Chemistry of Natural Compounds, Vol. 39, No. 4, 2003

STRUCTURE OF CYCLOGALEGINOSIDE E FROM *Astragalus galegiformis*

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UDC 547.918.547.926

The new cycloartane glycoside cyclogaleginoside E, 20S,24R-cycloartan-3β,6α,16β,25-tetraol-3-O-β-D-xylopyranoside-25-O-β-D-glucopyranoside, was isolated from stems of Astragalus galegiformis *L. Its structure was established using enzymatic and total acid hydrolysis, mass spectrometry, and PMR and ^{13}C NMR.*

Key words: *Astragalus galegiformis* L., cycloartanes, cyclogaleginoside E, two-dimensional NMR spectroscopy (^{1}H and ^{13}C, HSQC, COSY, HMBC).

In continuation of the study of cycloartanes from stems of *Astragalus galegiformis* (Leguminosae) [1], we isolated known cyclogaleginosides [2, 3] and a new cycloartane glycoside, cyclogaleginoside E (**1**). The chemical structure was studied using mass spectrometry, PMR and ^{13}C NMR including 2D homonuclear (^{1}H—^{1}H) COSY (Correlated Spectroscopy) and heteronuclear (^{13}C—^{1}H) HSQC (Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear Multiple-Bond Correlation).

Mass spectrometric data ($[M - 1]^+$, *m/z* 783.6) and preliminary results from PMR (two strong-field 1H doublets, seven methyl singlets) and ^{13}C NMR were consistent with a triterpenoid cycloartane compound. The PMR and ^{13}C NMR of compound **1** have signals for two anomeric protons at 4.26 and 4.43 ppm and two anomeric C atoms at 107.48 and 99.04 ppm. Table 1 contains data that confirm that **1** is a bioside containing two terminal ionic pyranose saccharides with axial anomeric protons. One of the units is D-xylose; the other, D-glucose. Enzymatic and total acid hydrolysis of glycoside **1** confirm these conclusions by showing the presence of D-xylose and D-glucose. The aglycon was identified as cyclogalegigenin (**2**) [2].

Table 1 lists the PMR and ^{13}C NMR of glycoside **1**. It can be seen that the chemical shifts (CS) of the carbinol C-3 and C-25 were affected by glycosylation and resonate at 89.91 and 78.93 ppm. Thus, the sugars are bonded to the genin at C-3 and C-25.

The locations of the carbohydrates were confirmed using HMBC spectra. The doublet for the anomeric proton of D-glucose at 4.43 ppm correlates with the singlet at 78.93 ppm. This unambiguously indicates that the D-glucose is located on C-25. The doublet for the anomeric proton of D-xylose at 4.26 ppm correlates with the doublet at 89.91 ppm. This indicates that it is bonded to C-3 because the CS of this line in other instances, in particular, upon glycosylation of C-6, undergoes a significantly smaller shift of 78-80 ppm.

The structure of cyclogaleginoside E was studied in more detail using the NMR methods noted above and literature data [3-11]. Table 1 contains assignments for the spectral lines of glycoside **1**; Table 2, progenin **3** and aglycon **2**. Literature data for the CS of C atoms in the cycloartane genin cyclosiversigenin are given in Table 2 for comparison [10]. We used CD_3OD and not the more frequently used Py-d_5 as the solvent for the NMR spectra of these compounds. Therefore, some comments are necessary regarding the procedure for making the assignments and the results.

The most characteristic lines in the PMR spectrum are two strong-field 1H doublets for the cyclopropane protons at 0.38 and 0.54 ppm with a geminal spin—spin coupling constant (SSCC) of 4.2 Hz. The doublet at comparatively weak field is distorted owing to weak through-space couplings.

