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CYCLOTANOSIDE, A NEW CYCLOARTANE GLYCOSIDE FROM FLOWERS OF *Astragalus tanae*

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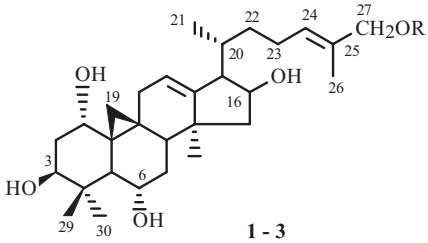
The new cycloartane glycoside cyclotanoside and the triterpene saponin astragaloside VIII were isolated from flowers of Astragalus tanae *Sosn. Their structures were established as 9β,19-cyclolanost-12,24E-dien-1α,3β,6α,16β,27-pentaol-27-*O-β-*D-(6′-*O-*acetoxy)-galactopyranoside and 3-*O-α-*L-rhamnopyranosyl-(1→2)-*O-β-*D-xylopyranosyl-(1→2)-*O-β-*D-glucuronopyranosyl-soyasapogenol B.*

Keywords: *Astragalus tanae* Sosn., cycloartanes, cyclotanoside, cyclotanogenin, astragaloside VIII.

The genus *Astragalus* L. (Leguminosae) comprises 2455 plant species, 72 of which occur in Georgia [1]. Extracts purified of lipophilic substances and pure compounds from *Astragalus* plants exhibit high biological activity [2, 3]. A hypoazotemic and diuretic medicine was created from *A. falcatus* Lam. [2]. Extracts from other *Astragalus* species exhibit cardiotonic [4], hypocholesterolemic, and antioxidant [5] activity. Several of them cause leukocyte proliferation [2].

A. tanae Sosn. is endemic to Georgia and typically has high flavonoid and triterpenoid contents. Several phenolic components of the flowers were studied [6]. Herein, the isolation and structure elucidation of the cycloartane glycoside cyclotanoside (**1**) and a saponin (**4**) are described.



1 - 3

1: R = Gal*p*-*O*-Ac
2: R = H; **3:** R = Gal*p*

Compounds **1** and **4** were isolated by column chromatography over SiO_2. Cycloartane glycoside **1** was obtained as white needle-like crystals. The molecular formula $C_{37}H_{58}O_{11}$ was established by HR-TOF-MS (*m*/*z* 679.4989 $[M + H]^+$). The IR spectrum showed a broad absorption band at ν_{max} 3580–3300 cm^{-1}, indicative of OH groups, and an absorption band at ν_{max} 3045 cm^{-1}, characteristic of a cyclopropane methylene.

The PMR spectrum of **1** (Table 1) contained resonances for cyclopropane methylene at δ 0.55 and 0.65 ppm (d, J = 4.2 Hz); one secondary and four tertiary methyls at δ 0.76–1.25 ppm; and methylene and four oxidized methine protons belonging to the carbohydrate part (δ 3.54–3.86 ppm). The ^{13}C NMR spectrum (Table 1) exhibited resonances for 37 C atoms including 6 primary, 9 secondary, 15 tertiary, and 7 quaternary ones.

A resonance corresponding to an acetyl methyl at δ 2.01 ppm, the existence of which was confirmed by a resonance at δ 168.5 ppm in the ^{13}C NMR spectrum, was observed in addition to the aforementioned resonances. The resonance of D-galactose C-6′ was shifted to weak field at δ 66.5 ppm, which indicated that the acetyl was situated on this C atom.

