The Austrian Journal of Technical and Natural Sciences, No 1–2
ISSN 2310-5607

PREMIER Publishing
ppublishing.org

DOI:10.29013/AJT-24-1.2-24-27

CYCLOASCIDOSIDE D FROM *ASTRAGALUS MUCIDUS*

Artikbaeva Baxtibike Rasbergenovna [1], ***Naubeev Temirbek Khasetullaevich*** [1]

[1] Department "Oil & Gas Technology", Karakalpak State University

Cite: *Artikbaeva B.R., Naubeev T.Kh. (2024). Cycloascidoside D from Astragalus Mucidus. Austrian Journal of Technical and Natural Sciences 2023, No 11-12.* https://doi.org/10.29013/AJT-24-1.2-24-27

Abstract

A new cycloartane glycoside, cycloascidoside D which is determined as 3-O-β-D-(2-OAc)-xylopyranoside-24R-cycloartane-3β,6α,16β,24, 25-pentaol, was isolated from the aerial part of the plant *Astragalus mucidus* Bunge (Leguminosae).

Structure of this glycoside was proven by chemical transformations and on the basis of ^{1}H and ^{13}C NMR spectra, which were interpreted using 2D NMR.

Keywords: *Triterpenoids, cycloartanes, cycloasgenin C, glycosides, cycloascidoside D, Leguminosae, Astragalus, ^{1}H, ^{13}C NMR spectra, HMBC*

Introduction

In continuation of our studies of cycloartane triterpenoids from plants of the genus *Astragalus* (Leguminosae) (Naubeev, T. Kh., Uteniyazov, K.K., Isaev, M.I., 2011). We determined the structure of a new cycloartane glycoside, cycloascidoside D (**1**), obtained from the aerial part of *Astragalus mucidus* Bunge. Its structure is discussed in the article.

The ^{1}H NMR spectrum of cycloascidoside D shows single-proton doublets of the AX system at 0.29 and 0.58 ppm with SSCC2J = = 4.5 Hz along with signals of seven methyl groups in the range of 1.05–1.86 ppm, allowing us to classify the compound under discussion as triterpenoids of the cycloartane series (Naubeev, T. Kh., Uteniyazov, K.K., Isaev, M.I., 2011; Naubeev, T. Kh., Zhanibekov, A.A., Isaev, M.I., 2012; Naubeev, T. Kh., Zhanibekov, A.A., Uteniyazov, K.K., Bobakulov, Kh.M., Abdullaev, N.D., 2014; Naubeev, T. Kh., Ramazonov, N. Sh., 2021; Naubeev, T. Kh., Uteniyazov, K.K., Ramazonov, N. Sh., 2022).

Indeed, genin **2**, identified with cycloasgenin C, was isolated from the products of acid hydrolysis of glycoside **1** (Naubeev, T. Kh., Uteniyazov, K.K., Isaev, M.I., 2011; Naubeev, T. Kh., Zhanibekov, A.A., Isaev, M.I., 2012).