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0009-3130/03/3904-0373$25.00

TABLE 1. PMR and ^{13}C NMR Data for Cyclogalaginoside E

Atom	^{13}C	^{1}H	HMBC (^{13}C-^{1}H)
1	33.30	1.53 td (10.6; 2.5); 1.20 m	2, 5, 19
2	30.58	1.92 m; 1.66 dq (12.5; 3.3)	1, 3, 5
3	89.91	3.176 dd (8.5; 1.4)	1, 2, 5, 29, 30, XI
4	43.15		2, 3, 5, 6, 29, 30
5	54.66	1.36 d (9.8)	6, 19, 29, 30
6	69.60	3.45 ddd (9/8; 5; 4)	5, 7, 8
7	38.85	1.42 m; 1.31 m	5, 8
8	48.50	1.82 dd (12.2; 4.3)	6, 7, 19, 28
9	21.91		7, 8, 11, 19
10	30.58		1, 5, 8, 19
11	27.17	2.03 ddd (10; 5; 5); 1.22 m	8, 19
12	34.26	1.83 m; 1.72 m	11, 17, 18
13	47.85		8, 15, 18
14	47.47		8, 15, 16, 17, 18, 28
15	49.23	1.96 dd (13.5; 7.8); 1.48 dd (13.5; 4.6)	28
16	74.42	4.64 ddd (7.8; 7.1; 4.6)	15, 17
17	56.31	2.22 d (7.1)	15, 18, 21, 22
18	21.40	1.41 s	17
19	32.18	0.54 d (4.1); 0.38 d (4.1)	5, 8, 11
20	88.93		17, 21, 22*, 23
21	24.84	1.28 s	18, 22
22	38.00	2.20 m; 1.74 m	17, 21
23	24.27	2.19 m; 1.98 m	22
24	86.38	3.90 dd - 8.8; 6.3	22**, 23**, 26, 27
25	78.93		22, 23, 24, 26, 27, G1
26	25.01[a]	1.24[a] s	27
27	23.51[a]	1.30[a] s	26
28	20.90	0.95 s	8, 15
29	28.73	1.29 s	3, 5, 30
30	16.55	1.02 s	3, 5, 29
		β-D-Xyl*p* (X)	
X1	107.48	4.26 d (7.5)	3, X2, X5
X2	75.50	3.185 dd (8.5; 7.5)	X5
X3	77.95	3.27 d (8.5)	X2, X4, X5
X4	71.29	3.44 td (8.5; 5.2)	X2, X5
X5	66.70	3.81 dd (11.4; 5.2); 3.16 dd (11.8; 8.5)	X1, X3, X4
		β-D-Glc*p* (G)	
G1	99.04	4.43 d (7.6)	25, G2, G3
G2	75.04	3.17 dd (8.8; 7.6)	G1
G3	77.63	3.35 d (8.8)	(G1, G4, G6)***
G4	71.80	3.285 d (8.8)	G3, G5, G6
G5	77.63	3.24 dd (8.8; 5.5)	(G1, G4, G6)***
G6	62.84	3.813 d (11.6); 3.63 dd (11.6; 5.5)	G2

Chemical shifts of C atoms experiencing a glycosylation effect are underlined; arbitrary assignment of PMR and ^{13}C NMR signals owing to the assignment of the strong-field PMR signal to 26-Me [5] (a); *coupled only to the H-22 weak-field multiplet; **coupled only to the H-22 and H-23 strong-field multiplets; ***arbitrary assignment.

The strong-field shift of the methyl singlets compared with those in the literature [4-9] for Py-d_5 solutions, which is especially evident for the axial methyls situated through two bonds from the hydroxyl, can be explained for the PMR spectra by a solvent effect, i.e., the lack of deshielding by the magnetically anisotropic pyridine ring [10]. However, this effect does not completely explain the ^{13}C NMR spectra because the contribution of magnetic anisotropy to the CS of C atoms, as a rule, is insignificant. Nevertheless, the 2D heteronuclear correlation spectra make unambiguous assignments possible, with the exception, of course, of the 26- and 27-methyls.

The PMR spectrum also contains a characteristic multiplet at 4.64 ppm for H-16 of the five-membered ring. A similar multiplet for cyclocephalogenin and related compounds was not analyzed [8]. It was described for askendosides as a quartet with SSCC 7.3 Hz [9]. For cycloastragenol, it is a quartet of doublets (qd) with SSCC 7.5 and 2 Hz. For cyclastragenol glycoside, asernestioside C, it is a triplet of doublets (td) with SSCC 8 and 6 Hz [5]. For cyclogaleginoside E, this multiplet to a first approximation is a doublet of doublets of doublets (ddd) with SSCC 7.8, 7.1, and 4.6 Hz.