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TABLE 1. PMR (400 MHz) and ^{13}C NMR Spectral Data (100 MHz) for **1** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_H	δ_C	DEPT	HMBC (^1H–^{13}C)
1	3.85 (br.s)	73.8	CH	2, 3, 10, 19
2	1.80, 2.0 (m)	35.8	CH_2	1, 3, 4
3	3.19 (dd, J = 4.7, 12.1)	76.8	CH	2, 4, 29
4	–	41.2	C	–
5	1.69 (d, J = 8.01)	43.1	CH	4, 6, 10, 30
6	4.13 (m)	67.2	CH	5, 7, 8
7	1.51 (m); 1.69 (m)	23.0	CH_2	5, 6, 8
8	1.47 (dd, J = 4.3, 12.2)	48.2	CH	6,7, 14
9	–	20.7	C	–
10	–	29.2	C	–
11	1.36 (m); 2.29 (m)	22.1	CH_2	9, 12
12	5.2 (m)	121.3	CH	13, 17
13	–	144.8	C	–
14	–	46.3	C	–
15	1.59 (m); 2.13 (dd, J = 5.2, 12.5)	47.7	CH_2	14, 16
16	4.58 (m)	72.0	CH	15, 17
17	1.82 (m)	56.7	CH	12, 13, 16, 20
19	0.55; 0.65 (d, J_{AB} = 4.2)	28.6	CH_2	1, 10, 11
20	2.13 (m)	29.0	CH	17, 21, 22
21	1.07 (d, J = 6.8)	17.7	CH_3	17, 20, 22
22	1.25 (m); 2.10 (m)	37.6	CH_2	21, 23
23	1.50 (m); 2.13 (m)	25.1	CH_2	22, 24, 25
24	5.35 (m)	128.4	CH	22, 23, 25, 26, 27
25	–	131.4	C	–
26	1.25 (s)	14.9	CH_3	24, 25, 27
27	3.87 (2H, br.s)	69.0	CH_2	26, 1′
28	0.76 (s)	16.4	CH_3	8, 14, 13
29	0.98 (s)	20.2	CH_3	3, 4, 5, 30
30	0.89 (s)	13.7	CH_3	3, 4, 5, 29
$\underline{C}OCH_3$	–	168.5	C	–
$CO\underline{C}H_3$	2.01 (s)	12.9	CH_3	$\underline{C}O$
β-D-Gal*p*				
1′	5.18 (d, J = 8.0)	100.7	CH	27
2′	3.86 (dd, J = 8.0, 9.4)	73.6	CH	1′, 3′
3′	3.60 (dd, J = 9.4, 3.8)	74.7	CH	2′
4′	3.83 (m)	69.5	CH	2′
5′	3.71 (m)	77.2	CH	4′, 6′
6′	3.54; 3.75 (m)	66.5	CH_2	$\underline{C}O$, 4′, 5′

Correspondingly, glycoside **1** underwent alkaline hydrolysis to form progenin **3**, the PMR and ^{13}C NMR spectra of which had a resonance for the anomeric proton at δ 5.18 (d, J = 8.0 Hz, H-1′) and the anomeric C atom at δ 100.7. The NMR data (Table 1) confirmed that the compound was a monoside. It contained a D-galactose sugar in the pyranose form with an axial anomeric proton. Acid hydrolysis of **1** formed the genin **2** and D-galactose, which confirmed that it was present in glycoside **1**.

The structure of **1** was studied in more detail by comparing its NMR spectrum with those in the literature. Spectral shifts of the aglycon of **2** and progenin **3** were compared with those of the genins of mongholicoside I [7], kahiricoside II [8], and astragaloside VIII [9] (Table 2).

Glycosylation at C-3 and a closed skeleton in the glycoside side chain were excluded because the ^{13}C NMR spectrum of **1** lacked resonances at δ 84–88 ppm. A comparison of ^{13}C NMR spectra of the genin of kahiricoside II [8] and of **2** showed that the side chains had the same structure (Table 2).

Spectral data for **1** and its progenin **3** confirmed that the chemical shifts of C-6 and C-16 practically did not change as compared with those of kahiricoside II [8]. The resonance at δ 69.0 ppm in the ^{13}C NMR spectrum belonged to C-27 and was glycosylated, similar to mongholicosides I and II (δ 68.8 ppm) [7]. Thus, the sugar residue was apparently bonded to the genin in the aforementioned position. The chemical shift of C-1 (δ 73.8 ppm) was identical to that of mongholicoside I (Table 2) and confirmed that the OH was located in this position.

TABLE 2. ^{13}C NMR Spectral Data for Cyclotanogenin (**2**) and Progenin (**3**) and the Aglycons of Mongholicoside I [7], Kahiricoside II* [8], and Astragaloside VIII [9] (DMSO-d_6, δ, ppm)

