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Triterpenoids from the aerial part of *Astragsalus alopecias* Pall.

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ABSTRACT

The genus *Astragalus* (*Fabaceae*), in the Flora of Kazakhstan is represented by 307 species. *Astragalus alopecias* Pall. is one of these species growing wildly in the South Kazakhstan and using in folk medicine, but not investigated phytochemically. In the present study chromatographic separation were carried out and two individual triterpenoidal compounds were isolated. Their structures were established on the basis of ^{1}H and ^{13}C NMR spectroscopy methods and acidic hydrolysis, it is identified as β-D-glucopyranoside of β-sitosterin (**1**) and 3-O-β-D-glucopyranoside 6,16-di-O-acetyl-24R-cycloart-3β,6α,16β,24,25-pentaol (**2**). The compounds have isolated from the investigated plant for the first time.

Key words: *Astragalus alopecias*, β-sitosterin, cycloartane, cyclounifolioside A.

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INTRODUCTION

The Flora of Kazakhstan has richest unique sources of useful plants, in the first place wild plant species having medicinal properties and perspective for investigation of their phytochemical constituents and biological activity of metabolites [1]. *Astragalus* genus (Fabaceae) plants are one of popular means of folk medicine used in China, Mongolia, Korea and Japan. Tibetan medicine widely uses the *Astragalus* plants, which recommended for common strengthening of body, excretion of toxins, for regeneration of injured tissues. Traditional medicine of West Siberia, Far East and Transbaikalia use the plants as stimulator of immune system [2]. 307 species of *Astragalus* grow in the Flora of Kazakhstan [3]. As a part of our ongoing research of biological active compounds from *Astragalus* species of the South Kazakhstan, we investigate the plant *Astragalus alopecias* Pall.This paper reports the isolation and structure elucidation of two triterpenoid glycosides **1** and **2** (Figure 1) from EtOH extract of the aerial part of *Astragalus alopecias*. The structures of glycosides **1** and **2** were elucidated by extensive spectroscopic methods including 1D- and 2D-NMR experiments.

The ^{1}H NMR of compound **1** displayed a multiplet at δ 5.33 indicating the presence of an olefinic proton, at position H-6. The doublet (J = 7.7 Hz) centered at δ 5.03 suggested the presence of an anomeric proton in a glycoside having a β-linkage between the sugar and the aglycone moiety. The spectrum also showed two three proton singlets of the methyl groups, H-18 and H-19 at δ 0.58 and 0.86 respectively. The three proton doublets at δ 0.90 (J = 6.4 MHz), 0.81 (J = 6.8 MHz) and 0.77 (J = 6.9 MHz) indicated the presence of three methyl groups at C-20 (H-21) and C-25 (H-26, H-27), respectively. The multiplet at δ 3.85 could be assigned to H-3 of the aglycone moiety. The ^{13}C-NMR spectra of the compound **1** revealed the presence of 35 carbon atoms (Table 1). The chemical shift at δ 19.3 and 19.4 were assigned for the two separate terminal methyl groups (C-26 and C-27) linked at position 25 of the molecule. The carbon atoms of the angular methyl groups C-18, C-19 and C-21 resonate at δ 11.8, 19.8 and 18,9 accordingly. The carbon of C-29 methyl group resonates at δ 12.0 The olephinic carbon atoms C-5 and C-6 are appeared at δ 140.6 and 121.9. The third carbon atom of the aglycon has glycolisation effect and resonate at δ 78.7. Anomeric carbon atom of the sugar moiety are appeared at δ 102.4. On the basis of comparative analysis with literature data the isolated compound **1** were identified as β-D-glucopyranoside of β-sitosterin (Figure 1).

