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TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS. XLVIII. THE STRUCTURES OF CYCLOALPIGENIN B AND CYCLOALPIOSIDE B

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The structures of the new cycloartane methylsteroid cycloalpigenin B and its glycoside cycloalpioside B, isolated from Astragalus alopecurus *Pall. (Leguminosae) have been determined on the basis of chemical transformations with the assistance of ^1H and ^{13}C NMR spectroscopy and 2D NMR $^1H—^1H$ and $^1H—^{13}C$ correlations of chemical signals and IR, CD, and electron-impact mass spectrometry. Cycloalpigenin B is 20R,24S-epoxycycloartane-3β,12α,16β,25-tetraol. A transition from cycloalpigenin B to cycloalpigenin A has been achieved. Cycloalpioside B is 20R,24S-epoxycycloartane-3β,12α,16β,25-tetraol 3-O-β-D-xylopyranoside.*

Continuing investigations of the triterpenoids of *Astragalus alopecurus* Pall. (Leguminosae), we have established the structures of the new substances *2* and *6* [1], which we have called cycloalpigenin B (1) and cycloalpioside B (6), respectively.

The molecular formula of cycloalpigenin B, $C_{30}H_{50}O_5$, the presence in the strong-field region of the PMR spectrum of two one-proton doublets of an AB system at 0.35 and 0.47 ppm (Table 1), assigned to an isolated cyclopropane methylene, and the signals of seven methyl groups permitted us to assign the compound studied (1) to the cycloartane series [2, 3]. This was also shown by an absorption band at 3040 cm^{-1} in the IR spectrum of cycloalpigenin B, which is characteristic for the stretching vibrations of a methylene in a three-membered ring [4].

The mass spectrum of genin (1) showed the maximum peak of an ion with *m/z* 143, arising on the cleavge of the C-17 – C-20 bond and corresponding to the $C_8H_{15}O_2$ side-chain. As was to be expected, in the PMR spectrum of the substance under consideration a triplet signal of H-24 was easily traced at 3.97 ppm. These facts, in combination with a consideration of the ^{13}C NMR spectrum (Table 2), the assignmnt of the signals in which was made on the basis of J-modulation indices and 2D NMR $^1H—^1H$ and $^1H—^{13}C$ chemical shift correlation spectra, indicated a closeness of the structures of the side-chains of cycloalpigenins A (3) and B (1) (see Scheme in the following page).

Consequently, one of the oxygen atoms is present in a tertiary hydroxy group at C-25, and the other forms an epoxide ring in the side-chain, while the three remaining ones are represented by secondary hydroxy groups located in the polycyclic part of the molecule. In actual fact, the PMR spectrum of genin (1) showed at 3.45, 4.11, and 4.89 ppm the signals of protons geminal to hydroxy groups, which correlate in the two-dimensional spectrum with the signals of secondary carbinol carbon atoms (77.93, 72.76, and 72.83 ppm).

The acetylation of cycloalpigenin B with acetic anhydride in pyridine gave the diacetate (2). In the PMR spectrum of the diacetate (2) the signal of one of the protons geminal to hydroxy groups had scarcely changed its position, while the other two had undergone downfield shifts. The magnitudes of the chemical shifts amd their multiplicities and SSCCs were appropriate for 3α-H and 16α-H [3]. This means that cycloalpigenin B contains 3β,16β-hydroxy groups, and derivative (2) is the 3,16-diacetate. The same facts indicate the absence of an α-glycol system in the molecule.