C atom	**2**	**3**	[7]	[8]	[9]
1	73.6	73.8	73.6	32.4	38.8
2	36.8	36.8	36.8	30.2	26.7
3	77.0	77.0	73.8	89.1	78.9
4	42.0	42.0	40.5	42.6	44.3
5	43.0	43.1	39.6	54.1	56.4
6	68.3	68.3	20.7	68.1	18.4
7	26.5	26.4	24.7	38.5	33.3
8	48.0	48.3	48.0	46.9	39.9
9	21.8	21.8	20.7	21.3	47.7
10	30.0	29.9	30.5	29.2	36.5
11	25.9	26.0	25.9	26.4	24.0
12	122.8	122.8	32.8	33.3	122.4
13	144.8	144.8	45.4	45.8	144.8
14	46.0	46.2	46.8	47.0	42.4
15	47.8	48.0	48.2	49.2	26.4
16	73.2	73.2	72.7	71.3	28.6
17	56.7	56.7	56.5	57.0	38.0
18	–	–	19.1	19.0	45.3
19	28.3	28.5	30.2	29.9	46.7
20	29.0	30.0	29.6	30.8	30.9
21	18.1	18.1	17.9	18.2	42.4
22	36.5	36.5	36.3	36.8	75.5
23	26.5	26.0	25.9	25.6	23.0
24	128.0	128.0	126.4	125.9	62.0
25	132.8	132.8	135.4	135.8	15.6
26	14.9	14.9	13.7	14.0	17.0
27	69.1	68.9	68.8	68.3	25.6
28	20.3	20.3	20.2	29.0	28.6
29	26.5	26.5	25.2	16.7	33.3
30	13.5	13.5	14.1	20.2	21.1
		27-*β*-D-Gal*p*		27-*β*-D-Glc*p*	
1′		100.3		107.0	
2′		73.7		76.0	
3′		74.6		78.8	
4′		71.8		71.9	
5′		77.3		78.2	
6′		62.7		63.1	
$\underline{C}O$-CH_3			170.6		
CO-$\underline{C}H_3$			21.0		

*Spectra of kahiricoside II were taken in C_5D_5N.

The structure of the genin of glycoside **1** was proposed as 9*β*,19-cyclolanost-12,24*E*-dien-1*α*,3*β*,6*α*,16*β*,27-pentaol (**2**). The PMR and ^{13}C NMR spectral data for **2** did not agree with analogous data for known genins. Therefore, the compound was new and was called cyclotanogenin.

The location of the carbohydrate was confirmed by HMBC spectral data. The doublet for the D-galactose anomeric proton at δ 5.18 ppm had a correlation peak with the singlet at δ 69.0 ppm, which indicated that the D-galactose was located on C-27 [7] (Fig. 1).

A multiplet at δ 4.58 ppm in the PMR spectrum was characteristic of H-16 in the five-membered ring. The 2D heteronuclear correlation spectrum allowed the resonance at δ 72.0 ppm to be attributed to C-16; the multiplet at δ 1.82 ppm, to H-17; the doublet of doublets (dd) with SSCC 12.5 and 5.2 Hz at δ 2.13 ppm and the multiplet at δ 1.59 ppm, to the H-15 protons. In turn, this allowed resonances at δ 47.8 and 56.7 ppm to be assigned to C-15 and C-17, respectively.

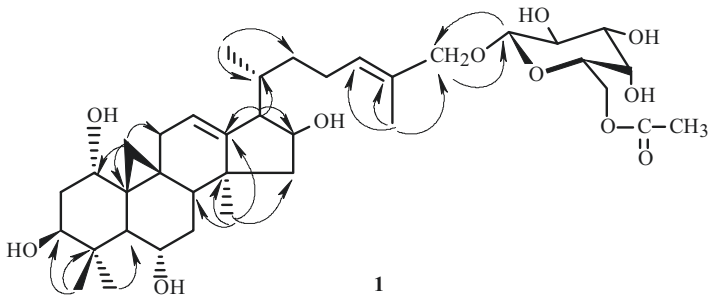

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Fig. 1. HMBC correlations in **1**.

The resonance at δ 3.85 ppm (br.s, H-1) correlated with that at δ 73.8 ppm, indicating that the C-1 H atom was equatorial with the 1*S* absolute conformation. The resonance of H-3 at δ 3.19 ppm (doublet of doublets with SSCC 4.7 and 12.1 Hz) correlated with that at δ 76.8 ppm, indicating that the C-3 H atom was axial with the 3*R* absolute conformation.

The resonance of H-5 at δ 1.69 ppm interacted strongly with H-6 (δ 4.13 ppm) according to the correlation spectrum.

Table 1 presents data for the coupling of H atoms through two and more C atoms based on the HMBC spectrum.

The placement of an OH on C-6 was confirmed by COSY (H-6/H-5) and HMBC (H-5/C-6, H-6/C-5) correlations. The *E*-stereochemistry of the C-24 double bond was determined from ROESY correlations of H-24 (δ 5.35 ppm) and H-27 (δ 3.87 ppm). Analogously, the configurations of the C-6 and C-16 hydroxyls were α and β, respectively.

The experimental results led to the conclusion that cycloartane glycoside **1** had the structure 9β,19-cyclolanost-12,24*E*-dien-1α,3β,6α,16β,27-pentaol-27-*O*-β-D-(6′-*O*-acetoxy)-galactopyranoside. This structure has not been reported and was called by us cyclotanoside (Fig. 1).

