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TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS. XLVII. STRUCTURES OF CYCLOALPIGENIN A AND CYCLOALPIOSIDE A

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The structures of two new cycloartane triterpenoids, cycloalpigenin A and cycloalpioside A, isolated from Astragalus alopecurus *Pall. (Leguminosae) have been established on the basis of chemical transformations together with ^1H, ^{13}C, and 2D $^1H-^1H$ and $^1H-^{13}C$ chemical shift correlation NMR spectroscopy and IR, mass, and CD spectra. Cycloalpigenin A is 3β,16β,25-trihydroxy-20R,24S-epoxycycloartan-12-one, and cycloalpioside A is cycloalpigenin A 3-O-β-D-xylopyranoside.*

We have previously [1] reported on the structures of cycloalpigenin D and cycloalpioside D, isolated from *Astragalus alopecurus* Pall. (Leguminosae). In the present paper we consider the structures of another two components of this plant – substances (**1**) and (**5**) in [1] – having the natures of a genin and a glycoside, which we have now called cycloalpigenin A (**1**) and cycloalpioside A (**3**), respectively.

The elemental composition of genin (**1**), $C_{30}H_{48}O_5$, the presence in the PMR spectrum at 0.36 and 0.70 ppm of two one-proton doublets of an AB system with the SSCC ^2J = 4 Hz (Table 1), characterizing an isolated cyclopropane methylene, and also the signal of seven methyl groups in the high field served as arguments for assigning the new compound to the cycloartane triterpenoids [2, 3]. The signals of quaternary carbon atoms at 19.99 and 28.14 ppm and of a methylene carbon atom at 31.00 ppm in the ^{13}C NMR spectrum of cycloalpigenin A (Table 2) are also characteristic for a 9,19 three-membered ring. In agreement with this, the IR spectrum of genin (**1**) showed an absorption band that was assigned to the stretching vibrations of a CH_2 group of a cyclopropane ring [4].

In the mass spectrum of cycloalpigenin A the peak of an ion with *m/z* 143 was observed. This fact permitted the conclusion that the side-chain of genin (**1**) contained a 20,24-epoxy-25-hydroxy function, as in cyclosieversigenin [3, 5], cyclogalegigenin [3, 6] and cycloalpigenin D [1]. This was also shown by a one-proton triplet at 3.89 ppm (^3J = 8 Hz) in the PMR spectrum of genin (**1**), belonging to H-24, and by the chemical shifts of the C-20–C-27 atoms of the side-chain in the ^{13}C NMR spectrum (Tables 1 and 2). The ^1H and ^{13}C NMR spectra were interpreted with the aid of the indices of the 2D $^1H-^1H$, $^1H-^{13}C$ chemical shift correlation NMR and J-modulation ^{13}C NMR spectra.

The IR spectrum of the new genin also contained the absorption bands of hydroxy groups (3550-3300 cm^{-1}) and of a six-membered cyclic ketone (1705 cm^{-1}). The presence of a ketonic carbonyl group was shown additionally by the signal of the corresponding carbon atom in the ^{13}C NMR spectrum at 211.15 ppm.

Thus, it followed from the elemental composition $C_{30}H_{48}O_5$ in the light of the results presented that two of the five oxygen atoms were present in a side-chain and the other three were included in the polycyclic moiety of the molecule and formed one carbonyl and two hydroxy groups.

In the PMR spectrum of the genin, protons geminal to to the hydroxy groups resonated at 3.50 ppm (dd, $^3J_1$ = 12 Hz, $^3J_2$ = 4 Hz) and 4.87 ppm (q, 1:3:3:1, $^3J_1 = {}^3J_2 = {}^3J_3$ = 8 Hz), showing the secondary nature of these groups. The chemical shifts, multiplicities, and SSCCs of these protons permitted their identification as 3α-H and 16α-H, respectively [3]. In the 2D NOE NMR (NOESY) spectrum, cross-peaks of the paired protons H_2-11, H_2-15, and H_2-19 and of the H-16 and

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TABLE 1. Chemical Shifts (δ, ppm), Multiplicities, and SSCCs (J, Hz) of the Protons of Cycloalpigenin A (**1**), Quisquagenin (**2**), and Cycloalpioside A (**3**) (C_5D_5N, 0 – TMS)

