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CYCLOASCAULOSIDE A FROM *Astragalus caucasicus* LEAVES

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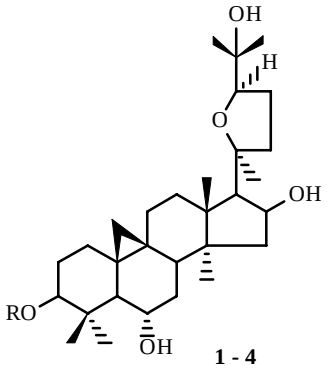
The new cycloartane glycoside cycloascauloside A with the structure 20S,24R-epoxycycloartan-3β,6α,16β,25-tetraol 3-O-[α-L-rhamnopyranosyl(1→6)]-β-D-(2′-O-acetyl)-glucopyranoside was isolated from leaves of Astragalus caucasicus *Pall. The structure was established based on IR, PMR, and ^{13}C NMR spectra and physicochemical properties of the compound itself and the products of its chemical transformations.*

Key words: *Astragalus caucasicus*, cycloascauloside, cyclogalegigenin, cycloartane.

In continuation of research on isoprenoids from plants of the genus *Astragalus* [1], we isolated from leaves of *A. caucasicus* Pall. (Leguminosae L.) compounds called cycloascaulosides A, B, and C. Herein the structure of cycloascauloside A is reported.

The PMR spectrum of cycloascauloside A (**1**) contained two 1H doublets coupled in an AB-type system at 0.39-0.54 ppm with SSCC ^2J = 4.5 Hz in addition to signals for seven methyls at 0.80-1.78 ppm. This enabled **1** to be considered as a cycloartane triterpenoid [1-3]. This conclusion was confirmed by absorption bands in the IR spectrum at 3045 and 1452 cm^{-1} and the preparation of cyclogalegigenin (**2**) upon acid hydrolysis of **1** and its Smith decomposition [2-4]. The carbohydrate part of the hydrolysate contained D-glucose and L-rhamnose in a 1:1 ratio according to paper (PC) and gas chromatography [5].



1 - 4

1: R = α-L-Rha*p*-(1→6)-β-D-(2-O-Ac)-Glc*p*

2: R = H

3: R = α-L-Rha*p*-(1→6)-β-D-Glc*p*

4: R = β-D-Glc*p*

The IR spectrum of **1** exhibited absorption bands at 1755 and 1240 cm^{-1} (ester); the PMR spectrum, a 3H singlet at 1.98 ppm belonging to an acetyl. As expected, the ^{13}C NMR spectrum of **1** contained signals for C atoms of one acetyl at 19.70 and 170.02 ppm.

Glycoside **1** treated with dilute base solutions gave progenin **3**, which underwent partial hydrolysis. The resulting products included cyclogalegigenin and glycoside **4**, acid hydrolysis of which gave D-glucose and genin **2**. Therefore, D-glucose was directly bound to cyclogalegigenin.

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TABLE 1. ^{13}C NMR Spectrum of **1** and **2** (0 = TMS, δ, ppm, C_5D_5N)

C atom	**1**	**2**	C atom	**1**	**2**
1	32.70	32.50	24	85.00	85.00
2	29.60	31.50	25	70.10	70.10
3	88.10	78.20	26	27.50*	27.20*
4	42.70	42.40	27	28.10*	28.00*
5	53.80	53.30	28	20.30	20.17
6	68.40	68.20	29	29.18	29.40
7	37.80	38.00	30	16.00	16.20
8	46.30	47.00	$\underline{C}OCH_3$	170.02	
9	20.30	20.80	$CO\underline{C}H_3$	19.70	
10	29.60	29.90		-*D*-Glc*p*	
11	26.20	26.30	1′	104.60	
12	33.70	33.40	2′	79.00	
13	45.50	45.30	3′	78.50	
14	46.30	46.60	4′	72.00[a]	
15	47.40	47.00	5′	77.80	
16	73.30	73.10	6′	67.60	
17	58.80	58.10		-*L*-Rha*p*	
18	21.66	21.63	1″	101.00	
19	30.25	30.90	2″	72.00[a]	
20	86.90	86.70	3″	71.70	
21	28.80	28.40	4″	74.83	
22	34.70	34.80	5″	68.90	
23	26.17	26.10	6″	17.80	

Signals marked with the same letters overlap; with asterisks, are assigned arbitrarily. Signals were assigned according to the literature [8].

The production of a less polar compound from **1** by absolute acetone and anhydrous copper sulfate suggested that the OH groups on C-2′ and C-3′ were free in the rhamnosyl units [6].

Enzymatic hydrolysis of **3** produced genin **2** and a biose, which was identical to rutinose [7].

The ^{13}C NMR spectra of **1** and **2** (Table 1) indicated that only one carbinol C atom of the genin of **1** experienced a glycosylation effect and resonated at 88.1 ppm (C-3). This means that the carbohydrate substituent was a biose and was bonded to C-3 of the genin.

The similarity of the chemical shifts for C-20 and C-24 in the ^{13}C NMR spectra of **1** and **2** and the production of a genin with constants identical to cyclogalegigenin [1] indicated that the side chain of the glycoside had the 20*S*- and 24*R*-configuration.

Chemical shifts (CS) of C atoms in the carbohydrate units (Table 1) indicated that the CS of C-2 and C-6 of the D-glucose changed most. This was consistent with C-2 and C-6 of the D-glucose being substituted. The production of rutinose from **3** indicated that rhamnose was 1→6-bonded to D-glucose whereas the acyl substituent was located on C-2 of the D-glucose. This was confirmed by the CS of this C atom (Table 1).

