https://doi.org/10.29013/AJT-22-7.8-42-47

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TRITERPENE GLYCOSIDES *ASTRAGALUS* AND THEIR GENINS XCV. CYCLOASCIDOSIDE B FROM *ASTRAGALUS MUCIDUS*

Abstract. Structure of the novel cycloartane glycoside, cycloascidoside B isolated from the aerial parts of *Astragalus mucidus* Bunge (*Leguminosae*) is determined as 3-O-β-D-(2-OAc)-xylopyranoside, 6,25-di-*O*-β-*D*-glycopyranosides-24R-cycloartan-3β,6α,16β,24,25-pentanol. Structure of this glycoside had been proven based on chemical transformations and spectral data of NMR1H, ^{13}C.

Keywords: *Astragalus mucidus* Bunge, *Leguminosae*, cycloartan triterpenoids, cycloascidoside A, B and E, cycloasgenin C, spectra NMR^{1}H, ^{13}C, DEPT.

Continuing of our investigation of isoprenoid compounds from *Astragalus* (*Leguminosae*) plants and their chemical transformation [1], from the aerial part of *Astragalus mucidus* Bunge we have isolated a novel cycloarrtane glycoside named by us as cycloascidoside B (**1**). In the article we give proof of the chemical structure of the isolated glycoside.

NMR^{1}H spectrum of the novel glycoside **1** in high field at δ 0.07 and 0.45 have two single-proton doublets is discernible with specific germinal spin-spin coupling constant (SSCC) ^{2}J = 4 hz and signal of seven methyl groups at δ 0.83 – 1.86. These data indicate that the isolated compound is cycloartane type triterpene glycoside [2–5].

NMR^{1}H and ^{13}C spectrum have singlet signal of three proton units at 1.91 and signals of carbon atoms at 21.18 and 170.07. These data confirm that cycloascidoside B contains one acetyl group (table 2).

Acidic hydrolysis of glycoside **1** gives genine (**2**) identified with cycloasgenin C [1–8]. Sugar fraction of the yield of acidic hydrolysis contains *D*- glucose and *D*- xylose (lyxosazone) which are determined by paper chromatography method (PC) in the presence of samples of different carbohydrates.

Alkaline hydrolysis of cycloascidoside B (**1**) gives glycoside **3** identified with cycloascidoside E (**3**). Consequently, cycloascidoside B (**1**) is monoacetate of cycloascidoside E (figure 1) [8].

Partial hydrolysis of glycoside **3** gives cycloasgenin **2** and progenins **4** and **5**.

