



Section 1. Chemistry

DOI:10.29013/AJT-26-5.6-3-8



A NEW CYCLOARTANE TRITERPENE TRIDESMOSIDE FROM ASTRAGALUS MUCIDUS

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Cite: Artikbaeva B.R., Naubeev T.Kh. (2026). A New Cycloartane Triterpene Tridesmoside from *Astragalus mucidus*. *Austrian Journal of Technical and Natural Sciences* 2026, No 5 – 6. <https://doi.org/10.29013/AJT-26-5.6-3-8>

Abstract

From the aerial parts of *Astragalus mucidus* (Leguminosae), a new cycloartane glycoside – cycloasidoside C (**1**) – was isolated. Its structure was determined as 3-O-β-D-(3-OAc)-xylopyranoside, 6,25-di-O-β-D-glucopyranoside-24R-cycloartane-3β,6α,16β,24,25-pentol. The structure of the isolated compounds was established based on chemical transformations and ¹H, ¹³C NMR spectra (2D HMBC, HSQC, COSY and DEPT).

Keywords: cycloartane triterpenoids, cycloasidoside C, cycloasgenin C, *Astragalus mucidus*, ¹H and ¹³C NMR

Introduction

Plants and microorganisms remain the most attractive and often uncontested producers of low-molecular bioregulators, also called secondary metabolites. One of the main pathways for the biosynthesis of secondary metabolites is isoprene, which leads to the formation of compounds known as “isoprenoids”. Among natural compounds that have found application in medicine, veterinary and agriculture, isoprenoids occupy a very significant place. It is difficult to overestimate the role of steroid hormones (isoprenoids) in the regulation of the physiological functions of the human body, animals and insects.

In terms of the pace of development, the chemistry of cycloartane-type triterpenoids occupies a very significant place among plant isoprenoids. This class of natural compounds has developed rapidly over the past quarter century and, as a result, the number of compounds with an established structure is approaching a thousand. The source of cycloartane glycosides in our region are plants of the genus *Astragalus* (Leguminosae) (Kaypnazarov T.N., Ramazanov N.Sh., et al. 2020). In the flora of Uzbekistan, this genus is represented by 239 species. We are conducting a selective study of plants of the genus *Astragalus* for isoprenoid content.

Results and discussion

Continuing the study of triterpene glycosides of the cycloartane-type of plants of the genus *Astragalus* (Leguminosae), we investigated the aerial part of *Astragalus mucidus* Bunge for the content of these compounds. The new cycloartane-type glycoside – cycloascidoside C (**1**). Herein the evidence of the structure of these glycosides is provided.

The IR spectrum of compound **1** exhibited absorption bands at 3324 and 2913 cm^{-1} , characteristic of hydroxyl groups and the CH_2 group of a cyclopropane ring, respectively.

In the ^1H NMR spectrum of compound **1**, two one-proton doublets of an AX system were observed at δ 0.18 and 0.54 ppm with a coupling constant of $^2J = 4.2$ Hz, which are characteristic of a 1,1,2,2-tetrasubstituted three-membered ring. In addition, signals corresponding to seven methyl groups appeared at δ 0.97, 1.08, 1.31, 1.39, 1.52, 1.53 and 1.84 ppm. Furthermore, four signals of methine protons at δ 3.44 (dd, $J = 11.6, 4.4$ Hz), 3.67 (dd, $J = 10.6, 2.4$ Hz), 3.69 (td, $J = 9.2, 3.8$ Hz), and 4.66 (td, $^3J_1 = ^3J_2 = 7.8$ Hz, $^3J_3 = 5.4$ Hz) indicated the presence of secondary alcohol functionalities.

The carbon atoms of the cyclopropane ring, C-9, C-10, and C-19, resonated in the ^{13}C NMR spectrum at δ 21.94, 28.87, and 30.41 ppm, respectively. These data allowed us to classify compound **1** as a cycloartane-type triterpenoid (Gvazava L. N. and Kikoladze V.S., 2012; Shameem S.A., Banday A.H., et al., 2022; Aslanipour B., Gülcemal D., et al. 2017).