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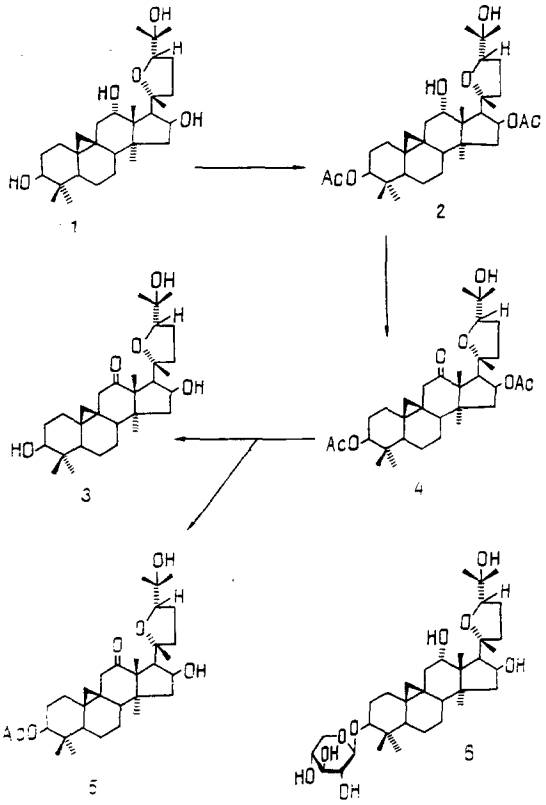
TABLE 1. Chemical Shifts (δ, ppm), Multiplicities, and SSCC (J, Hz) of the protons of Cycloalpigenins A (3) and B (1) and Their Derivatives (0 – TMS)

Compound	Positions of the protons							
	H-3	H-12/2H-11	H-16	H-17	2H-19	H-24	CH_3 groups	OAc
1	3.45 dd (10.5; 5.5) [3.26 dd (11; 4.5)]	4.11 dd (10; 6) [3.86 dd (9; 6)]	4.89 a (7; 7; 7) [4.56 td (8; 6)]	3.15 d (7) [2.63 d (8)]	0.35; 0.47 d (4) [0.41; 0.52 d (4.4)]	3.97 t (7) [3.82 t (7)]	1.02; 1.14; 1.23; 1.32; 1.47; 1.50; 1.74 [0.79; 0.95; 0.99; 1.14; 1.24; 1.26; 1.44]	— —
2	4.71 dd (11; 5)	4.16 m	5.61 td (8; 6)	3.06 d (8)	0.34; 0.46 d (4)	4.01 t (7)	0.88; 0.94; 1.16; 1.34; 1.40; 1.43; 1.64	2.03; 2.11
3	3.50 dd (11; 4)	2.06; 2.74 d (20)	4.87 a (8; 8; 8)	3.19 d (8)	0.36; 0.70 d (4)	3.89 t (8)	0.71; 1.06; 1.20; 1.29; 1.57; 1.58; 1.61	—
4	4.72 dd (11; 4.5)	1.91; 2.64 d (20)	5.61 a (8; 8; 8)	3.18 d (8)	0.35; 0.66 d (4)	3.83 dd (9; 6)	0.64; 0.89; 0.93; 1.28; 1.42; 1.58; 1.64	2.06; 2.16
5	4.72 dd (11.5; 4.5)	2.02; 2.69 d (20.1)	4.86 m	3.17 d (8.7)	0.34; 0.65 d (4.3)	3.89 t (8)	0.70; 0.88; 0.93; 1.29; 1.56; 1.56; 1.60	2.05

*The spectra were taken in deuteropyridine or deuterochloroform. The indices given in square brackets were obtained with the use of deuterochloroform. SSCC is given in round brackets. The signals of the methyl groups had a singlet nature.



The diacetate (2) was subjected to Jones oxidation [6] and gave a monoketo derivative (4). The IR spectrum of the ketone (4) contained an absorption band at 1710 cm^{-1}, which is characteristic for a six-membered cyclic ketone. Consequently, the keto function in compound (4) and the corrresponding hydroxy group in the genin (1) could be present in ring B or C at C-6, C-7, or C-12. Positions 1 and 11 were ruled out by the absence of conjugation of the keto function with the cyclopropane ring. The multiplicity of the signal of the proton geminal to the remining unidentified hydroxy group in he PMR spectrum of genin (1) (doublet of doublets at 4.11 ppm) permitted us to exclude position 6 and 7 from further consideration, as well.

In actual fact, resonance signals at 1.91 and 2.64 ppm in the PMR spectrum of the ketone (4) were one-proton doublets interconnected in the manner of an AB system, which is characteristic for a methylene isolated by a keto function. This unambiguously determined the position of the keto function at C-12. As confirmation of this we may give the negative Cotton effect on the CD curve of compound (4) [$\Delta\varepsilon = -2.64$ (286 nm)] due to the keto function [7].