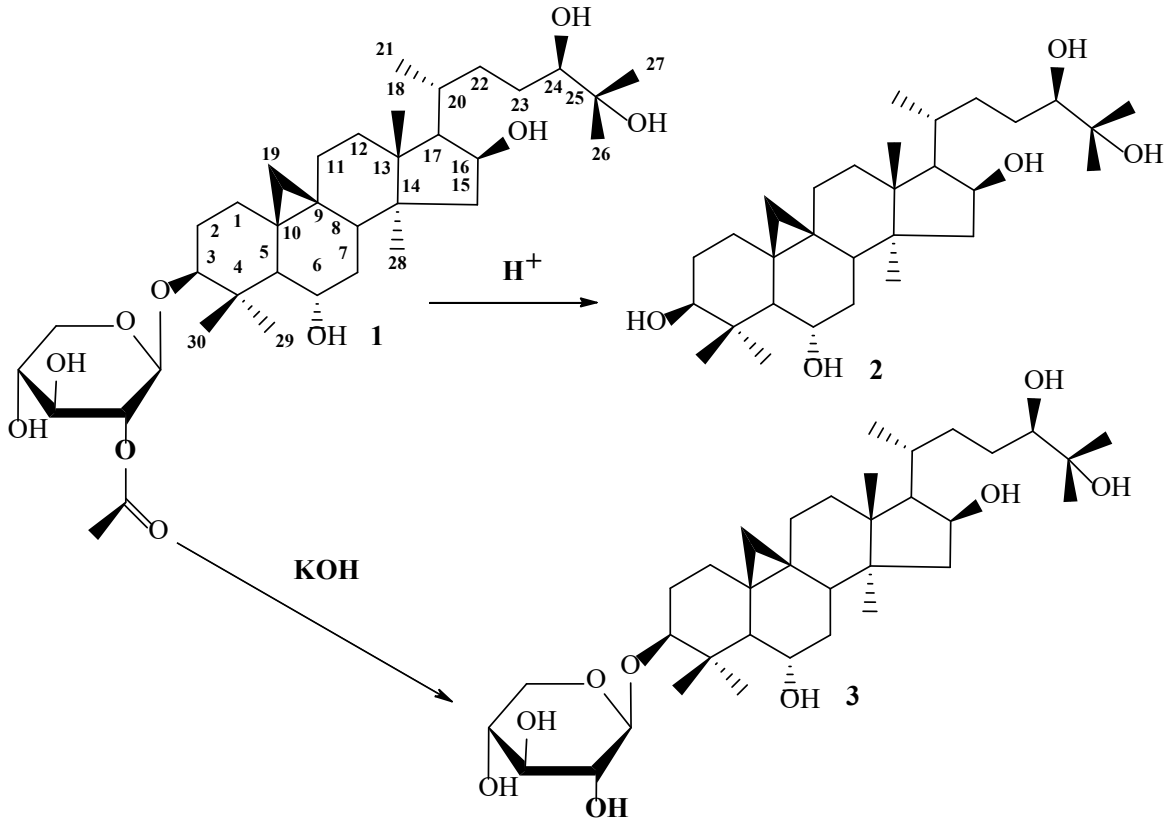
D-xylose was detected in the carbohydrate part of the acid hydrolyzate after neutralization and concentration by paper chromatography (PC). The presence of signals of one anomeric proton and one anomeric carbon atom at 4.64 ppm and 104.92 ppm in the ^{1}H and ^{13}C NMR spectra suggests that the discussed compound **1** is of glycosidic nature and is a monoside. A set of chemical shifts of carbon and hydrogen atoms, as well as the coupling constant of protons of the monosaccharide residue indicate the pyranose form,

$^4C_1$-conformation and β-configuration of D-xylose. The same conclusion emerges from the chemical shifts of the corresponding carbon atoms. Comparative analysis of the ^{13}C NMR spectra of the new glycoside **1** and cycloasgenin C unambiguously determines the position of D-xylose at C-3.

Chemical correlation in the HMBC spectrum of the glycoside was observed between the signals of H-3 (δ 3.66 ppm) and the anomeric carbon atom of D-xylose (δ 104.92 ppm), as well as between the signals of the anomeric proton of the monosaccharide (δ 4.64 ppm) and the C-3 atom (δ 89.20 ppm) of the aglycone.

These facts also indicate the location of D-xylose at C-3.

Figure 1. *Acid and alkaline hydrolysis of cycloascidoside D (1)*



In the ^1H NMR spectrum of the glycoside there is **1** three-proton singlet at 2.02 ppm indicates that this glycoside contains one acetyl group. As expected, the ^{13}C NMR spectrum of new glycoside **1** shows signals from the carbon atoms of one acetyl group at 21.23 and 170.02 ppm.

Alkaline hydrolysis of cycloascidoside D leads to the formation of glycoside **3**, identified with 3-O-β-D-xylopyranoside cycloasgenin C (Naubeev, T. Kh., Uteniyazov, K.K., Isaev, M.I., 2011; Naubeev, T. Kh., Zhanibekov, A.A., Isaev, M.I., 2012; Naubeev, T. Kh., Zhanibekov, A.A., Uteniyazov, K.K., Bobakulov, Kh.M., Abdullaev, N.D., 2014). The place of the attachment of the acetyl group was determined by comparative study of the ^{13}C NMR and ^1H spectra and compounds **1** and **3**.

Table 1. *Chemical shifts of carbon atoms of compounds 1 and 3 (C_5D_5N, δ, ppm, J/Hz, 0-TMS)*

C/H	1		3 [2]	C/H	1		3 [2]
	δ_C	δ_H	δ_C		δ_C	δ_H	δ_C
1	32.95	1.22 m, 1.65 m	32.97	20	32.10	1.92 m	32.11
2	30.85	2.50 m, 1.93 m	30.85	21	19.44	1.12 d (6.3)	19.46