The ^1H NMR spectrum of compound **1** also showed a three-proton singlet at δ 2.05 ppm, indicating the presence of a single acetate group in the glycoside molecule. As expected, the ^{13}C NMR spectrum of the new glycoside **1** displayed signals at δ 21.71 and 170.57 ppm, corresponding to the carbon atoms of the acetyl moiety.

Acid hydrolysis of compound **1** yielded cycloasgenin C (Figure 1) as the aglycone (Kucherbaev K.Dzh., Uteniyazov K. K., Kachala V. V., et al., 2002; Isaev M. I., Gorovits M. B., et al., 1982). In addition, paper chromatography of the hydrolysate, followed by comparison with authentic standards, revealed the presence of D-glucose and D-xylose.

Alkaline hydrolysis of cycloascidoside C (**1**) resulted in the formation of glycoside **3**,

which was identified as cycloascidoside E (**3**) (Figure 1). Therefore, cycloascidoside C (**1**) can be regarded as a monoacetyl derivative of cycloascidoside E (**3**) (Naubeev T. X., Janibekov A. A., Kucherbaev K.Dzh., 2017).

The position of the acetyl group attachment was established through a comparative analysis of the ^1H and ^{13}C NMR spectra of compounds **1** and **3**.

As shown in table 1, the transition from glycoside **1** to glycoside **3** is accompanied by significant changes in the chemical shifts of the carbon atoms C-2 (-3.27 ppm), C-3 ($+1.15$ ppm), and C-4 (-1.89 ppm) of the xylopyranosyl residue.

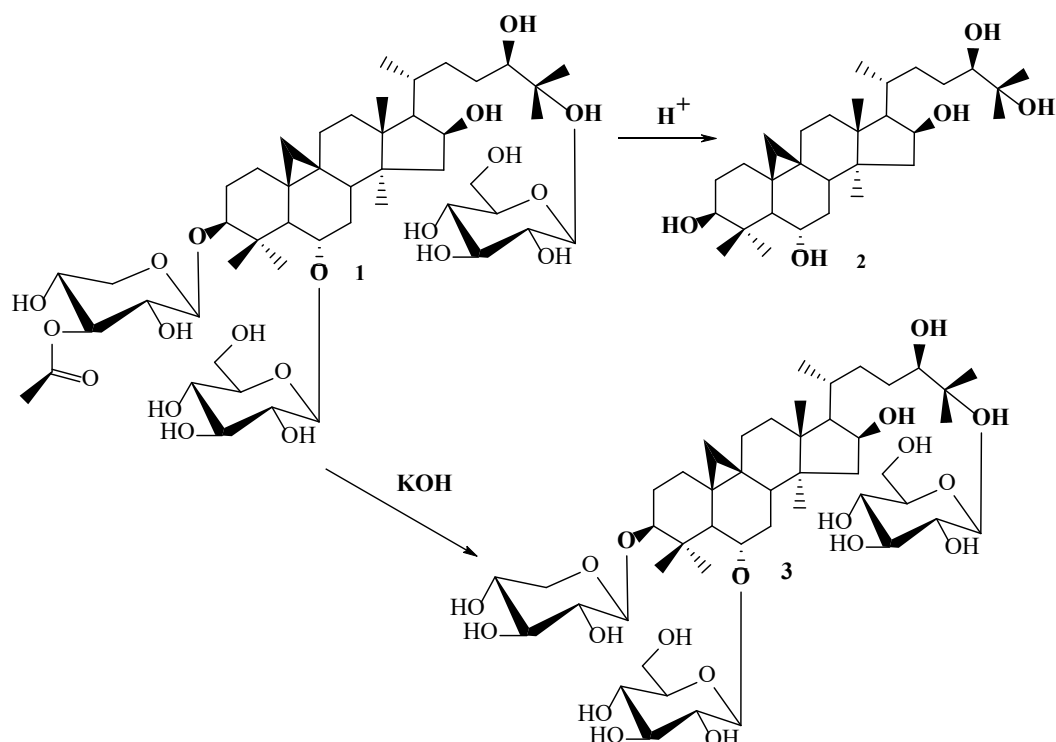
Comparison of the ^{13}C NMR signals of the anomeric carbon atoms in compound **1** and cycloascidoside E (**3**) confirmed the location of the acetyl group at the C-3 position of the xylopyranose residue (Table 1).