Positions of the protons	Compound		
	1	2	3
1α-H	1.49 td (12; 4)		
1β-H	1.00		
2-H2	1.83; 1.92		
3α-H	3.50 dd (12; 4)	3.52 m	3.46 dd (12; 4.5)
5α-H	1.22		
6α-H	1.54		
6β-H	0.77 q[a] (12; 12; 12)		
7α-H	0.93 q[a] (12; 12; 12)		
7β-H	1.30		
8β-H	1.73 dd (12; 4)		
11-H2*	2.05; 2.75 d (20)		2.04; 2.74 d (20)
15α-H	1.86		
15β-H	2.10 dd (12; 8)		
16α-H	4.87 q (8; 8; 8)	5.02 qd (8; 8; 8; 2.5)	4.83 m
17α-H	3.19 d (8)	2.52 d (8)	3.18 d (8.5)
18-CH3	1.58 s		1.57 s
19-H2	0.36; 0.70 d (4)	0.31; 0.58 d (4)	0.33; 0.66 d (4)
21-CH3	1.61 s		1.60 s
22-H2	2.95 td (12; 6); 1.71	3.11 q (10; 10; 10)	2.94 m
23-H2	2.33 m; 1.97	2.37 m	
24-H	3.89 t (8)	3.89 dd (8; 6)	
26-CH3	1.29 s*	[0.93; 1.08;	1.29 s*
27-CH3	1.57 s*	1.21; 1.29;	1.57 s*
28-CH3	0.71 s	1.33; 1.45;	0.70 s
29-CH3	1.20 s	1.58 s]	1.32 s
30-CH3	1.06 s		1.04 s

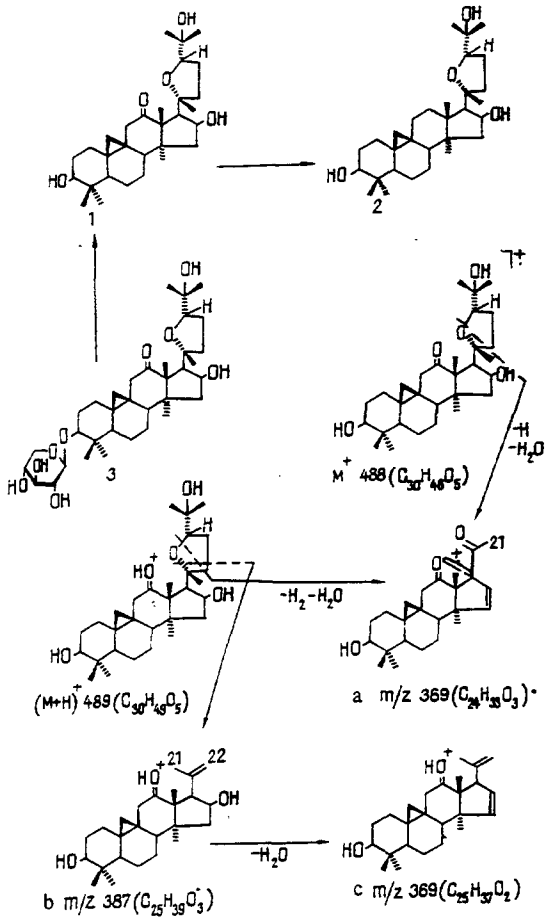
*Signals asigned ambiguously.

[a]1:3:3:1 quartet with broadened lines. The chemical shifts given without multiplicities and SSCCs were determined from 2D $^1H-^1H$, $^1H-^{13}C$ chemical shift correlation NMR spectra. The anomeric proton of the β-D-xylopyranose residue resonated at 4.82 ppm in the form of a doublet with ^3J = 7 Hz.

H-17 protons were detected, showing the spatial propinquity of these protons and confirming the α-orientation of H-16. Consequently, the cycloalpigenin A molecule contained 3β- and 16β-hydroxy groups. This conclusion was in full agreement with the 2D $^1H-^{13}C$ chemical shift correlation (COSY) spectrum, in which the H-3 and H-16 signals correlated with the signals of secondary carbinol carbon atoms resonating at 77.59 and 73.01 ppm, respectively.