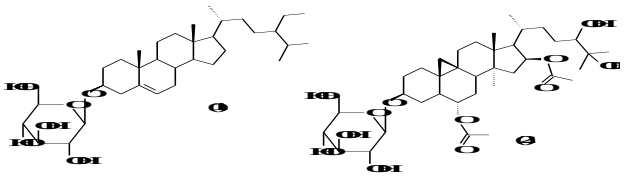

Figure 1: Structures of the isolated triterpenoid glycosides from *Astragalus alopecias*

Table 1: ^{13}C NMR data of the compound 1 in $CDCl_3$

Carbon Number	δ (ppm)	Carbon Number	δ (ppm)
1	37,4	19	19.8
2	30.2	20	36.3
3	78.7	21	18.9
4	39.2	22	34.1
5	140.6	23	26.2
6	121.9	24	45.9
7	32.0	25	29.3
8	31.9	26	19.3
9	50.2	27	19.4
10	36.8	28	23.2
11	21.1	29	12.0
12	39.8	Glc-1	102.4
13	42.4	Glc-2	75.3
14	56.7	Glc-3	78.6
15	24.4	Glc-4	71.6
16	28.4	Glc-5	78.1
17	56.1	Glc-6	62.5
18	11.8		

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Table 2: NMR data of the compound 2 in DMSO-d_6

Carbon Number	DEPT	HSQC		HMBC	COSY
		^{13}C δ(ppm)	^{1}H δ(ppm)		
1	CH_2	45.18	1.39; 1.42 (t, 2H)	C-2	H-2
2	CH_2	33.21	2.33; 2.35 (t, 2H)	C-4	H-1, H-3
3	CH	87.48	3.06 (t, 1H)		H-2
4	C	45.17	–		–
5	CH	60.90	3.19 (s, 1H)		H-6
6	CH	70.28	4.10		H-5, H-7
7	CH_2	31.08	1.47 1.49 (dt, 2H)	C-6, C-8	H-6, H-8
8	CH	45.84	1.73 (t, 1H)		H-7
9	C	25.75	–		–
10	C	45.17	–		–
11	CH_2	30.78	1.47; 1.51 (t, 2H)	C-13, C-19	H-12
12	CH_2	45.84	1.74; 1.76 (t, 2H)		H-11
13	C	49.07	–		–
14	C	60.90	–		–
15	CH_2	54.09	1.82; 1.83 (d, 2H)		H-16
16	CH	73.68	4.93 (m, 1H)		H-15, H-17
17	CH	69.16	1.55 (d, 1H)		H-16, H-20
18	CH_3	19.44	0.91 (s, 3H)	C-12, C-13, C-20, C-28	–
19	CH_2	31.08	0.21; 0.57 (d, 2H)		–
20	CH	25.76	3.01 (m, 1H)		H-17, H-21, H-22
21	CH_3	19.58	0.81 (d, 2H)		H-20
22	CH_2	32.53	1.99; 2.09 (m, 2H)		H-20, H-23
23	CH_2	44.68	1.99; 2.09 (m, 2H)		H-22, H-24
24	CH	74.35	2.88 (m, 1H)		H-23
25	C	72.39	–		–
26	CH_3	27.20	1.31 (s, 3H)	C-27	–
27	CH_3	29.47	1.20 (s, 3H)	C-26	–
28	CH_3	15.36	0.72 (s, 3H)	C-8, C-13, C-14	–
29	CH_3	22.16	1.06 (s, 3H)		–
30	CH_3	31.58	1.04 (s, 3H)		–
Ac(6)	CH_3	25.76; 109.68	2.45 (s, 3H)		–
Ac(16)	CH_3	25.09; 109.68	3.10 (s, 3H)		–
1'	CH	106.27	4.17 (d, 1H)		2'
2'	CH	77.41	3.58 (m, 1H)		1', 3'
3'	CH	84.39	4.06 (m, 1H)	C-5'	2', 4'
4'	CH	70.28	4.01 (m, 1H)	C-3', C-5'	3', 5'
5'	CH	84.39	3.19 (m, 1H)		4', 6'
6'	CH_2	70.27	4.08; 4.10 (dd, 2H)	C-4'	5'

Compound **2** was isolated as white powder. The ^{1}H-NMR spectrum of **2** (Table 2) showed signals due to a cyclopropane methylene at *δ* 0.14 and *δ*. 0.52. Seven methyl group signals were observed at *δ* 0.7-0.91. The ^{1}H NMR spectrum of the compound **2** showed signals at *δ* 1.98 and *δ* 2.00 ascribable to two acetoxy groups. Presence of the anomeric proton signal at *δ* 4.81 in ^{1}H NMR spectrum indicate that the compound **2** has one sugar moiety. On the basis of ^{1}H and ^{13}C NMR it were established, that a) the aglycon part was determined as 24R-cycloartan- 3β,6α,16β,24,25-pentaol; b) compound **2** contains two acetyl group.