Thus, the doublet of doublets at 4.11 ppm in the PMR spectrum of genin (1) related to the proton geminal to the hydroxy group at C-12, and the SSCCs of 10 and 6 Hz determined the β-axial orientation of this proton and, consequently, the α-orientation of the hydroxy group in the same position [8]. This was also shown by the fact that the H-17 signal had undergome a downfield shift in comparison with that of cyclosieversigenin and was observed at 3.15 ppm, which is posible because of γ-*gauche* interaction of this proton with the hydroxy group at C-12, having the analogous orientation [5]. Such interaction was also realized between H-17 and the keto function at C-12. In the PMR spectrum of cycloalpigenin A the H-17 signal was traced at 3.19 ppm.

The chemical shifts of the C-20-C-24 carbon atoms in the ^{13}C NMR spectrum of cycloalpigenin B differed appreciably from those for the 20R,24S-epoxycycloartane-16β,25-diols. At the same time, cycloalpigenins A (3) [5] and D [1], isolated from the same plant, have the 20R,24S- stereochemistry. Starting from biogenetic considerations and assuming that cycloalpigenin B has the same stereochemistry of the chiral centers of the side-chain, the observed differences in the chemical shifts of the carbon atoms under consideration must be ascribed to the influence of the hydroxy group at C-12. Cycloalpigenin B contains 3β,12α,16β-hydroxy groups in the steroid part of the molecule, and the difference between cycloalpigenins A (3) and B (1) consists only in the nature of the oxygen function at C-12. Starting from this, we made an attempt to achieve a transition from cycloalpigenin B to cycloalpigenin A. For this, the ketonic diacetate (4) was subjected to alkaline hydrolysis. Two compounds, (3) and (4), were isolated from the hydrolysis products. From its spectral characteristics and physicochemical constants, product (3) was identical with cycloalpigenin A. The production of cycloalpigenin A from cycloalpigenin B confirmed the structure (1) that had been established.

TABLE 2. Chemical Shifts of the Carbon Atoms of Cycloalpigenins A (3) and B (1) and of Derivatives of the Latter (δ, ppm, C_5D_5N, 0 – TMS)

C atom	Compound				
	1	2	3	4	6
1	32.60	31.79	32.84	32.04	32.36
2	31.28	26.53	31.00[a]	26.86	30.12[a]
3	77.93	80.33	77.59	79.93	88.47
4	41.10	40.22	40.95	40.01	41.35
5	47.74	47.58	47.05	46.65	47.65
6	21.58	21.10	20.71	20.20	21.30
7	25.98	26.13	26.54	26.14	26.03
8	48.96	48.52	47.62	47.13	48.90
9	19.93	19.99	19.99	20.09	19.94
10	26.95	27.15	28.14	27.76	26.81
11	38.80	37.57	45.91	45.89	38.76
12	72.76	72.47	211.25	210.81	72.77[b]
13	49.90	51.85	60.95	61.98	49.85
14	50.84	46.69	47.54	47.45	50.76
15	46.49	43.10	46.02	43.75	46.44
16	72.83	75.61	73.01	73.94	72.77[b]
17	52.29	52.44	49.85	49.66	52.20
18	21.95	21.68	14.98	15.07	21.97
19	30.35	30.00	31.00[a]	30.55	30.12[a]
20	87.51	85.99	87.22	84.82	87.53
21	26.18	27.34	28.74	28.61	25.75
22	38.40	39.65	36.02	39.51	38.38
23	26.92	25.84	25.92	26.11	26.29
24	83.54	84.94	82.32	83.94	83.48
25	70.76	70.46	70.67	70.20	70.78
26	26.59*	25.56*	27.26*	25.47*	27.03*
27	27.45*	27.02*	28.22*	28.26*	27.47[c]*
28	21.20	20.42	20.78	20.71	21.19
29	27.40	26.13	26.12	26.22	27.47[c]
30	14.89	15.46	14.79	15.32	15.50
		Acetate residues		*β-D*-Xyl*p* residue	
		21.12		21.08	107.55
		21.37		21.56	75.54
		170.03		170.18	78.57
		170.50		170.46	71.22
					67.09

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

From its spectral parameters, the other saponification product (5) contained one acetyl group, at C-3.