The signals of the anomeric carbon atom of the xylopyranosyl residue attached to the aglycone at C-3 appear at δ 108.16 and 107.62 ppm in the ^{13}C NMR spectra of cycloascidoside C (**1**) and cycloascidoside E (**3**), respectively. The chemical shifts of the anomeric carbon atoms remained essentially unchanged. Comparison of the carbon chemical shifts for cycloascidoside C (**1**) and cycloascidoside E (**3**) showed that, in the former, the signal of carbon C-2' of the xylose residue experienced an upfield shift of 3.27 ppm, confirming the presence of an acetyl substituent within the xylose moiety. The magnitude of this upfield shift indicates that the acetyl group is located on the adjacent carbon atom, C-3'.

This conclusion is further supported by the upfield shift of the C-3' signal by 1.15 ppm. In the ^1H NMR spectrum of cycloascidoside C (**1**), the proton geminal to the acetylated carbon resonates as a triplet at δ 5.58 ppm with coupling constants of $^3J_1 = ^3J_2 = 8.7$ Hz.

Based on these data, we concluded that the acetyl group in cycloascidoside C (**1**) is attached to the C-3' position of the xylose residue.

In the anomeric proton region of the ^1H NMR spectrum of cycloascidoside C (**1**), three signals were observed at δ 4.80 ppm (H-1, d, β -D-Xylp), δ 4.97 ppm (H-1', d, β -D-Glcp), and δ 5.23 ppm (H-1'', d, β -D-Glcp). The coupling constants of the anomeric protons were $^3J = 7.9, 7.7, \text{ and } 7.8$ Hz, respectively, indicating the β -configuration of all sugar residues.

Figure 1. Acidic and alkaline hydrolysis of cycloascidoside C (1)

In the ^{13}C NMR spectrum of cycloasgenin C (2), the signals corresponding to carbon atoms C-3, C-6, and C-25 were observed at δ 78.39, 68.33, and 72.74 ppm, respectively.

In the spectrum of cycloascidoside C (1), the corresponding carbon signals appeared at δ 89.41, 79.68, and 81.38 ppm (Table 1).

Table 1. ^{13}C NMR spectral data of cycloascidoside C (1), aglycone 2 and cycloascidoside E (3) ($\text{C}_5\text{D}_5\text{N}$, δ , ppm, J/Hz, TMS as internal standard)

Atom C	DEPT	1	2	3	Atom C	DEPT	1	2	3
1	CH_2	32.53	32.83	32.20	27	CH_3	24.74	26.19	24.22
2	CH_2	29.68	31.51	30.22	28	CH_3	20.34	20.34	19.83
3	CH	89.41	78.39	88.55	29	CH_3	28.73	29.41	28.52
4	C	42.75	42.51	42.65	30	CH_3	17.04	16.22	16.63
5	CH	52.96	54.05	52.48	3-O-β-D-Xylp				
6	CH	79.68	68.33	79.16	1	CH	108.16		107.62
7	CH_2	34.92	38.69	34.23	2	CH	72.31		75.58
8	CH	46.18	47.28	45.62	3	CH	79.65		78.50
9	C	21.94	21.33	21.38	4	CH	69.33		71.22
10	C	28.87	29.62	28.71	5	CH_2	67.59		67.02
11	CH_2	26.70	26.42	26.26	Ac		21.71		
12	CH_2	33.59	33.22	33.14	Ac		170.57		
13	C	46.25	45.76	45.66	6-O-β-D-Glcp'				
14	C	47.38	47.00	46.89	1	CH	105.63		105.13
15	CH_2	48.66	48.86	48.05	2	CH	75.87		75.58
16	CH	72.13	71.76	71.75	3	CH	79.45		79.16
17	CH	57.65	57.28	57.15	4	CH	71.81		71.75

