		78.87	
4	CH	71.23	4.09 m		71.69	
5	CH_2	67.03	3.56 dd (11.3, 10), 4.22 dd (11, 5.1)		67.43	
			β-D-Glc*p*			
1	CH	105.18	4.79 d (7.8)			105.13
2	CH	75.59	3.91 dd (8.9, 7.8)			75.63
3	CH	79.16	4.11 t (8.9)			79.19
4	CH	71.83	4.07 t (8.9)			71.82
5	CH	78.09	3.77 ddd (9.2, 5.1, 2.7)			78.04
6	CH_2	63.12	4.19 dd (11.6, 5.4), 4.35 dd (11.6, 3)			63.23

*Chemical shifts of protons are given relative to HMDS.

Paper chromatography (PC) of the carbohydrate part of the stepwise hydrolysate of the new glycoside detected D-glucose and D-xylose according to a comparison with authentic samples and biogenetic considerations.

PMR and ^{13}C NMR spectra of **1** showed resonances for two anomeric protons at δ 4.71 and 4.79 and two anomeric C atoms at δ 107.63 and 105.18, respectively. Therefore, **1** was a bioside. The set of chemical shifts for the C and H atoms and the SSCC of protons in the monosaccharide units indicated that the terminal monosaccharides had the *β*-D-pyranoside form.

This meant that **1** was a bisdesmoside glycoside. A comparison of ^{13}C NMR spectra of **1** and cycloasgenin C showed that C-3 and C-6 were glycosylated.

A difference PMR spectrum from measurement of the one-dimensional nuclear Overhauser effect (NOE) with irradiation of the D-xylose anomeric H atom (δ 4.71) exhibited a negative NOE for the H-3 resonance [7], indicating that the D-xylose was located on C-3 of the genin. Therefore, the D-glucose was bonded to the genin at the C-6 hydroxyl. As a consequence, progenin **3** was identified as cycloasgenin C 3-*O*-*β*-D-xylopyranoside [6, 8].

The ^{13}C NMR spectrum of **4** showed that it was cycloasgenin C 6-*O*-*β*-D-glucopyranoside, which is reported for the first time.

Thus, the experimental results led to the conclusion that cycloascidoside A had the structure 24*R*-cycloartan-3*β*,6*α*,16*β*,24,25-pentaol-3-*O*-*β*-D-xylopyranoside-6-*O*-*β*-D-glucopyranoside.

EXPERIMENTAL

General comments have been published [9]. Descending PC was performed on FN-1 paper using *n*-BuOH:Py:H_2O (6:4:3). Monosaccharides on chromatograms were detected by spraying with anilinium phthalate with subsequent heating at 110°C.

Thin-layer chromatography (TLC) and column chromatography were carried out over KSK silica gel. TLC plates were prepared from silica gel of particle size 5/40 μ containing 13% gypsum. The solvent systems were $CHCl_3$:MeOH (1, 9:1; 2, 6:1) and $CHCl_3$:MeOH:H_2O (3, 70:12:1).

NMR spectra were taken in Py-d_5 on a UNITYplus 400 spectrometer (Varian). ^{13}C NMR spectra were obtained with full C–H decoupling and under DEPT conditions. Two-dimensional spectra of **1** were taken using standard Varian programs. Chemical shifts of protons of **1**–**4** are given relative to HMDS; of C atoms, relative to those of the *β*-C atoms of Py-d_5 (δ 123.493 vs. TMS).

Isolation and Separation of Isoprenoids from the Aerial Part of *Astragalus mucidus*. The air-dried aerial part of the plant (1.5 kg) that was collected in June 2008 in Namangan Oblast, Uzbekistan (Kutirbulag Ridge), was extracted exhaustively by MeOH (5 × 8 L). The MeOH extracts were evaporated to a syrupy consistency and diluted with twice the volume of H_2O. The aqueous solution was worked up first with $CHCl_3$ and then *n*-BuOH. The BuOH extract was evaporated to dryness. The dry residue was chromatographed over a column of silica gel with elution by system 1 to isolate compound 9 (365 mg, 0.024%, here and henceforth yields are calculated per air-dried raw material). Elution of the column by system 2 afforded compound 11 (6.852 g, 0.457%).

D-3-*O*-Methyl-*chiro*-inositol. Compound 9, $C_7H_{14}O_6$, mp 189–191°C (MeOH), identified as D-3-*O*-methyl-*chiro*-inositol [10, 11].

PMR spectrum of D-3-*O*-methyl-*chiro*-inositol (400 MHz, C_5D_5N, δ, ppm, J/Hz, 0 = HMDS): 3.81 (s, CH_3O), 4.01 (1H, t, $^3J_1 = {}^3J_2 = 9$), 4.51 (1H, t, $^3J_1 = {}^3J_2 = 9.4$), 4.61 (1H, dd, $^3J_1 = 5.6$, $^3J_2 = 2.9$), 4.64 (1H, dd, $^3J_1 = 5.6$, $^3J_2 = 2.9$), 4.67 (1H, dd, $^3J_1 = 4$, $^3J_2 = 2.9$), 4.69 (1H, dd, $^3J_1 = 3.8$, $^3J_2 = 2.9$).

^{13}C NMR spectrum (100 MHz, C_5D_5N, δ, ppm, 0 = TMS): 85.77, 74.63, 74.13, 73.70, 73.03, 72.23, 60.71.