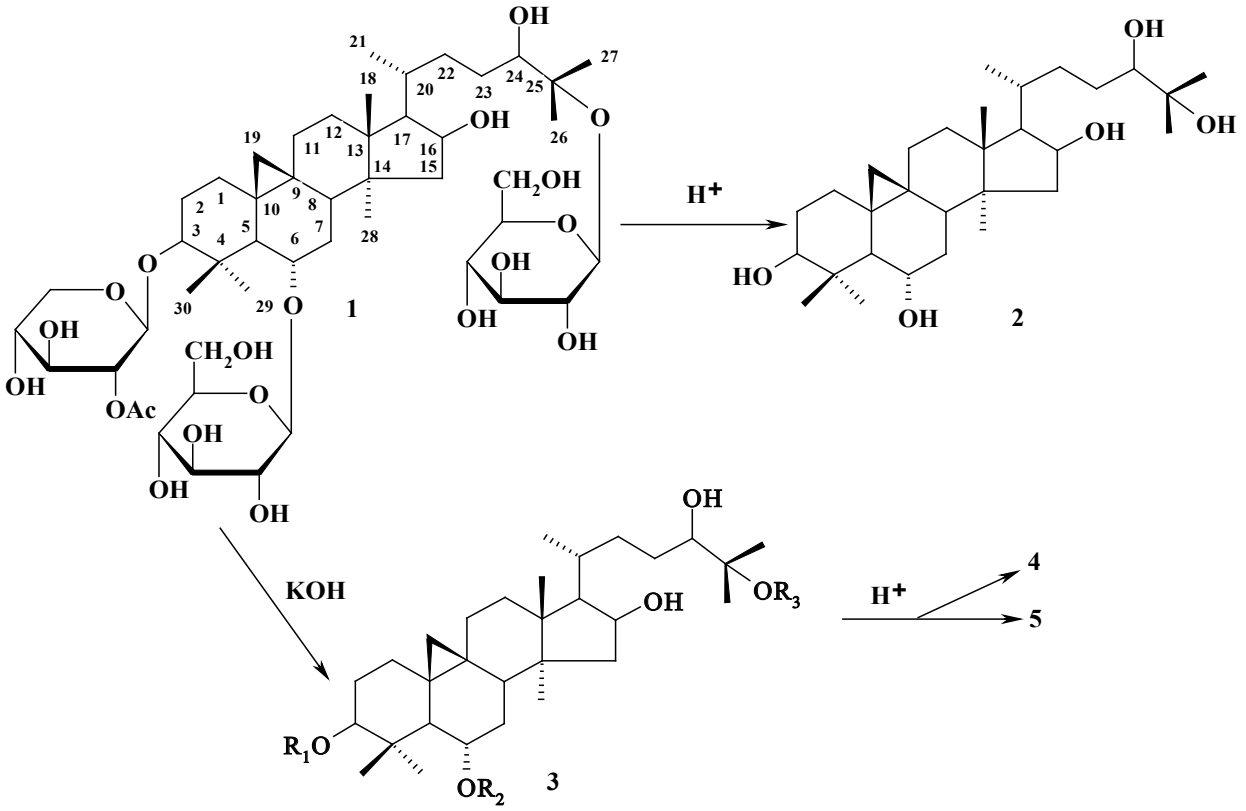
On basis of physical and chemical constants, spectral data and results of TLC analysis monoside

5 was identified as 3-*O*-β-*D*- xylopyranoside of cycloasgenin C [3], and bioside **4** was identified as cycloascidoside A (figure 1) [1].

NMR ^{1}H and ^{13}C spectra of cycloascidoside B (**1**) have signals of three protons at 4.67; 4.81; 5.08 and signals of three anomeric carbon atoms of monosaccharide residue at 104.96; 104.62 and 98.57.

Thereby, the above mentioned datum confirm that the cycloascidoside B (1) is trioside.

Comparative analysis of ^{13}C NMR spectrum data of cycloasgenin C (**2**) and cycloascidoside B (**1**) gives evidence that carbon atom C-3, C-6 and C-25 of cycloascidoside B (**1**) are endured glycosilation and resonate at 88.90; 79.14 and 80.86. These data shows that sugar residues are connected to genin through hydroxyl groups at C-3, C-6 and C-25 carbon atoms.



3. R_1=Xylp, R_2=R_3=Glcp
4. R_1=Xylp, R_2=Glcp, R_3=H
5. R_1=Xylp, R_2=R_3=H

Figure 1. Acidic and alkaline hydrolysis of cyclolastioside B (1)

Position of acetyl group in the molecule of isolated glycoside were found on basis of comparative analysis of ^{1}H and ^{13}C NMR spectra of compounds **1** and **3**. Comparative analysis of chemical shifts of anomeric carbon atoms in ^{13}C NMR spectra confirms position of acetyl group in the molecule of cycloascidoside B (**1**) at C-2^{1} atom of xylose (table 2). In ^{13}C NMR spectrum of cycloascidoside B (**1**) anomeric carbon atom C-1^{1} resonates at 104.96. Comparing of these data in cycloascidoside E (**3**) shows that atom C-1^{1} of xylose in cycloascidoside B (**1**) have endured diamagnetic displacement (shift) to 1.41. This data shows that acetyl residue is located in molecule of xylose. Value of upfield shift of carbon atom C-1^{1} indicates that acetyl group attached to carbon atom C-2^{1}. This conclusion is also proved out by

upfield shift of signal C-3[1] to 2,93. Abovementioned data let us to make conclusion that acetyl group in cycloascidoside B (**1**) is attached at C-2[1] of xylose.

Analysis of ^{1}H and ^{13}C NMR spectra (table 2) of glycoside **1** shows, that it has three monosaccharide residue and is trioside.