EXPERIMENTAL

NMR spectra were recorded in DMSO-d_6 or CD_3OD on a Bruker Avance 400 spectrometer. PMR spectra were recorded at 400 MHz; ^{13}C NMR spectra, at 100 MHz. The internal standards were residual solvent resonances. DEPT and 2D NMR spectra (HSQC, COSY, HMBC, ROESY) used standard Bruker pulse programs. HR-TOF-MS were measured using a Finnigan Model 4600 quadrupole system spectrometer. TLC used SiO_2 60 F254 plates (Merck); paper chromatography (PC), FN-11 (Whatman). TLC and PC used solvent systems $CHCl_3$–MeOH–H_2O (6:4:1, 1), C_5H_5N–C_6H_6–*n*-BuOH–H_2O (3:1:5:3, 2), and $CHCl_3$–MeOH–H_2O (70:23.5:2, 3). TLC spots were detected by spraying with phosphotungstic acid (25%) in MeOH followed by heating at 110°C for 2–3 min. Sugars were detected on PC using anilinium biphthalate.

Plant Material. *A. tanae* was collected in the Tana River gorge, eastern Georgia, in June 2013. Specimens of *A. tanae* were defined at the Department of Pharmacobotany, I. Kutateladze Institute of Pharmacochemistry, Tbilisi, Georgia and are preserved under herbarium No. 13076.

Extraction and Isolation. Air-dried ground flowers (0.7 kg) were extracted with EtOH (80%). The obtained extract was evaporated to an aqueous residue. A resinous precipitate was separated by filtration and did not contain flavonoids and triterpenoids according to TLC analysis. The filtrate was washed with $CHCl_3$. This caused white needle-like crystals that turned out to consist of two compounds to precipitate at the interface of the liquids. Compounds **1** (74 mg) and **4** (32 mg) were obtained by chromatography of this mixture over a column of SiO_2.

9β,19-Cyclolanost-12,24*E*-dien-1α,3β,6α,16β,27-pentaol-*O*-β-D-(6′-*O*-acetoxy)-galactopyranoside (Cyclotanoside, 1). White crystals, mp 271–274°C (MeOH). IR spectrum (KBr, ν, cm^{-1}): 3580–3300 (OH), 3045 (cyclopropane-ring CH_2). Table 1 lists the PMR and ^{13}C NMR spectral data. FAB-MS *m/z* 679.923 $[M + H]^+$ (calcd for $C_{37}H_{59}O_{11}$, 679.861).

Acid Hydrolysis of 1. A solution of **1** (15 mg) in MeOH was treated with HCl solution (0.5%), hydrolyzed under reflux at 80°C on a water bath for 1 h, cooled, and extracted with EtOAc. The extract was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness. The aqueous fraction was neutralized with AV-17 anion exchanger (OH-form). The sugar was identified by PC using system 2 and comparison with authentic sample.

9β,19-Cyclolanost-12,24*E*-dien-1α,3β,6α,16β,27-pentaol (cyclotanogenin, 2). White crystals, mp 249–252°C (MeOH). IR spectrum (KBr, ν, cm^{-1}): 3600–3200 (OH), 3040 (cyclopropane-ring CH_2). Table 2 lists the ^{13}C NMR spectral data. FAB-MS *m/z* 475.674 $[M + H]^+$ (calcd for $C_{29}H_{47}O_5$, 475.535).

Alkaline Hydrolysis of 1. Compound **1** (12 mg) was hydrolyzed by KOH solution (0.5%) at 25°C for 1 h. The reaction mixture was neutralized and purified over a column of SiO_2 to afford **3**.

9β,19-Cyclolanost-12,24*E*-dien-1α,3β,6α,16β,27-pentaol-*O*-β-D-galactopyranoside (3). White crystals, mp 277–280°C (MeOH). IR spectrum (KBr, ν, cm^{-1}): 3580–3200 (OH), 3043 (cyclopropane-ring CH_2). Table 2 lists the ^{13}C NMR spectral data. FAB-MS *m/z* 637.815 $[M + H]^+$ (calcd for $C_{35}H_{57}O_{10}$, 637.8157).