The heteronuclear 2D correlation spectrum makes it easy to assign lines in the ^{13}C NMR spectrum at 74.42 ppm to C-16. The homonuclear 2D correlation spectrum identified resonances for protons involved in the coupling to H-16: a doublet (d) with SSCC 7.1 Hz at 2.22 ppm for H-17, a doublet of doublets (dd) with SSCC 13.5 and 7.8 Hz at 1.964 ppm, and a doublet of doublets (dd) with SSCC 13.5 and 4.6 Hz at 1.48 ppm, to protons H-15. This, in turn, enables the assignment in the ^{13}C NMR spectrum of lines at 49.23 (overlapped by CD_3OD lines) and 56.31 ppm to C-15 and C-17, resectively.

The signal for H-3 at 3.176 ppm, which correlates with the signal at 89.91 ppm in the ^{13}C NMR spectrum, is a doublet of doublets with SSCC 8.5 and 1.4 Hz. This is consistent with an axial H on C-3 with absolute configuration 3*R*.

The axial proton on C-2 has CS 1.66 ppm. The similarity of the geminal SSCC to the axial—axial SSCC with H-3 and H_a-1 leads to degeneration of the corresponding multiplet into a quartet of doublets with apparent SSCC 12.5 (effective average of ^2J and two $^3J_{aa}$) and 3.3 Hz ($^3J_{ae}$). The absorption of the equatorial proton on C-2 is partially overlapped by lines of H-15. The corresponding CS can be found only by using the 2D spectrum.

The C-1 protons resonate at 1.53 ppm as a broad triplet of doublets with SSCC 10.6 and 2.5 Hz and at 1.20 ppm as a multiplet overlapped by signals of H-11.

According to the correlation spectrum, the signal at 3.4-3.5 ppm is a superposition of multiplets of D-glucose H-4 and genin H-6. We used in the analysis the SSCC with H-5, which is 9.8 Hz and was obtained from the doublet splitting of H-5, which resonates at 1.36 ppm and couples most strongly with H-6.

Proton H-8 resonates at 1.82 ppm as a doublet of doublets with SSCC 12.2 and 4.3 Hz. This indicates that it is axial and has absolute configuration 8*S*.

The signals for H-7 in the PMR are overlapped by those of methyls. The correlation spectrum yields the CS. However, it does not make it possible to analyze the multiplets with negative geminal and positive vicinal SSCC.

Proton H-24, which is found at 3.90 ppm, resonates as a doublet of doublets with SSCC 8.3 and 6.1 Hz, which is typical of a first-order spectrum, owing to coupling with C-23 protons. The signal is broadened owing to coupling with C-22 protons (effective SSCC ^5J = 1 Hz). This was confirmed by correlation spectra.

The multiplet for Hα-11, which arises due to geminal and two vicinal couplings, is slightly overlapped by the weak-field doublet of doublets of H-15. However, the lines coalesce because of the similarity of the sum of the vicinal SSCC and the geminal SSCC. This significantly complicates analysis of the multiplet. The strong-field signal of Hβ-11 is overlapped by the H-1 multiplet and a methyl signal. Analogously, the multiplets of both H-12 protons are overlapped by signals of H-8 and H-22. The weak-field signals of H-22 and H-23 are superimposed on each other. The high-field signal of H-23 is overlapped by signals of H-15 and Hβ-2. Therefore, the CS of H-11, H-12, H-22, and H-23 can only be found using 2D spectra.

The resonance lines of all sugar protons were shifted to strong field compared with the results obtained for the analogous glycosides in Py-d_6 because the PMR spectra of the studied glycoside were recorded in CD_3OD. Nevertheless, the lines can be fully assigned if correlation spectra, especially heteronuclear, are used. Moreover, the CS of the sugar C atoms experience practically no solvent effect.

Doublets for the anomeric protons at 4.43 ppm (7.6 Hz) and 4.26 ppm (7.5 Hz) are related to lines at 99.04 and 107.48 ppm. This unambiguously assigns these signals to the anomeric C atoms of the glucose and xylose, respectively.