The PMR spectrum of compound (**1**) contained another pair of one-proton doublets of an AB system, at 2.05 and 2.75 ppm, with the SSCC ^2J = 20 Hz, assigned to a methylene isolated by a keto function. In the light of the multiplicity of the H-3 signal, this fact showed that the keto function was present in ring C. Since the ketone was not conjugated with the cyclopropanre ring, this could show unambiguously that the keto function was located at C-12. In actual fact a negative Cotton effect was observed in the circular dichroism curve at 286 nm ($\Delta E = -2.64$), which confirmed the conclusion about the position of the keto function [3, 7].

The closeness of the chemical shifts of the C-20–C-25 atoms of cycloalpigenin A (**1**) to those of quisquagenin (**2**) and cyclosieversigenin (**4**) in comparison with the analogous indices of cyclogalegigenin (**5**) (Table 2), and also biogenetic considerations based on the fact that we have isolated cycloalpigenin D, with 20R,24S stereochemistry, from the plant under investigation [1], permitted us to assign the 20R,24R-configuration to the chiral center of the side-chain of cycloalpigenin A. As was to be expected, in the PMR spectrum of the genin (**1**) the signal of one of the protons at C-22 was shifted downfield, as in other genins having the 20R,24S stereochemistry, and was observed at 2.95 ppm [3]. It must be mentioned that the chemical shifts of the other atoms of the tetrahydrofuran ring also agreed well with those of cyclosieversigenin [8] and of



quisquagenin [9]. The deviation of the chemical shift of C-22 observed for cycloalpigenin A (+1.02; +1.21) is obviously a consequence of the influence of the carbonyl group at C-12. This influence is not so considerable on the chemical shifts of the other carbon atoms of the side-chain (Table 2).

In confirmation of these considerations, we effected a transition from cycloalpigenin A (**1**) to quisquagenin (**2**). For this, ketone (**1**) was reduced and modified by the Wolff–Kizhner [–Kishner] method [10]. From the reaction products we isolated genin (**2**), identical from its physicochemical constants and its ^1H and ^{13}C NMR and mass spectra with the quisquagenin obtained from *Astragalus quisqualis* Bunge [9].

Thus, cycloalpigenin A has the structure of 3β,16β,25-trihydroxy-20R,24S-epoxycycloartan-12-one.

The keto function at C-12 exerts a considerable influence on some parameters of the NMR spectrum and the electron-impact mass spectrum of cycloalpigenin A.

The H-17 signal in the PMR spectrum of genin (**1**) experiences a considerable downfield shift (+0.67 ppm) in comparison with that of quisquagenin and cyclosieversigenin and is observed at 3.19 ppm. In all probability this takes place as a consequence of a γ-gauche interaction of the protons concerned with the keto function at C-12.

The keto function at C-12 also introduces substantial modifications into the course of the mass spectrometric fragmentation of cycloalpigenin A under electron impact. The mass spectra of triterpenoids of the cycloartane [6, 11, 15], dammarane [12, 13], and cucurbitane [14] series, each containing a side chain with a 20,24-epoxy-25-ol function, are characterized by the maximum peak of an ion with *m/z* 143. This ion arises on the cleavage of the C-17–C-20 bond and corresponds to a $C_8H_{15}O_2$ side-chain. In the spectrum of cycloalpigenin A the peak of the ion under consideration has an intensity of 28.9%. The maximum peak in the spectrum of this compound, with *m/z* 369, is due to two ions: *c* ($C_{25}H_{37}O_2$) and *a* ($C_{24}H_{33}O_3$) in a ratio of 4:1. The pathways for the appearance of these ions are shown in the scheme. The ions *a*, *b*, and *c* are specific for the fragmentation of cycloalpigenin A under electron impact and are due to the keto function at C-12. The same function also explains the protonated molecular ion with *m/z* 489, the subsequent fragmentation of which leads to the ions *b* and *c*. Ion *a* may arise both from the molecular ion and from the protonated molecular ion.

