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DETERMINATION OF CHEMICAL STRUCTURE OF CYCLOLEHMANOSIDE A FROM ASTRAGALUS LEHMANNIANUS

Abstract. In the present work, the determination of chemical structure of the novel cycloartane glycoside, cyclolehmanoside A is given. Determination of chemical structure was carried out by using of chemical reaction (acidic hydrolysis) and spectral methods (1D and 2D NMR spectroscopy). Novel cycloartane glycoside, cyclolehmanoside A was isolated from aerial parts of *Astragalus Lehmannianus* Bunge (*Leguminosae*) by column chromatography on silica, and its chemical structure was established as 3-O-β-D-xylopyranoside, 6,16-di-O-β-D-glucopyranoside-24S-cycloartane-3β,6α,16β,24,25-pentaol.

Keywords: *Astragalus Lehmannianus* Bunge, *Leguminosae*, cycloartane triterpenoids, cyclolehmanoside A, cyclocanthogenin, ^{1}H, ^{13}C, DEPT NMR spectra, HMBC.

Introduction. Investigation of isolation methods and structural determination of biological active substances from different medicinal plants has great importance for creation of modern high effective natural medicines [1].

Cycloartane line triterpenoidal compounds, cycloartanes are wide spread in plants. At present it is known, that cycloartanes have perspective biological activities. Therefore, investigation of cycloartanes has theoretical and practical value. Cycloartanes were first discovered in astragalus plants. Astragalus plants are good sources of these biological active substances. More 200 cycloartanes have been isolated from the plants of this genus [2]. About 239 astragalus species grow in Uzbekistan [3]. There are 307 species in Kazakhstan [4,5]. Twelve species of astragalus – *Astragalus alopecias*, *A. contortuplicatus, A. filicaulis, A. flexus, A. floccosifolius, A. frigidus, A. glycyphyllus, A. onobrychis, A. sieversianus, A. tribuloides, A. ugamicus, A. u1iginosus* growing in Kazakhstan are used in folk medicine [6]. Roots of *Astragalus membranaceus* are used as diuretic, as gastric and intestinal means, for treating of spleen diseases, during metabolic derangements in Chinese, Korean and Tibetan medicines. It has been determined, that cycloartane glycoside, askendoside D isolated from *Astragalus taschkendicus* has positive action to regulation of heart function and myocardium. Cyclosiversioside F from *Astragalus sieversianus* has hypotensive, anti-inflammatory actions, sedative, analgesic and antitumor activities [2,4,7,8].

The aim of our investigation is determination of chemical structure of the cycloartane glycoside, cyclolehmanoside A isolated from the aerial parts of *Astragalus Lehmannianus* Bunge, growing in Karakalpakstan.

Astragalus Lehmannianus Bunge grows in the Middle Asia (Kyzylkum, Karakum), Kyzyl-Orda region, Near Aral sea regions. The plant is good eaten by cattle.

Materials and methods. *Astragalus Lehmannianus* Bunge was collected from Karakalpakstan (Sultanuzdag) in May 2007.

Plates with silica (0.005-0.043 mm) containing 10% of plaster and plates Silufol UV-254 (Czechia) were used. Column chromatography was carried out on silica 0.1-0.08 and 0.16-0.1 mm. Cycloartanes

were detected by spraying of 20% methanol solution of phosphotungstic acid following heating at 120ºC during 5-10 min. Paper chromatography was conducted on «FN-11» using solvent system *n*-BuOH-C_5H_5N-H_2O (6:4:3). Monosaccharides were detected by spraying of aniline phthalate following heating at 110ºC.

Next solvent systems were used for elution of the column and for thin layer chromatography (TLC):

1) chloroform-MeOH (9:1);

2) chloroform-MeOH (6:1);

3) **chloroform-MeOH-H_2O (70:12:1);**

4) chloroform-MeOH-H_2O (70:28:3);

5) chloroform-MeOH-H_2O (60:35:5).

NMR spectrum were obtained on UNITYplus 400 (Varian) in CD_3OD. ^{13}C NMR spectrum were obtained at full suppression of C-H interaction and at the DEPT conditions.

Air dried aerial parts of the plant (1.2 kg) were exhaustive extracted with methanol (MeOH). The obtained MeOH extract was evaporated until thick condition and added twice volume of water. The obtained water solution was treated at first with chloroform, after with *n*-BuOH. *n*-BuOH extract was evaporated and obtained dry residue (68 g) was chromatographed on silica column using solvent systems 4 and 5 for elution. Compound **1** (75 mg) was isolated from *n*-BuOH extract**.**

Acidic hydrolysis of compound 1 (Fig.1). Compound 1 (35 mg) was dissolved in 10 ml of MeOH containing 0.5% sulfuric acid and boiled in water bath during 1 hour. Then the reaction mixture was diluted with 20 ml of water and MeOH was evaporated. Laid down precipitate was filtered, washed with water and dried. Filtrate was neutralized with barium carbonate. After neutralization in the filtrate D-xylose and D-glucose were detected by using of paper chromatography method in comparison with standard substances. The obtained precipitate was set to the chromatographic column and eluated with system 1. Genin 2 was isolated in the result of the column chromatography. Genin 2 was identified as cyclocanthogenin by comparing with standard substance on TLC and on basis of ^1H NMR data.