Atom C	DEPT	1	2	3	Atom C	DEPT	1	2	3
18	CH ₃	19.08	18.84	18.47	5	CH	78.66		78.09
19	CH ₂	30.42	30.40	30.33	6	CH ₂	63.57		63.08
20	CH	32.06	31.68	31.55	25-O-β-D-Glcp''				
21	CH ₃	19.31	19.16	18.80	1	CH	99.21		98.69
22	CH ₂	35.40	34.90	34.96	2	CH	76.11		75.35
23	CH ₂	29.16	29.44	29.25	3	CH	79.04		78.71
24	CH	78.74	80.65	78.94	4	CH	72.24		71.77
25	C	81.38	72.74	80.56	5	CH	79.24		78.20
26	CH ₃	22.02	26.03	21.50	6	CH ₂	63.23		62.74

Therefore, glycosylation effects were observed only for carbon atoms C-3 (+11.24 ppm), C-6 (+11.35 ppm), and C-25 (+8.64 ppm), whereas the chemical shifts of carbon atoms C-16 and C-24 remained essentially unchanged.

The ¹³C NMR spectrum of cycloascidoside C (**1**) exhibited signals of three anomeric carbon atoms resonating at δ 108.16, 105.63, and 99.21 ppm, respectively. These data indicate that the D-xylose residue is attached at C-3 of the aglycone, while two D-glucose residues are linked at C-6 and C-25.

In the ¹³C NMR spectrum of cycloascidoside C (**1**), three signals were observed in the region characteristic of anomeric carbon atoms, indicating that the compound is a triglycoside. Analysis of the COSY and TOCSY spectra allowed the identification of distinct spin systems corresponding to the sugar residues and enabled determination of the monosaccharide composition of the carbohydrate moiety based on chemical shift values and coupling constants.

These assumptions were further confirmed by HMBC correlations between H-1 of the xylose residue (δ 4.80 ppm) and C-3 of the aglycone (δ 89.41 ppm), H-1 of a glucose residue (δ 4.97 ppm) and C-6 of the aglycone (δ 79.68 ppm), and H-1 of the second glucose residue (δ 5.23 ppm) and C-25 of the aglycone (δ 81.38 ppm). Additionally, an HMBC correlation was observed between H-3' of the xylose residue (δ 5.58 ppm) and the acetyl carbonyl carbon (δ 170.57 ppm), providing conclusive evidence for the positions of the sugar residues and the acetyl group.

Based on the obtained experimental data, the new cycloartane glycoside, cycloascidoside C (**1**), was identified as

24R-cycloartane-3β,6α,16β,24,25-pentaol 3-O-β-D-(3-O-acetyl)-xylopyranoside, 6,25-di-O-β-D-glucopyranoside.

Isolation and purification of isoprenoids from the aerial parts of *Astragalus mucidus* Bunge were carried out as previously described (Naubeev T. Kh., Uteniyazov K. K., Isaev M. I., 2011). The aerial parts of *Astragalus mucidus* (1.5 kg), collected in the Namangan region of the Republic of Uzbekistan, were air-dried and finely ground. The plant material was extracted five times with 8 l portions of methanol. The combined methanolic extracts were concentrated under reduced pressure using a rotary evaporator at 40–50 °C to a syrup-like consistency and then mixed with twice their volume of water. The resulting aqueous residue was successively extracted with chloroform and then with *n*-butanol. The *n*-butanol extract was evaporated to dryness under reduced pressure.