TABLE 2. Chemical Shifts of the Carbon Atoms of Compounds (**1**)–(**5**) (δ, ppm, C_5D_5N, 0 – TMS). Difference between the Chemical Shifts of the C20–C25 Carbon Atoms of Cycloalpigenin A (**1**) and the Corresponding Atoms of Genins (**2**) (quisquagenin), (**4**) (cyclosieversigenin) and (**5**) (cyclogalegigenin)

C Atom	Compound							
	1	2	Δδ(1—2)	3	4	Δδ(1—4)	5	Δδ(1—5)
1	32.84	32.42		32.61	32.72		32.78	
2	31.00[a]	31.33		30.49	31.30		31.43	
3	77.59	77.99		88.03	78.21		78.30	
4	40.95	41.13		41.20	42.28		42.43	
5	47.05	47.56		47.49	53.86		53.99	
6	20.71	20.33[a]		20.75	68.27		68.35	
7	26.54	26.60		25.70	38.69		38.73	
8	47.62	48.20		47.60	47.21		47.20	
9	19.99	21.33		20.02	20.84		21.01	
10	28.14	29.98		26.43[a]	29.80		29.79	
11	45.91	26.47[b]		45.79	26.29[a]		26.48	
12	211.25	33.53		211.22	33.31		33.76	
13	60.95	45.13		60.93	44.89		46.50	
14	47.54	47.08[c]		47.18	46.09		46.80	
15	46.02	47.08[c]		45.96	46.69		49.02	
16	73.01	73.48		73.01	73.35		72.84	
17	49.85	58.55		49.82	58.26		56.49	
18	14.98	21.92		14.96	21.51		21.23	
19	31.00[a]	30.61		30.86	31.00		30.88	
20	87.22	87.28	—0.06	87.21	87.17	+0.05	86.62	+0.60
21	28.74	28.57	+0.20	25.70	28.46	+0.28	26.32	+2.42
22	36.02	35.00	+1.02	36.00	34.81	+1.21	37.53	—1.51
23	25.92	26.47[b]	—0.55	26.43[a]	26.29[a]	—0.23	24.30	+1.62
24	82.32	81.79	+0.53	82.30	81.57	+0.57	84.90	—2.58
25	70.67	71.24	—0.57	70.68	71.19	—0.52	70.28	+0.39
26	27.26*	27.18*		27.26[b*]	27.04*		26.87*	
27	28.22*	28.22*		27.84*	28.09*		28.10*	
28	20.78	20.33[a]		20.45	20.17		20.49	
29	26.12	26.20		27.26[b]	29.28		29.41	
30	14.79	14.60		15.38	16.16		16.11	
				β-D-Xylp residue				
1				107.50				
2				75.55				
3				78.58				
4				21.23				
5				67.10				

Note. The signals marked with the same letters are superposed on one another, and those marked with an asterisk have been assignd ambiguously.

We have previously observed a fall in the intensity of the peak at *m/z* 143 in the mass spectra of certain 16-keto derivatives [6, 11].

The PMR spectrum of the glycoside (**3**), containing one-proton doublets at 0.33 and 0.66 ppm showed that the new glycoside also belonged to the cycloartane series.

The acid hydrolysis of this glycoside gave a genin identified as cycloalpigenin A (**1**). D-Xylose was detected in the carbohydrate part of the hydrolysate by paper chromatography. GLC [15] showed that cycloalpioside A contained one D-xylose residue.

The ^1H and ^{13}C NMR spectra of glycoside (**3**) (Tables 1 and 2) contained signals from one D-xylose residue, which confirmed the monoside nature of of cycloalpioside A. The anomeric proton of the D-xylose residue resonated at 4.82 ppm in the form of a doublet with the SSCC ^3J = 7 Hz. This fact showed that the pentose had the pyranose form, the $^4C_1$ conformation, and the β-configuration [16]. A similar conclusion followed from the chemical shifts of the carbon atoms of the monosaccharide residue.

A comparative study of the ^{13}C NMR spectra of cycloalpigenin A and cycloalpioside A showed a considerable downfield shift of the C-3 signal in the spectrum of the glycoside, which was observed at 88.03 ppm, and this unambiguously determined the position of the β-D-xylopyranose at the same carbon atom.

Thus, cycloalpioside A has the structure of 3β,16β,25-trihydroxy-20R,24S-epoxycycloartan-12-one 3-O-β-D-xylopyranoside.

EXPERIMENTAL

For general observations, see [15, 17]. The following solvent systems were used: 1) chloroform–methanol (20:1); and 2) *n*-butyl alcohol–pyridine–water (6:4:3).