^1H NMR data of cyclocanthogenin (400 MHz, C_5D_5N): 0.34 and 0.62 (2H-19, d, ^2J=4), 1.05 (CH_3, s), 1.11 (CH_3-21, d), 1.38, 1.44, 1.49, 1.90, 1.90 (5xCH_3, s), 3.67 (H-3, dd), 3.94 (H-24, dd), 4.74 (H-6, td), 4.76 (H-16, td) (Table 1).

Results and discussion.

The ^1H NMR spectrum of the compound 1 showed signals due to cyclopropane methylene at δ0.58 and 0.35 (each 1H, d, J=4Hz) and signals of methyl groups at δ 0.99-1.29. These data indicate that the compound 1 is triterpenoid of cycloartane line (Table 1) [8,9].

Acidic hydrolysis of the compound 1 gives genin 2 identified with cyclocanthogenin [1,7,8]. D-xylose and D-glucose were detected in carbohydrate part of the hydrolysate by using of paper chromatography method in comparison with standard substances (Fig.1).

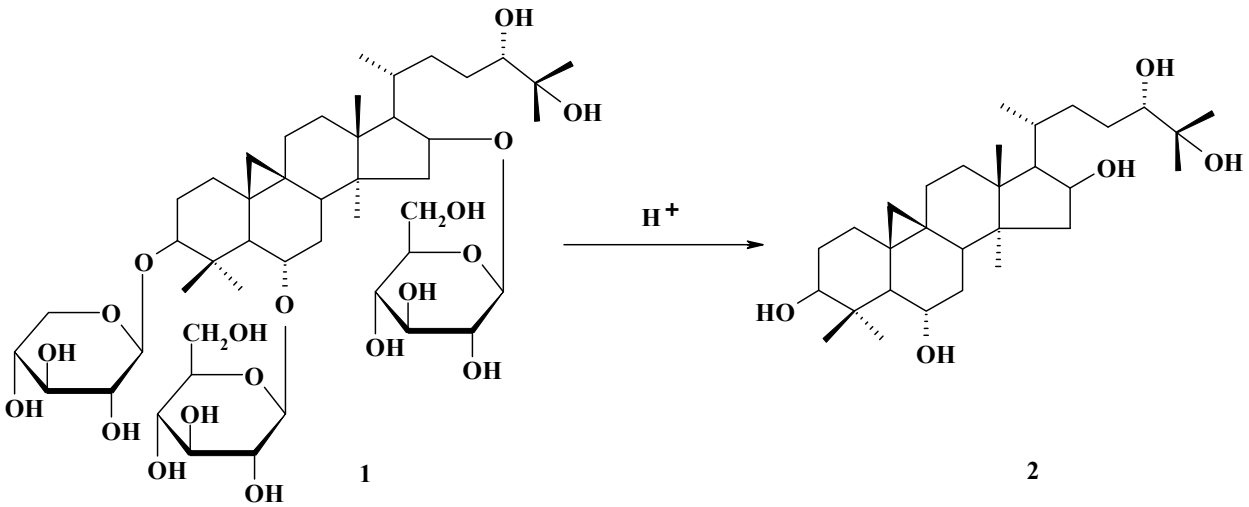

Figure 1 - Acidic hydrolysis of compound 1

Table 1 – ^{1}H, ^{13}C NMR and DEPT data of cyclolehmanoside A (1) (CD_3OD, δ, J/Hz, 0-TMS) and cyclocanthogenin (2) (C_5D_5N, δ, J/Гц, 0-TMS)

Атом С	DEPT	1		2
		δ_C	δ_H	δ_C
1	CH_2	31.36	1.29, 1.60	32.54
2	CH_2	30.42	1.72, 1.98	31.17
3	CH	90.22	3.24	78.10
4	C	42.13	-	42.18
5	CH	53.10	1.68	53.73
6	CH	78.21	3.59	68.04
7	CH_2	34.40	1.65, 1.95	38.33
8	CH	46.11	1.90	46.94
9	C	21.13	-	21.02
10	C	30.11	-	29.71
11	CH_2	26.29	1.37, 1.94	26.17
12	CH_2	33.27		32.95
13	C	46.66	-	45.47
14	C	47.49	-	46.67
15	CH_2	47.94	1.40, 2.13	48.16
16	CH	82.23		71.75
17	CH	58.23		57.11
18	CH_3	18.59	1.15	18.03
19	CH_2	27.55	0.35 и 0.58	29.09
20	CH	30.03		28.44
21	CH_3	17.85	1.0	18.77
22	CH_2	36.82		32.81
23	CH_2	29.90		27.67
24	CH	78.59		76.99
25	C	72.59	-	72.88
26	CH_3	25.55	1.18	25.48
27	CH_3	26.20	1.21	26.07
28	CH_3	19.66	0.99	19.93
29	CH_3	28.41	1.29	29.34
30	CH_3	16.80	1.05	15.87
β-D-Xyl*p*				
1	CH	106.51	4.42	
2	CH	75.12	3.24	
3	CH	77.55	3.33	
4	CH	71.83	3.50	
5	CH_2	67.33	3.21, 3.85	
β-D-Glc*p*				
1	CH	104.78	4.48	
2	CH	76.43	3.26	
3	CH	78.04	3.37	
4	CH	71.32	3.31	
5	CH	77.55	3.27	
6	CH_2	62.80	3.69, 3.85	
β-D-Glc*p*				
1	CH	105.05	4.65	
2	CH	76.43	3.29	
3	CH	77.90	3.43	
4	CH	71.83	3.35	
5	CH	77.05	3.36	
6	CH_2	62.80	3.69, 3.85	