3-*O*-L-Rhamnopyranosyl-(1→2)-*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucuronopyranosyl-soyasapogenol B (astragaloside VIII) (4). White crystals, mp 222–224°C (MeOH), $[\alpha]_D^{20}$ −17.3° (*c* 0.4, EtOH–H_2O, 1:1). IR spectrum (KBr, ν, cm^{-1}): 3410 (OH), 1726. ^1H NMR spectrum (400 MHz, CD_3OD, δ, ppm, J/Hz): 1.64 (m, H_a-1), 1.02 (m, H_b-1), 2.15 (m, H_a-2), 1.82 (m, H_b-2), 3.32 (dd, J = 8.6, 3.1, H-3), 0.95 (m, H-5), 1.63 (m, H_a-6), 1.38 (m, H_b-6), 1.57 (m, H_a-7), 1.41 (m, H_b-7), 1.58 (m, H-9), 1.88 (m, H-11), 5.24 (br.s, H-12), 1.78 (m, H_a-15), 1.04 (m, H_b-15), 1.75 (m, H_a-16), 1.32 (m, H_b-16), 2.05 (dd, J = 13.6, 3.8, H-18), 1.74 (m, H_a-19), 0.96 (m, H_b-19), 1.44 (m, H_a-2), 1.33 (m, H_b-21), 3.39 (m, H-22), 1.20 (3H, s, Me-23), 1.23 (3H, s, Me-24), 0.89 (3H, s, Me-25), 0.98 (3H, s, Me-26), 1.13 (3H, s, Me-27), 0.83 (3H, s, Me-28), 0.92 (3H, s, Me-29), 1.02 (3H, s, Me-30), 1.27 (3H, d, J = 6.4, Rha Me), 5.19 (d, J = 8.1, GlcA H-1′), 4.92 (d, J = 7.6, Xyl H-1″), 4.12 (d, J = 1.2, Rha H-1‴). ^{13}C NMR spectrum (100 MHz, CD_3OD, δ, ppm): 39.6 (C-1), 26.8 (C-2), 91.9 (C-3), 44.5 (C-4), 57.4 (C-5), 19.2 (C-6), 34.2 (C-7), 40.6 (C-8), 48.8 (C-9), 37.0 (C-10), 24.7 (C-11), 122.3 (C-12), 144.6 (C-13), 43.3 (C-14), 26.5 (C-15), 29.9 (C-16), 38.4 (C-17), 46.4 (C-18), 47.4 (C-19), 31.4 (C-20), 42.1 (C-21), 76.8 (C-22), 63.6 (C-23), 22.8 (C-24), 16.1 (C-25), 17.3 (C-26), 25.2 (C-27), 20.2 (C-28), 32.3 (C-29), 28.9 (C-30), 105.1 (Glc A C-1′), 78.3 (C-2′), 78.8 (C-3′), 73.6 (C-4′), 77.5 (C-5′), 169.6 (C-6′), 100.5 (Xyl C-1″), 79.4 (C-2″), 78.5 (C-3″), 70.3 (C-4″), 66.7 (C-5″), 100.7 (Rha C-1‴), 70.9 (C-2‴), 70.7 (C-3‴), 72.8 (C-4‴), 68.1 (C-5‴), 16.9 (C-6‴).

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REFERENCES

1. *Flora of Georgia* [in Russian], Vol. VII, Metsniereba, Tbilisi, 1981, 516 pp.
2. M. D. Alaniya, E. P. Kemertelidze, and N. F. Komissarenko, *Flavonoids from Several Astragalus Species of the Flora of Georgia* [in Russian], Metsniereba, Tbilisi, 22, 152 pp.
3. E. P. Kemertelidze, M. D. Alaniya, K. G. Shalashvili, T. G. Sagareishvili, and N. Sh. Kavtaradze, *Original Medicines of Flavonoid-bearing Plants of Georgia* [in Russian], Izd. Natsional′noi Akademii Gruzii, Tbilisi, 2016, 121 pp.
4. M. D. Alania, *Allergol. Immunol.*, **7** (5), 575 (2006).
5. M. D. Alania, K. G. Shalashvili, N. Sh. Kavtaradze, T. G. Sagareishvili, and M. G. Sutiashvili, in: *Abstract Book of the VIIIth International Symposium "Phenolic Compounds: Fundamental and Applied Aspects"*, Moscow, Russia, 2012, pp. 11–15.
6. M. D. Alaniya and N. F. Chkadua, *Chem. Nat. Compd.*, **36**, 537 (2000).
7. Y. Z. Zhu, S. H. Lu, Y. Okada, M. Takata, and T. Okoyama, *Chem. Pharm. Bull.*, **40** (8), 2230 (1992).
8. M. M. Radwan, N. A. El-Sebakhy, A. M. Asaad, S. M. Toaima, and D. G. I. Kingston, *Phytochemistry* **65**, 2909 (2004).
9. I. Kitagawa, H. Wang, and M. Yoshikawa, *Chem. Pharm. Bull.*, **31** (2), 716 (1983).