Comparison of the ^{1}H and ^{13}C NMR spectrums of **2** to datum of glycosides isolated from *Astragalus unifoliolatus* allowed to identified **2** as cyclounifolioside A, which has the structure 3-O-β-D -glucopyranoside 6,16-di-O-acetyl-24R-cycloart-3β,6α,16β,24,25-pentaol (Figure 1).

Literature reviews show that various biological active substances such as triterpenoids, flavonoids, alkaloids, polysaccharides were isolated from *Astragalus* plants [5]. As determined by systematical investigations basic secondary metabolites of *Astragalus* plants are represented by cycloartane and oleanane line triterpenoids which have wide spectra of biological activity [2]. It was determined that β-sitosterin have hypocholesterinemic activity and used for prophylaxis and treatment of atherosclerosis [6]. It is known using of β-sitosterin for treating and alleviation of prostate hyperplasia symptoms [7]. There are many datum in literature about the cytotoxic activity of the cycloartane glycosides to different lines of cancer cells. MeOH extract of leaves of *Combretum quadrangulare* containing cycloartane glycosides, methylquadrangularates B and D showed strong cell proliferative activity against murine colon 26-L5 carcinoma cells [8].

Cycloartane glycosides from *Cimicifuga racemosa* have strong cytotoxic activity to cancer cell lines *HepG2*, *HL-60*, *R-HepG2*, *HSC-2*, *HGF*, *MCF-7* [9]. Authors [9] marked that some changes of cytotoxic action of the compound caused by insignificant structural difference of the aglykon part. So, 3-O-α-L-arabinopyranoside of cimigenol hasn't the significant activity against HSC-2 cells even at 400 μM concentration, but its derivative, 3-O-α-L-arabinopyranoside of 25-O-methylcimigenol, lowers the quantity of the living cells depending of the dose (its IC_{50} 30 μM). Actein is major secondary methabolite of *Cimicifuga racemosa*, has more high cytotoixic activity than 27-deoxyactein. Among cimiacerohenic derivarives, cimiracemosides F, G, H and (*22R,23R,24R*)-12β-acetoxi-16β,23:22,25-diepoxi-23,24-dihydroxi-9β,19-cyclolanostan-3β-il 3-O-α-L-arabinopyranoside the cytoytoxic activity of 7(8)-dehydrosaponines is higher than appropriate saturated saponins. It is remarkable, that cimiracemoside G showed 15 time higher activity against *HSC-2* cancer cells than against *HGF* [9]. All cycloartane glycosides isolated from *Astragalus kahirikus* compounds exhibited very weak cytotoxicity against the A2780 ovarian cancer cell line [10]. Evaluation of the cytotoxic activity of isolated from *Astragalus eremophillus* cycloartane glycosides in MCF7 and U937 cell lines showed the inhibition slightly the growth (controlling the cell cycle) and/or to induce death process in U937 cell line, the most susceptible cell line. By the results of evaluation found that the eremophilosides A and K are the most effective to induce cell death, the first by necrosis while the latter by apoptosis [11].

The isolated triterpenoid glycosides 1 and 2 were previously isolated from *Astragalus cruciatus* Link. [12] and *Astragalus unifoliolatus* Bunge [4]. Preliminary *in vitro* determination of cytotoxic activity of alcohol extract obtained from the aerial parts of *Astragalus alopecias* Pall carried out. It was determined that the EtOH extract from *A. alopecias* doesn't inhibit the proliferation of cancer cells *HeLa*.

EXPERIMENTAL

General: NMR spectra were acquired on a JNM-ECA 400 MHz spectrometer using standard pulse sequence at ambient temperature. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz.

Plant material: *Astragalus alopecias* Pall was collected from Khoja-Togay village, South Kazakhstan region, in June 2015.

Extraction and isolation: Air-dried and grinded plant material (223.74 g) was extracted with EtOH (2x2 L) at room temperature. After filtration, the solvent was removed by rotary evaporation yielding (25.74 g) of EtOH extract. The EtOH extract was set to column with silica gel for isolation of the triterpenoid substances by elution with the solvent system CH_3Cl-MeOH (4:1). Fractions 21-30 containing compound **1** were concentrated and allowed to stand at room temperature for few days. A yellowish powder was purified by preparative TLC. Compound **2** was isolated from fractions 53-69.

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