A total of 75 g of the dry *n*-butanol residue was subjected to column chromatography on silica gel. Elution with a chloroform-methanol-water solvent system (70:23:3) afforded 150 mg of cycloascidoside C (**1**), corresponding to a yield of 0.01% based on the air-dried plant material.

Cycloascidoside C (1**)**, C₄₉H₈₂O₂₀, white amorphous powder, mp 292–294 °C (from CH₃OH).

¹H NMR spectrum of cycloascidoside C (600 MHz, C₅D₅N, δ ppm, J/Hz, TMS as internal standard): δ 0.18 and 0.54 (each d, J = 4.2 Hz, 2H, H-19), 0.97 (s, CH₃-28), 1.08 (3H, d, J = 6.4 Hz, CH₃-21), 1.31, 1.39, 1.52, 1.53, and 1.84 (each s, 5 × CH₃), 2.05 (3H, s, OAc), 3.44 (1H, dd, J=11.6, 4.4, H-3), 3.56 (1H, dd, J=10.5, 2.5, H-5a, β-D-Xylp), 3.67

(dd, $J=10.6, 2.4$, H-24), 3.69 (1H, td, $J=9.2, 3.8$, H-6), 3.82 (1H, m, H-5'', Glcp), 3.90 (1H, dd, $J=8.4, 7.8$, H-2'', Glcp), 3.92 (1H, dd, $J=8.9, 7.8$, H-2', Glcp), 3.94 (ddd, $J=9.2, 5.1, 2.7$, H-5', Glcp), 4.04 (1H, dd, $J=8.8, 7.8$, H-2, Xylp), 4.07 (1H, t, $J=8.9$, H-4', Glcp), 4.08 (1H, t, $J=8.5$, H-4', Glcp), 4.09 (1H, t, $J=8.8$, H-3', Glcp), 4.16 (1H, t, $J=8.8$, H-3'', Glcp), 4.17 (1H, dd, $J=11.3, 5.4$, H-6'', Glcp), 4.21 (1H, dd, $J=11.7, 5.5$, H-6', Glcp), 4.34 (1H, dd, $J=11.5, 5.2$, H-5e, Xylp), 4.35 (1H, dd, $J=11.7, 5.1$, H-6', Glcp), 4.49 (1H, td, $J=7.5, 7.6, 4.8$, H-4, Xylp), 4.56 (1H, dd, $J=11.8, 3$, H-6'', Glcp), 4.66 (td, $J=7.8, 5.4$, H-16), 4.80 (1H, d, $J=7.9$, H-1, Xylp), 4.97 (1H, d, $J=7.7$, H-1', Glcp), 5.23 (1H, d, $J=7.8$, Glcp, H-1''), 5.58 (1H, t, $J=8.7$, H-3, Xylp).

The ^{13}C NMR spectral data of cycloascidoside C are presented in Table 1.

Acid hydrolysis of Cycloascidoside C. Glycoside **1** (50 mg) was dissolved in 20 mL of 50% aqueous methanol containing 0.5% sulfuric acid, and the solution was refluxed for 1 h. After completion of the reaction, water was added, the methanol was removed under reduced pressure, and the reaction products were extracted with *n*-butanol. The butanolic extract was washed with water and evaporated to dryness.

Subsequent column chromatography using solvent system chloroform-methanol (25:1) afforded 10 mg of cycloasgenin C (**2**) as a white amorphous powder, $\text{C}_{30}\text{H}_{52}\text{O}_5$, mp 250–252 °C (from acetone).

Paper chromatography (PC) in solvent system *n*-butanol-pyridine-water (6:4:3) revealed the presence of D-glucose and D-xylose in the hydrolysate.