^{1}C, ^{13}C, and 2D $^{1}H-^{1}H$, $^{1}H-^{13}C$ chemical shift correlation NMR spectra were taken on a Bruker AM-400 instrument. ^{13}C NMR spectra were recorded with complete decoupling of C–H interactions and J-modulation. All the spectra were recorded by the standard Bruker programs.

The mass spectra and elemental compositions of the ions were meaured a MKh-1310 instrument at an ionizing potential of 50 V and temperatures of 130-170°C.

The CD curve was obtained on a Jasco spectropolarimeter.

For the isolation and separation of the triterpenoids of *Astragalus alopecurus* Pall., see [1].

Cycloalpigenin A (1) – substance (1) in [1] –, $C_{30}H_{48}O_5$, mp 223-226° (from methanol), $[\alpha]_D^{24}-43.2 \pm 2°$ (sec 0.37; methanol). IR spectrum (ν, KBr, cm^{-1}): 3550-3300 (OH), 3040 (CH_2 of a cyclopropane ring), 1705 (C = O at C-12). CD (s 0.1; ethanol) ΔE = −2.64 (286 nm). Mass spectrum (*m/z*, %): $(M + H)^+$ 489 (4.7), M^+ 488 (3.5), 473 (15.8), 470 (2.6), 455 (5.3), 445 (2.6), 437 (7.0) 430 (42.1), 419 (3.5), 415 (5.3), 412 (52.6), 397 (9.6), 393 (42.1), 387 (89.4), 379 (6.1), 375 (14.4), 369 (100), 351 (57.9), 343 (7.9), 333 (7.9), 325 (15.8), 311 (8.8), 288 (39.5), 271 (14.0), 269 (14.9), 261 (10.5), 187 (21.1), 143 (28.9). For the ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2.

Cycloalpioside A (3) – substance (5) [1], $C_{35}H_{56}O_9$, mp 287-288°C (from methanol), $[\alpha]_D^{27}$ −15.1 ± 2°C (*c* 0.53; pyridine). IR spectrum (ν, KBr, cm^{-1}): 3520-3200 (OH), 3040 (CH_2 of a cyclopropane ring), 1720 (C=O at C-12). For the ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2.

Quisquagenin (2) from Cycloapigenin A (1). Cycloalpigenin A (70 mg) in 0.5 ml of *n*-butyl alcohol was treated with hydrazine hydrate (0.1 ml), and the mixture was boiled for 5 days, further 0.1-ml portions of hydrazine hydrate being added from time to time. The course of the reaction was monitored by TLC. After evaporation of the solvent, the residue was dried. The reaction products were dissolved in 1 ml of absolute dimethyl sulfoxide, 130 mg of potassium tertiary butanolate was added, and the mixture was left at room temperature for 4 h. After this, it was diluted with water and treated with chloroform. The chloroform extract was washed with water and evaporated. The residue was chromatographed on a column, with elution by chloroform. This led to the isolation of 6 mg of genin (2), $C_{30}H_{50}0_4$ mp 230-232°C (from methanol), $[\alpha]_D^{24}$ +32 ± 2°C (*c* 0.5; chloroform), additionally identified as quisquagenin [9] from its mass and ^{1}H and ^{13}C NMR spectra. The ^{1}H and ^{13}C NMR spectra are given in Tables 1 and 2.

Cycloalpigenin A (1) from (3). Glycoside (3) (50 mg) in 50 ml of methanol was boiled until all the solid had dissolved. The solution was then treated with 0.12 ml of concentrated sulfuric acid, and heating was continued until the hydrolysis of the glycoside was complete. After this, the reaction mixture was evaporated to small volume and diluted with water, and the methanol was evaporated off. The precipitate that deposited was filtered off, washed with water, and dried. It was then chromatographed on a column, with elution by system 1. This gave 20 mg of the genin, identified as cycloalpigenin A (1) by the usual means.

The aqueous filtrate was concentrated to small volume and was boiled for 2 h. After cooling, the solution was neutralized with the anion-exchange resin ARA-8p and it was chromatographed on FN-11 paper in system 2 in the presence of markers; D-xylose was detected.

It was established by the GLC method that cycloalpioside A contained one D-xylose residue.

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