Table 1.– ^{13}C NMR data of aglycone part of cycloascidoside B (1), cycloasgenin C (2), cycloascidoside E (3), cycloascidoside A (4) and 3-*O*-β-*D*- xylopyranoside of cycloasgenin C (5) (C_5D_5N, δ, J/Hz)

Atom C	DEPT	1	2 [3]	3	4	5 [3]
1	CH_2	32.01	32.83	32.20	32.20	32.97
2	CH_2	29.83	31.45	30.22	30.18	30.85
3	CH	88.90	78.41	88.55	88.55	89.20
4	C	42.18	42.45	42.65	42.64	43.20
5	CH	52.37	54.05	52.48	52.49	54.61
6	CH	79.14	68.35	79.16	79.16	68.40
7	CH_2	34.35	38.62	34.23	34.24	38.93
8	CH	45.64	47.27	45.62	45.59	47.50
9	C	21.40	21.34	21.38	21.41	21.84
10	C	28.63	29.67	28.71	28.73	29.71
11	CH_2	26.17	26.43	26.26	26.29	26.79
12	CH_2	33.08	33.28	33.14	33.14	33.64
13	C	45.74	45.78	45.66	45.75	46.19
14	C	46.80	47.00	46.89	46.99	47.41
15	CH_2	48.00	48.83	48.05	48.19	49.20
16	CH	71.70	71.83	71.75	71.69	72.20
17	CH	57.17	57.31	57.15	57.02	57.70
18	CH_3	18.51	18.80	18.47	18.50	19.27
19	CH_2	30.02	30.40	30.33	28.16	30.46
20	CH	31.46	31.66	31.55	31.63	32.11
21	CH_3	18.72	19.10	18.80	18.82	19.46
22	CH_2	34.86	34.86	34.96	34.82	35.31
23	CH_2	29.18	29.43	29.25	29.32	29.86
24	CH	78.88	80.58	78.94	80.58	81.07
25	C	80.86	72.71	80.56	72.67	73.16
26	CH_3	21.58	25.86	21.50	25.95	26.41
27	CH_3	24.13	26.22	24.22	26.10	26.64
28	CH_3	19.80	20.31	19.83	19.86	20.70
29	CH_3	28.44	29.34	28.52	28.53	29.36
30	CH_3	16.49	16.12	16.63	16.64	17.18

Anomeric protons of monosaccharide residue are resonated in ^{1}H NMR spectra of glycoside 1 at δ 4.67 (H-1 of residue of β-*D*-xylopyranoses, d, ^{3}J = 8 Hz), δ 4.81 (H-1 of residue of β-*D*- glucopyranose, d, ^{3}J =7.5 Hz) and δ 5.08 (H-1 of residue of β-*D*-glucopyranose, d, ^{3}J=7.6 Hz) (table 2). It means that monosaccharide residues in the glycoside have pyranose form, $^{4}C_1$- conformation and β-configuration of chemical structure. This conclusion is also confirmed by chemical shift value of carbon atoms of monosaccharide residues in ^{13}C NMR spectra of cycloascidoside B. Mentioned values of ^{13}C NMR also point at terminal character of both monosaccharide residues. Accordingly, cycloascidoside B is trisdesmoside glycoside.

Table 2. – ^{13}C NMR data of carbohydrate part of cycloascidoside B (1), cycloascidoside E (3), cycloascidoside A (4) and 3-*O*-β-*D*- xylopyranoside of cycloasgenin C (5) (C_5D_5N, δ, J/Hz)

Atom C	**3-O-β-D-Xylp**			
1	104.96	106.37	107.63	108.12
2	76.13	75.57	75.59	76.13
3	75.52	78.45	78.51	79.02
4	71.23	71.22	71.23	71.74
5	66.98	66.99	67.03	67.55
Ac	21.18			
	170.07			
6-O-β-D-Glc*p*				
1	104.62	105.54	105.18	
2	75.52	75.57	75.59	
3	79.00	79.13	79.16	
4	71.70	71.77	71.83	
5	77.95	78.14	78.09	
6	63.02	63.07	63.12	
25-O-β-D-Glc*p*				
1	98.57	98.45		
2	75.23	75.40		
3	78.57	78.54		
4	71.79	71.77		
5	78.05	78.24		
6	62.69	62.66		

According to comparative analysis of ^{13}C NMR spectra of compounds 1 and 2 location of residue of D-xylopyranose found be at C-3, and location of D-glucopyranose residues at C-6 and C-25. From table 1, signal of carbon atom C-3 have downfield shift to 10.49, and signals of C-6 and C-25 carbon atoms have downfield shift to 10.79 and 8.15 comparatively with cycloasgenin C.