C/H	1		3 [2]	C/H	1		3 [2]
	δ_C	δ_H	δ_C		δ_C	δ_H	δ_C
3	89.20	3.66 dd (11.5, 4.4)	89.20	22	35.30	1.26 m, 2.38 m	35.31
4	43.18	–	43.20	23	29.82	1.85 m; 1.95 m	29.86
5	54.60	1.76 d (8.7)	54.61	24	81.07	3.80 q	81.07
6	68.38	3.75 td (9, 9, 3.8)	68.40	25	73.14	–	73.16
7	38.92	2.17 dt (13.1, 4, 4)	38.93	26	26.41	1.53 s	26.41
8	47.48	1.99 dd (9.8, 5.2)	47.50	27	26.62	1.50 s	26.64
9	21.82	–	21.84	28	20.69	1.04 s	20.70
10	29.69	–	29.71	29	29.34	1.86 s	29.36
11	26.76	1.70 m, 1.24 m	26.79	30	17.17	1.36 s	17.18
12	33.61	1.68 m,	33.64	**β-D-Xyl*p***			
13	46.18	–	46.19	1	104.92	4.64 d (7.5)	108.12
14	47.39	–	47.41	2	76.65	5.36 dd (8.6, 7.8)	76.13
15	49.19	1.97 dd (5.2, 12); 2.44 dd (8, 12)	49.20	3	75.59	3.97 t (8.7)	79.02
16	72.16	4.53 td (7.5, 7.5, 4.8)	72.20	4	71.73	4.17 t (9)	71.74
17	57.67	1.72 m	57.70	5	67.56	3.68 dd (11.3, 10); 4.38 dd (11.2, 5.1)	67.55
18	19.24	1.41 s	19.27	Ac	21.23	2.02 s	
19	30.43	0.28 and 0.58 dd (4.5)	30.46	Ac	170.02		

The C-6, C-16, C-25 atoms of the glycoside **1** molecule in the ^{13}C NMR spectrum resonate at 68.38, respectively 72.17 and 73.14 ppm. These values practically coincide with those of the ^{13}C NMR spectra of cycloasgenin C and glycoside **3**. Consequently, the acetyl group is not located in the genin part of the cycloascidoside D molecule. Indeed, a one-proton doublet with a coupling constant of $^3J_1 = 9$ and $^3J_2$–8 Hz is noted in the ^1H NMR spectrum of the latter at 5.36 ppm, belonging to the proton geminal to the acetyl group.

Additional confirmation of the conclusion about the position of the acetyl group is provided by a comparative analysis of the ^{13}C NMR spectra of glycosides **1** and **3**.

As can be seen from Table 1, upon transition from glycoside **3** to glycoside **1**, the chemical shifts of carbon atoms C-1 (–2.84 ppm), C-2 (+0.52 ppm), C-3 (–2.87) of monosaccharide residue change significantly. The signs and values of these changes coincide well with the α- and β-influences of the acetyl group located at C-2 of the β-D-xylopyranoside ring, and unambiguously determine the location of the acetyl residue.

Thus, the presented experimental data allow us to conclude that the new triterpene glycoside of the cycloartane series, cycloascidoside D, has the structure 3-O-β-D-(2-OAc)-xylopyranoside-24R-cycloartane-3β,6α,16β,24,25- pentaol.

Experimental part. General Experimental Ptocedures (Naubeev, T. Kh., Uteniyazov, K.K., Isaev, M.I., 2011). The following solvent systems were used: 1) chloroform-methanol (9:1), 2) chloroform-methanol-water (70:12:1), 3) chloroform-methanol-water (70:23:3).

NMR spectra were recorded on a JNM-ECZ600R spectrometer (JEOL, Japan) at an operating frequency of 600 MHz, for ^1H in C_5D_5N solutions. TMS (0 ppm) was used as an internal standard in ^1H NMR spectra. In the ^{13}C NMR spectra, the chemical shift of the solvent (C_5D_5N, 150.35 ppm relative to TMS) was used as an internal standard.

Extraction and isolation. Isolation method of isoprenoids from the aerial parts *Astragalus mucidus* Bunge were given in (Naubeev, T. Kh., Uteniyazov, K.K., Isaev, M.I., 2011). When the column was eluted with a 70:12:1 system (chloroform-methanol-wa-

ter), 84 mg of cycloascidoside D was isolated (0.0056%, the yield here and below is given based on the weight of air-dried raw material).

Cycloascidoside D (1) – substance 1, $C_{37}H_{62}O_{10}$, m.p. 260–262°C (from methanol).

Acid hydrolysis of cycloascidoside D. Glycoside 1 (35 mg) was dissolved in 15 ml of a 0.5% methanol solution of sulfuric acid and boiled in a water bath for 1 hour, monitoring the progress of hydrolysis on TLC every 20 minutes. Then the reaction mixture was diluted with a threefold volume of water and the methanol was evaporated. The formed precipitate was filtered off, washed with water, and dried. The filtrate was neutralized with $BaCO_3$. After removing the precipitate, the solution was concentrated and D-xylose was found by using PC in the system n-butyl alcohol-pyridine-water (6:4:3) in comparison with known samples.