Cycloascidoside A (1). Compound 11, $C_{41}H_{70}O_{14}$, mp 297–299°C (MeOH). Table 1 gives the PMR and ^{13}C NMR spectra.

Partial Hydrolysis of 1. Glycoside **1** (500 mg) was dissolved in MeOH (125 mL) containing H_2SO_4 (0.25%), refluxed on a water bath for 5 h, and diluted with H_2O (50 mL). The MeOH was evaporated. The resulting precipitate was filtered off, washed with H_2O, and dried. The filtrate was neutralized. PC detected D-xylose and D-glucose by comparison with authentic samples.

The solid was chromatographed over a column with elution by system 1 to afford genin **2** (44 mg), $C_{30}H_{52}O_5$, mp 250–252°C (acetone), that was identified as cycloasgenin C [6, 8].

PMR spectrum of cycloasgenin C (400 MHz, C_5D_5N, δ, ppm, J/Hz, 0 = HMDS): 0.21 and 0.49 (d, $^2J = 4$, 2H-19), 0.92 (s, CH_3), 0.99 (d, $^3J = 6.4$, CH_3-21), 1.24, 1.29, 1.36, 1.38, 1.76 (s, 5 × CH_3), 3.54 (dd, $^3J_1 = 11.4$, $^3J_2 = 4.7$, H-3), 3.66 (dd, $^3J_1 = 10.3$, $^3J_2 = 2.3$, H-24), 3.68 (td, $^3J_1 = {}^3J_2 = 9.3$, $^3J_3 = 3.7$, H-6), 4.59 (td, $^3J_1 = {}^3J_2 = 7.7$, $^3J_3 = 4.9$, H-16). Table 1 gives the ^{13}C NMR spectrum of cycloasgenin C.

Continued elution of the column by system 3 isolated **3** (8 mg), $C_{35}H_{60}O_9$, mp 252–254°C (MeOH), that was identified as cycloasgenin C 3-*O*-*β*-D-xylopyranoside [6, 8].

PMR spectrum of progenin **3** (400 MHz, C_5D_5N, δ, ppm, J/Hz, 0 = HMDS): 0.15 and 0.43 (d, $^2J = 4$, 2H-19), 0.90 (s, CH_3), 0.98 (d, $^3J = 6.4$, CH_3-21), 1.21, 1.27, 1.36, 1.38, 1.86 (s, 5 × CH_3), 3.51 (dd, $^3J_1 = 11.8$, $^3J_2 = 4.6$, H-3), 3.59 (dd, $^2J = 11.2$, $^3J = 9.8$, D-xylose H-5a), 3.63 (td, $^3J_1 = {}^3J_2 = 9.7$, $^3J_3 = 3.6$, H-6), 3.66 (dd, $^3J_1 = 10.4$, $^3J_2 = 2.3$, H-24), 3.93 (dd, $^3J_1 = 8.8$, $^3J_2 = 7.5$, D-xylose H-2), 4.02 (t, $^3J_1 = {}^3J_2 = 8.5$, D-xylose H-3), 4.10 (td, $^3J_1 = {}^3J_2 = 8.5$, $^3J_3 = 5.4$, D-xylose H-4), 4.23 (dd, $^2J = 11.2$, $^3J = 5$, D-xylose H-5e), 4.58 (td, $^3J_1 = {}^3J_2 = 7.7$, $^3J_3 = 4.9$, H-16), 4.78 (d, $^3J = 7.5$, D-xylose H-1). Table 1 gives the ^{13}C NMR spectrum of **3**.

Further elution of the column by the same solvent isolated pure glycoside **4** (40 mg), $C_{36}H_{62}O_{10}$, mp 258–259°C (MeOH), that was cycloasgenin C 6-*O*-*β*-D-glucopyranoside.

PMR spectrum of progenin **4** (400 MHz, C_5D_5N, δ, ppm, J/Hz, 0 = HMDS): 0.15 and 0.50 (d, $^2J = 4$, 2H-19), 0.85 (s, CH_3), 0.98 (d, $^3J = 6.7$, CH_3-21), 1.277, 1.28, 1.36, 1.38, 1.82 (s, 5 × CH_3), 1.80 (d, $^3J = 9$, H-5), 3.48 (dd, $^3J_1 = 11.3$, $^3J_2 = 4.6$, H-3), 3.66 (dd, $^3J_1 = 10.3$, $^3J_2 = 2.3$, H-24), 3.74 (td, $^3J_1 = {}^3J_2 = 8.8$, $^3J_3 = 3.9$, H-6), 3.78 (m, D-glucose H-5), 3.90 (dd, $^3J_1 = 9$, $^3J_2 = 7.8$, D-glucose H-2), 4.08 (m, D-glucose H-3 and H-4), 4.18 (dd, $^2J = 11.6$, $^3J = 5.3$, D-glucose H-6), 4.33 (dd, $^2J = 11.6$, $^3J = 2.8$, D-glucose H-6′), 4.56 (td, $^3J_1 = {}^3J_2 = 7.5$, $^3J_3 = 4.8$, H-16), 4.82 (d, $^3J = 7.7$, D-glucose H-1). Table 1 gives the ^{13}C NMR spectrum of progenin **4**.

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