Anomeric carbon atoms of monosaccharide residues resonate at δ104.96 (C-1 of β-*D*-xylopyranoses), 104.62 (C-1 of β-D-glucopyranose) and 98.57 (C-1 of β-*D*-glucopyranose) in ^{13}C NMR spectra of cycloascidoside E. Chemical shift values of anomeric carbon atoms show that residue of *D*-xylose is attached to C-3, and residues of *D*-glucose are attached to C-6 and C-25 of genin.

Thereby, on basis of above described experimental data the chemical structure of isolated novel cycloartane line triterpene glycoside, cycloascidoside B is 3-*O*-β-*D*-(2-OAc)- xylopyranoside, 6,25- di-*O*-β-*D*-glycopyranosides-24R-cycloartan-3β, 6α,16β, 24, 25-pentaol.

Experimental part. General Experimental Procedures [1]. Following solvent systems were used: chloroform – methanol – water, 70:12:1 (1), chloroform – methanol, 9 : 1 (2), chloroform – methanol – water, 70 : 23 : 3 (3). NMR spectrum of the compounds were recorded on Unityplus 400 (Varian) referenced with respect to the residual solvent signal of C_5D_5N. ^{13}C NMR spectrum were recorded at complete suppression of C-H interaction and under DEPT conditions. Chemical shifts of protons of compositions **1**, **3** were described in reference to HMDS.

Extraction and isolation. Isolation method of isoprenoids from aerial part *Astragalus mucidus* Bunge were given in [1]. 150 mg (0.01%) of cycloascidoside B was isolated by elution of silica gel column with the system 3.

Cycloascidoside B (1), $C_{49}H_{82}O_{21}$, melting point. 280–282°C (from methanol). Spectra NMR1H (400 MHz, C_5D_5N, δ, ppm., J/hz): 0.07, 0.45 (each 1H, d, J=4, H-19), 0.83 (3H, c, Me-28), 0.95 (3H, d, J=6.4, Me-21), 1.24, 1.25, 1.37, 1.38, 1.86 (each 3H, c, Me-30, 18, 27, 26, 29), 1.91 (3H, s, OAc), 3.41 (1H, dd, J=11.6, 4.9, H-3), 3.57 (1H, dd, J=11.3, 10, H-5α, Xyl*p*), 3.68 (1H, td, J=9, 3.8, H-6), 3.78 (1H, ddd, J=9.2, 5.1, 2.7, H-5 Glc*p*), 3.84 (1H, m, H'-5, Glc*p*,), 3.86 (1H, dd, J=8.4, 7.8, H'--2, Glc*p*), 3.93 (1H, dd, J=8.9, 7.8, H-2, Glc*p*), 4.00 (1H, t, J=8.5, H-3, Xyl*p*), 4.04 (1H, t, J=8.8, H-3', Glc*p*), 4.06 (1H, t, J=8.9, H-4, Glc*p*), 4.08 (1H, t, J=8.9, H-4, Xyl*p*), 4.09 (1H, t, J=8.7, H'-4, Glc*p*), 4.11 (1H, t, J=8.9, H-3, Glc*p*), 4.20 (1H, dd, J=11.7, 5.5, H-6, Glc*p*), 4.20 (1H, dd, J=11, 5.1, H-5e, Xyl*p*), 4.38 (1H, dd, J=11.7, 3.1, H-6, Glc*p*), 4.40 (1H, dd, J=11.6, 2.6, H-6', Glc*p*), 4.52 (1H, td, J=7.6, 5.2, H-16), 3.76 (1H, dd, J=10.5, 2, H-24), 4.67 (1H, d, J=8, H-1, Xyl*p*), 4.81 (1H, d, J=7.5, H-1, Glc*p*), 5.08 (1H, d, J=7.5, Glc*p*, H-1), 5.40 (1H, dd, J=8.8, 8, Xyl*p*, H-2). For Spectra ^{13}C refer to table 1, 2.