TABLE 2. ^{13}C NMR Spectra of Progenin **3**, Aglycon **2**, and Cyclosiversigenin*

C atom	Progenin **3**	Aglycon **2**	Cyclosiversigenin
1	32.80	32.80	32.81
2	29.80	31.46	31.47
3	88.70	78.30	78.32
4	42.45	42.46	42.46
5	54.00	54.00	54.00
6	68.10	68.40	68.38
7	38.81	38.85	38.85
8	47.30	47.30	47.30
9	20.90	20.99	20.99
10	29.90	29.90	29.92
11	26.31	26.31	26.32
12	33.45	33.45	33.47
13	45.10	45.10	45.09
14	46.21	46.21	46.21
15	46.81	46.81	46.81
16	72.90	72.90	73.48
17	58.48	58.48	58.44
18	21.68	21.63	21.66
19	31.02	31.02	31.02
20	86.70	86.70	87.27
21	28.60	28.60	28.59
22	34.97	34.97	34.97
23	26.19	26.18	26.47
24	85.00	85.00	81.75
25	70.30	70.30	71.27
26	27.17[a]	27.17	27.17
27	28.21[a]	28.21	28.21
28	20.28	20.25	20.27
29	29.40	29.40	29.44
30	16.05	16.05	16.14
	β-D-Xyl*p* (X)		
X1	107.5		
X2	75.5		
X3	78.3		
X4	71.2		
X5	66.9		

*Data for cyclosiversigenin were taken from the literature [10].

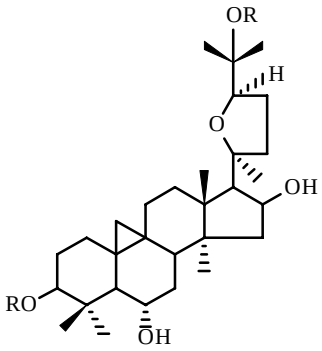
It should be noted that the solvent effect in this instance causes only a lack of deshielding by the magnetic anisotropy of the pyridine ring. However, the rotation of the side groups is hindered by the formation of H-bonds in CD_3OD. A typical example is the magnetic inequivalence of the glucose C-6 hydroxymethylene protons, which are seen in the PMR spectrum as two multiplets. These are a doublet at 3.81 ppm with a typical geminal SSCC of 11.6 Hz, which can be assigned to the *cis*-proton of glucose H-5, and a doublet of doublets at 3.63 ppm with SSCC 11.6 and 5.5 Hz. Despite the hindered rotation, the glucopyranose ring is not strained. The $^3J_{aa}$ SSCC throughout the whole ring is 8.8 Hz. Thus, the resonance lines appear as a simple doublet of doublets (H-2 and H-5) and triplets (H-3 and H-4), especially for H-3, which is not overlapped by other resonance lines.

Table 1 also gives data for the spin—spin coupling of C atoms that occurs through two and more chemical bonds and is obtained from HMBC spectra. Through-space coupling is an important property of the pure compound. In particular, in contrast with asernestioside C and cycloastragenol, for which C-19 couples only to H-5 and only for the aglycon [5], its coupling with H-5 and with H-8 and H-11 is clearly evident for cyclogaleginoside E. Furthermore, the H-19 protons of the cyclopropane ring of cyclogaleginoside E couple with C-9, C-10 (through two bonds), C-1, C-5, C-8, and C-11 (through three bonds), whereas for cyclocanthoside F coupling with C-5 and H-5 was not found [8].

The appearancce of coupling for C-21 and H_3-18, which occurs through five chemical bonds, is most interesting for C atoms of the five-membered rings of cyclogaleginoside E. Otherwise, the coupling is practically the same as those described for asernestioside and cycloastragenol [5], although for cyclogaleginoside E there is a distinction. The coupling occurs through three bonds for C-17—H-22 and C-20—H-23.

Acid hydrolysis of **1** forms cyclogalegigenin (**2**). Paper chromatography of the hydrolysate detected D-xylose and D-glucose according to comparison with authentic samples. Enzymatic hydrolysis of **1** by stomach juice of the snail *Helix plectotropis* forms progenin **3**, acid hydrolysis of which gives D-xylose and **2**. The structure of the progenin was established using PMR and ^{13}C NMR of the progenin (Table 2). The results show that D-glucose is bound to C-25 of the genin.

Based on the results, it can be concluded that the new cycloartane glycoside is cyclogaleginoside E and has the structure 20*S*,24*R*-cycloartan-3β,6α,16β,25-tetraol-3-O-β-D-xylopyranoside-25-O-β-D-glucopyranoside.