The CS of the L-rhamnose and D-glucose C atoms were consistent with the pyranose form of their rings, the α-configuration of the terminal monosaccharide, and the β-configuration of the D-glucose [9-11].

Thus, the structure of **1** can be represented as 20*S*,24*R*-epoxycycloartan-3β,6α,16β,25-tetraol 3-*O*-[α-L-rhamnopyranosyl(1→6)]-β-D-(2′-*O*-acetyl)-glucopyranoside.

EXPERIMENTAL

General Comments. TLC used Silufol plates. Column chromatography was carried out over KSK and L silica gel (Czech Rep.) (40-100 μm). Compounds on TLC were developed by spraying with methanolic (25%) phosphotungstic acid with

subsequent heating at 100-110°C for 2-3 min. The solvent systems were $CHCl_3$:CH_3OH (10:1, 1), $CHCl_3$:CH_3OH:H_2O (70:23.5:2, 2), and C_5H_5N:C_6H_6:C_4H_9OH:H_2O (3:5:1:3, 3). PMR and ^{13}C NMR spectra were recorded on Bruker AM-400 and BS-567 (Tesla) spectrometers in C_5D_5N. IR spectra were recorded on a UR-20 instrument in KBr. GC was carried out in a Chrom-5 instrument (Czech Rep.) in a glass column (1.5 m × 4 mm) packed with Chromaton-super impregnated with 5% silicone XE-60, column temperature 210°C, vaporizer 230°C, flame-ionization detector at 250°C, He carrier gas, flow rate 50 mL/min. Rotation angles were determined on a SU-2 instrument; melting points, on a Kofler block.

Isolation of Isoprenoids. Ground leaves of *A. caucasicus* (1 kg) were extracted three times with ethanol (80%) with heating. The combined extracts were evaporated to an aqueous residue that was worked up three times with $CHCl_3$ (0.2 L each). The $CHCl_3$ was evaporated. The aqueous residue was worked up with hot water, filtered, condensed, and chromatographed over a silica-gel column with elution by $CHCl_3$ and system 1 to afford total isoprenoids, which were separated over a silica-gel column. Compounds were eluted by system 2. Compounds A, B, and C were isolated. The yield of A was 120 mg.

Cycloascauloside A (1), $C_{44}H_{72}O_{16}$, mp 210-215°C (CH_3OH); $[\alpha]_D^{20}$ -4° (*c* 0.1, EtOH), IR spectrum (KBr, ν_{max}, cm^{-1}): 3400-3000 (OH), 3045 (CH_2-cyclopropane ring).

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.23, 0.50 (2H-19, d, ^2J = 4.2), 0.89, 1.17, 1.23, 1.24, 1.40, 1.57, 1.78 (7 × CH_3, s), 1.70 (d, J = 6, L-rhamnose CH_3), 1.98 (s, Ac CH_3), 3.55 (dd, $^3J_1$ = 12, $^3J_2$ = 5, H-3), 3.69 (sq, ^3J = 3.6, 9.6, 9.6, H-6), 3.83 (t, ^3J = 7, H-24), 4.70 (q, $^3J_1 = {}^3J_2 = {}^3J_3$ = 8, H-16) [12], 4.90 (d, J = 7.6, D-glucose anomeric proton), 5.89 (d, J = 0.9, L-rhamnose anomeric proton).

Cyclogalegigenin (2). Compound **1** (50 mg) was hydrolyzed by methanolic H_2SO_4 (10 mL, 25%) at 60°C for 2 h. The reaction mixture was diluted with water. The methanol was evaporated. The resulting precipitate was filtered off and dried. The resulting genin was purified over a column of silica gel with elution by system 2 to afford genin (28 mg), mp 196-197°C (CH_3OH), that was identified also by direct TLC comparison with an authentic sample [1].

The carbohydrate part of the hydrolysate (after evaporation) contained D-glucose and L-rhamnose according to PC (system 3). The remaining hydrolysate was reduced with sodium borohydride and acetylated using Ac_2/Py. The resulting polyol acetates were identified by comparing GC traces with those of authentic samples and detected rhamnite and dulcite acetates in a 1:1 ratio [5].

Smith decomposition [3] of glycoside (10 mg) also gave cyclogalegigenin [1].

Progenin 3 from 1. Glycoside (40 mg) was saponified with methanolic base (5 mL, 0.5%) at room temperature for 3 h. After the usual work up of the reaction products, chromatography of the glycoside part over a silica-gel column with elution by system 2 afforded progenin **3** (32 mg), mp 219-221°C (CH_3OH), $[\alpha]_D^{23}$ -121.5 ± 0.5° (*c* 0.5, CH_3OH).

Enzymatic Hydrolysis of 3. Progenin **3** (15 mg) was dissolved in CH_3OH, treated with an aqueous solution of rhamnodiastase, and held at 37°C for 12 h. After work up of the reaction products, the genin part contained the aglycon cyclogalegigenin (**2**). Rutinose was found in the carbohydrate part using PC and system 3 [7].

Partial Hydrolysis of 3. Progenin **3** (60 mg) was partially hydrolyzed in methanolic H_2SO_4 (10 mL, 0.25%) at 50°C for 1 h. After the usual work up of the reaction products, chromatography over a silica-gel column with elution by system 1 afforded **2** (5 mg), mp 195-196°C (CH_3OH). Continued elution of the column by system 2 afforded glycoside **4** (18 mg), mp 238-239°C (system 1), $[\alpha]_D^{23}$ +44 ± 0.20° (*c* 0.6, CH_3OH:$CHCl_3$, 1:1) and unreacted starting glycoside (25 mg), mp 210-221°C (system 1), $[\alpha]_D^{23}$ -121.5° (*c* 0.26, CH_3OH:$CHCl_3$, 1:1).

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