Acidic hydrolysis of cycloascidoside B. Glycoside **1** (30 mg) was dissolved in 10 ml of methanol that contains 0.5% of sulfuric acid, and boiled it in boiling-water bath for 1 hour. Then the reaction mixture was diluted with water up to 30 ml and MeOH was evaporated. Formed sediment was filtered, washed with water and dried. Filtrate was neutralized with $BaCO_3$. After removing of precipitate the solvent was concentrated and analyzed by TLC method using solvent system 3 and in comparison with samples *D*-glucose and *D*- xylose were found.

Residue was rechromatographed silica gel column by eluating with system 2. Genin **2** (15 mg) identified with cycloasgenin C in comparison with samples by TLC analysis and on basis of ^1H NMR spectrum.

Cycloasgenin C. ^1H NMR spectra (400 MHz, C_5D_5N, δ, ppm, J/hz): 0.21, 0.49 (each 1H, d, J=4, H-19), 0.92 (3H, s, Me-28), 1.00 (3H, d, J=6.4, Me-21), 1.25, 1.30, 1.36, 1.39, 1.77 (3H, c, Me-30, Me-18, Me-27, Me-26, Me-29), 3.55 (1H, dd, J=11.4, 4.7, H-3), 3.67 (1H, dd, J=10.4, 2.3, H-24), 3.69 (1H, td, J=9.4, 3.7, H-6), 4.60 (1H, td, J=7.8, 5.0, H-16).

Alkaline hydrolysis of cycloascidoside B. Cycloascidoside B (**1**) 50 mg was saponified in 20 ml of 0,5% methanol solution of potassium hydroxide. Reaction mixture was set at room temperature during 24 hour, then diluted with 25 ml of water. Then the mixture was neutralized with acetic acid. MeOH was evaporated and extracted with BuOH. The obtained residue was rechromatographed with silica gel. 30 mg of cycloascidoside E (**3**) was isolated by eluating with system 3, $C_{47}H_{80}O_{19}$, mp 276–278 °C (from MeOH). ^1H NMR spectra of cycloascidoside E (400 MHz, C_5D_5N, δ, ppm, J/Hz): 0.06, 0.45 (each 1H, d, J=4, H-19), 0.84 (3H, s, Me-28), 0.96 (3H, d, J=6.4, Me-21,), 1.23, 1.25, 1.38, 1.39, 1.91 (3H, s, Me-30, Me-18, Me-27, Me-26, Me-29), 3.40 (1H, dd, J=11.6, 4.3, H-3,), 3.56 (1H, dd, J=11.3, 10, H--5α Xyl*p*), 3.67 (1H, td, J=8.4, 4.3, H-6,), 3.77 (1H, dd, J=10.5, 2, H-24,), 3.78 (1H, ddd, J=9.2, 5.1, 2.7, Glc*p* H-5), 3.85 (1H, m, Glc*p* H'-5), 3.87 (1H, dd, J_1=8.4, 7.9, Glc*p* H'-2), 3.92 (1H, dd, J=8.6, 7.5, Glc*p* H--2), 3.93 (1H, dd, J=8.6, 7.5, Xyl*p* H-2), 4.03 (1H, t, J=8.6, Xyl*p* H-3), 4.05 (1H, т, J=8.9, Glc*p* H-4), 4.06 (1H, t, J=8.8, Glc*p* H'-3), 4.09 (1H, t, J=8.9, Xyl*p* H-4), 4.10 (1H, t, J=8.7, Glc*p* H'-4), 4.11 (1H, t, J=8.9, Glc*p* H-3), 4.20 (1H, dd, J=11.7, 5.5, Glc*p* H--6), 4.21 (1H, dd, J=11.5, 5.1, Xyl*p* H-5e), 4.35 (1H, dd, J=11.6, 3, Glc*p* H-6), 4.38 (1H, dd, J=11.6, 2.6, Glc*p* H'-6), 4.54 (1H, td, J=7.7, 5.3, H-16), 4.76 (1H, d, J=7.8, Glc*p* H-1). 4.79 (1H, d, J=7.5, Xyl*p* H-1), 5.06 (1H, d, J=7.8, Glc*p* H'-1). ^{13}C NMR spectrum of cycloascidoside E is given in tables 1, 2.