^1H NMR spectrum of cycloasgenin C (600 MHz, $\text{C}_5\text{D}_5\text{N}$, δ ppm, J/Hz, TMS as internal standard): δ 0.35 and 0.62 (d, $J = 4.0$ Hz, 2H, H-19), 1.06 (s, CH_3), 1.13 (d, $J = 6.4$ Hz, CH_3 –21), 1.40, 1.45, 1.52, 1.54 and 1.93 (each s, $5 \times \text{CH}_3$), 3.69 (dd, $J = 11.4$ Hz, H-3), 3.71 (dd, $J = 10.3, 2.3$ Hz, H-24), 3.82 (td, $J = 9.3, 3.7$ Hz, H-6) and 4.75 (td, $J = 7.7, 4.9$ Hz, H-16).

The ^{13}C NMR spectral data of cycloasgenin C are presented in Table 1.

Alkaline hydrolysis of Cycloascidoside C. Cycloascidoside C (**1**) (50 mg) was dissolved in 20 mL of a 0.5% methanolic KOH solution. The reaction mixture was kept at 25 °C for 24 h. The solution was then diluted with 35 mL of water, neutralized with acetic acid, and the methanol was removed under reduced pressure. The reaction products were extracted with *n*-butanol, after which the solvent was evaporated.

Chromatographic separation using solvent system chloroform-methanol-water (70:23:3) yielded 12 mg of cycloascidoside E (**3**), $\text{C}_{47}\text{H}_{80}\text{O}_{19}$, mp 276–278 °C (from CH_3OH).

Cycloascidoside E (3), $\text{C}_{47}\text{H}_{80}\text{O}_{19}$, white amorphous powder, mp 276–278 °C (from methanol).

^1H NMR spectrum of cycloascidoside E (600 MHz, $\text{C}_5\text{D}_5\text{N}$, δ ppm, J/Hz): δ 0.20 and 0.59 (each ^1H , d, $J = 4.2$ Hz, H-19), 0.83 (3H, s, Me-28) and 1.08 (3H, d, $J = 6.6$ Hz, Me-21), 1.38, 1.39, 1.52, 1.53, 2.06 (3H, s, Me-30, Me-18, Me-27, Me-26, Me-29), 3.56 (1H, dd, $J=11.6, 4.4$, H-3), 3.71 (1H, dd, $J=11.3, 10$, H-5 α Xylp), 3.83 (1H, td, $J=8.4, 4.2$, H-6), 3.85 (1H, m, Glcp H''-5), 3.92 (1H, ddd, $J=8.2, 5.7, 2.5$, Glcp H-5'), 3.93 (1H, dd, $J_1=8.3, 8$, Glcp H'-2), 3.91 (1H, dd, $J=8.6, 7.5$, Glcp H-2''), 3.93 (1H, dd, $J=8.6, 7.5$, Xylp H-2), 4.01 (1H, t, $J=8.2$, Xylp H-3), 4.09 (1H, m, Xylp H-4), 4.06 (1H, t, $J=8.7$, Glcp H-4'), 4.07 (1H, t, $J=8.6$, Glcp H-4''), 4.08 (1H, t, $J=8.7$, Glcp H-3'), 4.17 (1H, t, $J=9$, Glcp H-3''), 4.22 (1H, dd, $J=11.5, 5.1$, Xylp H-5e), 4.25 (1H, dd, $J=11.5, 5.5$, Glcp H-6''), 4.26 (1H, dd, $J=11.6, 5.4$, Glcp H-6'), 4.36 (1H, dd, $J=11.6, 5.1$, Glcp H-6), 4.56 (1H, dd, $J=11.8, 2.6$, Glcp H-6''), 4.67 (1H, td, $J=7.3, 6.5$, H-16), 4.86 (1H, d, $J=7.5$, Xylp H-1), 4.94 (1H, d, $J=7.7$, Glcp H-1'), 5.24 (1H, d, $J=7.8$, Glcp H-1'').

The ^{13}C NMR spectral data of cycloascidoside E (**3**) are presented in Table 1.

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submitted 15.06.2026;
accepted for publication 29.06.2026;
published 30.06.2026
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