In ^{1}H NMR spectrum anomeric protons of monosaccharide residues of the compound 1 are observed at δ 4.42 (H-1 of β-D-xylopyranose), δ 4.48 and δ 4.65 (H-1 of β-D-glucopyranoses) ^{3}J=7.4, ^{3}J=7.8 and ^{3}J=7.9 Hz accordingly. So, monosaccharide residues in the glycoside 1 have pyranose form, $^{4}C_{1}$-conformation and β-configuration. This conclusion is confirmed by values of chemical shifts of carbohydrate residue carbon atoms in ^{13}C NMR spectrum of 1 (Table 1).

Comparative analysis of ^{13}C NMR spectra of compound 1 and genin 2 showed that carbon atoms C-3, C-6 and C-16 have glycosidation shifts resonating at δ 90.22., δ 78.21 and 82.23 accordingly.

Anomeric carbon atoms of monosaccharide residues resonate at δ 106.51 (C-1 of β-D-xylopyranose), δ 104.78 and δ 105.05 (C-1 of β-D-glucopyranose) in the ^{13}C NMR spectrum of the compound 1. Value of chemical shifts of anomeric carbon atoms indicate, that D-xylose residue linked at C-3, and two D-glucose residues linked at C-6 and C-16 of genin.

In HMBC spectrum of 1 correlation peaks between H-1$^{'}$ of D-xylose (δ 4.42) and C-3 of aglycon (δ 90.22), H-1$^{'}$ of D-glucose (δ 4.48) and C-6 aglycon (δ 78.21), H-1$^{''}$ of D-glucose (δ 4.65) and C-16 aglycon (δ 82.23) are observed.

Thereby, fully analysis of the spectral data allow us to make conclusion, that isolated compound 1 is cycloartane glycoside having novel chemical structure 3-O-β-D-xylopyranoside,6,16-di-O-β-D-glucopyranoside-24S-cycloartane-3β,6α,16β,24,25-pentaol.

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ASTRAGALUS LEHMANNIANUS ӨСІМДІГІНЕН БӨЛІП АЛЫНҒАН ЦИКЛОЛЕХМАНОЗИД А ХИМИЯЛЫҚ ҚҰРЫЛЫМЫН АНЫҚТАУ

Аннотация. Осы жұмыста жаңа циклоартан гликозиді, циклолехманозид А –ның химиялық құрылымын анықтау берілген. Химиялық құрылым химиялық реакция (қышқылдық гидролиз) және спектральді әдістер (бірөлшемді және екіөлшемді ЯМР спектроскопия) көмегімен анықталды. Жаңа циклоартан гликозиді, циклолехманозид А *Astragalus Lehmannianus* Bunge (*Leguminosae*) өсімдігінің жер үсті бөлігінен силикагельде бағаналы хроматография әдісімен бөліп алынды және оның химиялық құрылымы 3-О-β-D-ксилопиранозид, 6,16-ди-О-β-D-глюкопиранозид-24S-циклоартан-3β,6α,16β,24,25-пентаол екендігі анықталды.

Түйін сөздер: *Astragalus Lehmannianus* Bunge, *Leguminosae*, циклоартан тритерпеноиды, циклолехманозид А, циклокантогенин, ^{1}H, ^{13}C, DEPT ЯМР спектр, HMBC.

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ОПРЕДЕЛЕНИЕ ХИМИЧЕСКОГО СТРОЕНИЯ ЦИКЛОЛЕХМАНОЗИДА А ИЗ ASTRAGALUS LEHMANNIANUS

Аннотация. В настоящей работе приводится определение химического строения нового циклоартанового гликозида, циклолехманозида А. Определение химического строения проводился при помощи химической реакции (кислотный гидролиз) и спектральными методами (одномерная и двумерная ЯМР спектроскопия). Новый циклоартановый гликозид, циклолехманозид А был выделен из надземной части *Astragalus Lehmannianus* Bunge (*Leguminosae*) колоночной хроматографией на силикагеле, а его химическое строение установлен как 3-О-β-D-ксилопиранозид, 6,16-ди-О-β-D-глюкопиранозид-24S-циклоартан-3β,6α,16β,24,25-пентаол.

Ключевые слова: *Astragalus Lehmannianus* Bunge, *Leguminosae*, циклоартановые тритерпеноиды, циклолехманозид А, циклокантогенин, ^{1}H, ^{13}C, DEPT ЯМР спектр, HMBC.

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