Partial hydrolysis of cycloascidoside E. Glycoside **3** (30 mg) was dissolved in 100 ml of methanol that contains 0.5% of sulfuric acid, and boiled it in boiling-water bath for 1 hour. The reaction mixture was diluted with water up to 30 ml and MeOH was removed by evaporation. Formed precipitation was filtered, washed with water and dried. Filtrate was

neutralized with $BaCO_3$. Filtrate was analyzed by TLC method using system 3 in comparison with samples and found *D*-glucose and *D*-xylose.

Residue was set to column with silica gel and was eluated with system 2. Genin **2** (7 mg) was isolated and identified with cycloasgenin C by TLC method and according to information of ^{1}H NMR spectrum.

Monoside **5** (8 mg) (3-*O*-β-*D*- xylopyranoside of cycloasgenin C), $C_{35}H_{60}O_9$, mp 252–254°C (from MeOH) and bioside **4** were isolated by eluting the silica gel column with system 1 [1; 3]. ^{1}H NMR spectra of progenin **5** (400 MHz, C_5D_5N, δ, ppm, J/Hz): : 0.30, 0.58 (each 1H, d, J=4, H-19), 1.05 (3H, s, Me-28), 1.13 (3H, d, J=6.4, Me-21), 1.36, 1.42, 1.51, 1.53, 2.02 (3H, s, Me-30, Me-18, Me-27, Me-26, Me-29), 3.60 (1H, dd, J=11.2, 9.8, Xyl*p* H-5*a*), 3.64 (1H, dd, J=11.7, 4.6, H-3), 3.67 (1H, td, J=9.7, 3.6 H--6), 3.75 (1H, dd, J=10.5, 2.4, H-24), 3.83 (1H, dd, J=8.8,7.5,Xyl*p*H-2),4.06(1H,t,J=8.6,Xyl*p*H-3),4.15 (1H, m, Xyl*p* H-4), 4.36 (1H, dd, J=11.3, 5, Xyl*p* H--5*e*), 4.71 (1H, td, J=7.7, 4.9, H-16), 4.92 (1H, d, J=7.5, Xyl*p* H-1). For spectra ^{13}C NMR spectra of progenin 5 is given in the (table 1).

Progenin 4. ^{1}H NMR spectra (400 MHz, C_5D_5N, δ, ppm, J/Hz): 0.06, 0.45 (each 1H, d, J=4.3, H-19), 0.85 (3H, s, Me-28), 0.97 (3H, s, J=6.4, Me-21,), 1.24, 1.26, 1.36, 1.39, 1.90 (3H, s, Me-30, Me-18, Me-27, Me-26, Me-29), 3.40 (1H, dd, J_1=11.6, 4.3, H-3), 3.56 (1H, dd, J=11.3, 10, Xyl*p* H-5*a*), 3.67 (1H, dd, J=10.5, 2.4, H-24), 3.68 (1H, td, J=8.4, 4.3, H-6), 4.57 (1H, td, J=7.5, 4.9, H-16), 3.77 (1H, ddd, J=9.2, 5.1, 2.7, Glc*p* H-5), 3.91 (1H, dd, J=8.9, 7.8, Glc*p* H-2), 3.92 (1H, dd, J=8.6, 7.5, Xyl*p* H-2), 4.02 (1H, t, J=8.6, Xyl*p* H-3), 4.09 (1H, m, Xyl*p* H--4), 4.11 (1H, t, J=8.9, Glc*p* H-3), 4.19 (1H, dd, J=11.6, 5.4, Glc*p* H-6), 4.22 (1H, dd, J=11.5, 5.1, Xyl*p* H-5*e*), 4.35 (1H, dd, J=11.6, 3, Glc*p* H-6'), 4.71 (1H, d, J=7.5, Xyl*p* H-1), 4.79 (1H, d, J=7.8, Glc*p* H--1). ^{13}C NMR spectra of the progenin **4** is given in the (table 1).

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