The residue was chromatographed on a column, eluting with a system 1. 10 mg of genin **2**, identified with cycloasgenin C $C_{30}H_{52}O_5$, m.p. 250–252°C (from methanol), which was identified as a true sample according to the ^1H and ^{13}C NMR spectrum and mobility on TLC.

^1H NMR spectrum of cycloasgenin C (400 MHz, C_5D_5N, δ, ppm, J/Hz, 0-HMDS): 0.21 and 0.49 (d, J=4, 2H-19), 0.92 (CH_3, s), 1.00 (d, J=6.4, CH_3–21), 1.25, 1.30, 1.36, 1.39, 1.77 (s, 5 × CH_3), 3.55 (dd, J=11.4, 4.7, H-3), 3.67 (dd, J=10.4, J=2.3, H-24), 3.69 (td, J=9.4, 3.7, H-6), 4.60 (td, J=7.8, 5.0, H-16) (Naubeev, T. Kh., Uteniyazov, K.K., Isaev, M.I., 2011).

Alkaline hydrolysis of cycloascidoside D. 25 mg of cycloascidoside D (1) was saponified with 20 ml of 0.5% methanol solution of potassium hydroxide. The reaction mixture was left at room temperature for a day. Then the methanol solution was diluted with a threefold volume of water, and the methanol was evaporated. The reaction product was extracted with butanol. The butanol extract was washed with water. The dry residue after distilling off the butanol was chromatographed on a silica gel column. Eluting with system 2, 15 mg of glycoside **3** was isolated, m.p. 252–254° C (from methanol), identified with 3-O-β-D-xylopyranoside cycloasgenin C (**3**) (Naubeev, T. Kh., Zhanibekov, A.A., Isaev, M.I., 2012).

^1H NMR spectrum of 3-O-β-D-xylopyranoside cycloasgenin C (**3**) (600 MHz, C_5D_5N, δ, ppm, J/Hz, 0-HMDS): 0.30 and 0.58 (2H-19, d, ^2J =4), 1.05 (CH_3, s), 1.13 (CH_3–21, d, ^3J=6.4), 1.36, 1.42, 1.51, 1.53, 2.02 (5xCH_3, s), 3.60 (H-5*a* of β-D-xylopyranose residue, dd, ^2J=11.2, ^3J=9.8), 3.64 (H-3, dd, $^3J_1$=11.7, $^3J_2$=4.6), 3.67 (H-6, td, $^3J_1$=$^3J_2$=9.7, $^3J_3$=3.6), 3.75 (H-24, dd, $^3J_1$=10.5, $^3J_2$=2.4), 3.83 (H-2 β-D-xylopyranose residue, dd, $^3J_1$=8.8, $^3J_2$=7.5), 4.06 (H-3 β-D-xylopyranose residue, t, $^3J_1$=$^3J_2$=8.6), 4.15 (H-4 of β-D-xylopyranose residue, m), 4.36 (H-5*e* of β-D-xylopyranose residue, dd, ^2J=11.3, ^3J=5), 4.71 (H -16, td, $^3J_1$=$^3J_2$=7.7, $^3J_3$=4.9), 4.92 (H-1 of β-D-xylopyranose residue, d, ^3J=7.5).

^{13}C NMR spectra of the 3-O-β-D-xylopyranoside cycloasgenin C (**3**) is given in the table 1.

References:

Naubeev, T. Kh., Uteniyazov, K.K., Isaev, M.I. (2011). *Chem. Nat. Compd.*,– 47.– 250 p.
Naubeev, T. Kh., Zhanibekov, A.A., Isaev, M.I. (2012). *Chem. Nat. Compd.*,– 48.– 813 p.
Naubeev, T. Kh., Zhanibekov, A.A., Uteniyazov, K.K., Bobakulov, Kh.M., Abdullaev, N.D. (2014). *Chem. Nat. Compd.*,– 49.– 1048 p.
Naubeev, T. Kh., Ramazonov, N. Sh. (2021). *Austrian Journal of Technical and Natural Sciences*,– № 7–8.– 48 p.
Naubeev, T. Kh., Uteniyazov, K.K., Ramazonov, N. Sh. (2022). *Austrian Journal of Technical and Natural Sciences*,– № 7–8.– 42 p.

submitted 07.02.2024;
accepted for publication 26.02.2024;
published 18.03.2024

Contact: timan05@mail.ru