1: R = β-D-Xyl*p*, R_1 = β-D-Glc*p*;
2: R = R_1 = H;
3: R = β-D-Xyl*p*, R_1 = H

EXPERIMENTAL

Cycloartanes were purified and separated by column chromatography over deactivated alumina and silica gel L 100/160 (Czech Rep.) and TLC over Silufol UV-254 plates using solvent systems $CHCl_3$:CH_3OH:H_2O (70:23.5:2, **1**) and ethylacetate:methanol:water (100:16.5:13.5, **2**). Paper chromatography of sugars used C_5H_5N:C_6H_6:C_4H_9OH:H_2O (3:1:5:3, **3**). Melting points were determined on a Kofler block. NMR spectra were obtained on Bruker AM-400 and DRX-500 instruments in CD_3OD; on BS-567 (Tesla), in C_5D_5N. Mass spectra were recorded in a Ribermag R-10-10H instrument. IR spectra were obtained on a UR-20 spectrophotometer in KBr disks.

Isolation. Air-dried ground raw material (4.5 kg, *Astragalus galegiformis* L. stems collected during flowering) was extracted with ethanol (80°). The alcohol was evaporated. The aqueous liquid was extracted with $CHCl_3$. The solvent was distilled off. The solid was reprecipitated from hot water, filtered, and dried to a syrupy mass (127 g) that was purified over a column of Al_2O_3 (diameter 4, height 60 cm). The total methylsteroids were eluted by CH_3OH (60%). The eluates were condensed to dryness to afford a light brown powder (33 g). A part of the powder (15 g) was fractionated over a silica-gel column (diameter 3.0, height 122 cm) by elution with $CHCl_3$ and then $CHCl_3$:CH_3OH mixtures with increasing CH_3OH concentration. Fractions eluted by $CHCl_3$:CH_3OH (1:1) yielded the total polar compounds (8 g). A part of this (4.3 g) was placed on a silica-gel column (diameter 2.8, height 70.0 cm) and eluted by $CHCl_3$:CH_3OH (10:1). Fractions were collected as the components eluted. The first fractions contained previously known glycosides [1, 3]. Condensation of a 200-mL fraction

afforded a crystalline solid that was purified by rechromatography over a silica-gel column (diameter 1.2, height 30 cm) to give a finely crystalline white powder **1**.

Cyclogaleginoside E (1), mp 187-188°C (CH_3OH). IR spectrum (KBr, ν, cm^{-1}): 3460-3380 (OH), 3045 (CH_2). PMR and ^{13}C NMR spectra (Table 1). Mass spectrum, *m*/*z*, %: 829.4 $[M + 2Na]^{2+}$ (100), 783.6 $[M - 1]^+$ (10), 727.4 (1.5), 685.4 (4), 669.2 (0.2), 651.4 $[(M - 1)^+$ - D-xylose] (0.9), 621.4 $[(M - 1)^+$ - D-glucose] (1.0), 489.4 $[(M - 1)^+$ aglycon] (0.25), 471 $[M^+$ aglycon - $H_2O]$ (0.05).

Acid Hydrolysis. Compound **1** (32 mg) was hydrolyzed by the literature method [2] to afford cyclogalegigenin (15 mg) [1, 2]. Paper chromatography of the hydrolysate using system **3** detected D-xylose and D-glucose (1:1) by comparison with authentic samples.

Enzymatic Hydrolysis. Compound **1** (100 mg) was treated with an aqueous solution (30 mL) of *Helix plectotropis* enzymes (50 mg) and toluene (2 drops), held at 38°C for 32 h, diluted with water (15 mL), and extracted with butanol (5 × 10). The solvent was evaporated. The solid was chromatographed over a silica-gel column with elution by system **1** to afford **3** (65 mg), $C_{35}H_{58}O_9$, mp 253-254°C ($CHCl_3$:CH_3OH, 1:1), $[\alpha]_D^{23}$ +32 ± 2° (*c* 1.0, pyridine). ^{13}C NMR spectrum (Table 2).

Acid Hydrolysis of the Monoside 3. Compound **3** (20 mg) was hydrolyzed as before [2] to afford cyclogalegigenin (12 mg) [1, 2]. Paper chromatography of the hydrolysate detected D-xylose by comparison with standards.

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