The experimental results presented enabled us to conclude that cycloalpigenin B had the structure of 20R,24S-epoxycycloartane-3β,12α,16β,25-tetraol.

In the PMR spectrum of the new glycoside (6), at 0.34 and 0.47 ppm we traced the signals of two protons characteristic for a cyclopropane methylene. On this basis, the glycoside under consideration was assigned to the cycloartane series [2, 3]. In actual fact, the acid hydrolysis of the glycoside gave a genin identified as cycloalpigenin B (1).

It was shown by the GLC method [9] that cycloalpioside B contained one xylose residue. The ^1H and ^{13}C NMR spectra of this glycoside, containing, in addition to the signals of of the genin moiety, the signals of one monosaccharide residue, also showed that the glycoside under study was a monoxyloside. The anomeric proton of the *D*-xylose residue resonated at 4.83 ppm in the form of a doublet with the SSCC J = 7.3 Hz, showing the pyranose form, $^4C_1$ conformation, and β-configuration of the monosaccharose residue [10]. The chemical shifts of the carbon atoms of the *D*-xylose residue confirmed the latter conclusion.

The signal of the C-3 atom in the ^{13}C NMR spectrum of glycoside (6) was subjected to a considerable (+10.54 ppm) downfield shift in comparison with that for cycloalpigenin B and was observed at 88.47 ppm. This fact unambiguously determined the position of attachment of the β-*D*-xylopyranose residue at C-3.

Thus, cycloalpioside B is 20R,24S-epoxycycloartane-3β,12α,16β,25-tetraol 3-O-β-*D*-xylopyranoside.

EXPERIMENTAL

For general observations, see [9, 11]. The following solvent systems were used: 1) benzene–ethyl acetate (2:1); 2) benzene–ethyl acetate (3:1); 3) chloroform–methanol (15:1).

^1H and ^{13}C NMR spectra were taken in deuteropyridine or deuterochloroform on Bruker AM 400 ands AC 299 instruments (δ, ppm, 0 – TMS). For interpretation we used the ^{13}C NMR spectra obtained under J-modulation conditions and also 2D ^1H—^1H and ^1H—^{13}C chemical shift correlation spectra taken on the same instruments.

CD spectra were obtained on a Jasco J-20 spectropolarimeter.

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

Cycloalpigenin B (1) – substance *2* [1], $C_{30}H_{50}O_5$, mp 210-211°C (from acetone), $[\alpha]_D^{26}$ + 18.7 ± 2° (c 0.64; methanol). ν_{max}^{KBr}, cm^{-1}: 3550-3230 (OH), 3040 (CH_2 of a cyclopropane ring). Mass spectrum, *m/z* (%): M^+ 490 (0.9), 472 (4.0), 457 (3.0), 454 (3.8), 439 (5.5), 436 (1.5), 429 (1.6), 421 (2.8), 413 (2.6), 403 (1.1), 396 (6.0), 395 (8.0), 377 (4.1), 353 (3.0), 327 (3.3), 312 (4.0), 143 (100), 125 (22.0).

Cycloalpigenin B 3,6-Diacetate (2) from (1). Cycloalpigenin B (200 mg) was acetylated with 1 ml of acetic anhydride in 2 ml of pyridine at room temperatures for two days. After evaporation of the solvent, the residue was chromatographed on a column, with elution by system 1. This gave 120 mg of the diacetate (2), $C_{34}H_{54}O_7$, mp 120-121°C (from methanol), $[\alpha]_D^{24}$ + 42.7 ± 2° (c 0.66; methanol). ν_{max}^{KBr}, cm^{-1}: 3480 (OH), 3040 (CH_2 of a cyclopropane ring), 1740, 1255 (ester group). Mass spectrum, *m/z* (%): M^+ 574 (0.6), 556 (1.6), 538 (0.4), 514 (8.0), 496 (4.2), 481 (3.2), 471 (2.9), 439 (5.3), 438 (5.3), 437 (5.0), 377 (5.0), 312 (4.0), 251 (4.2), 143 (100), 125 (17.2).

3β,16β,25-Trihydroxy-20R,24S-epoxycycloartan-12-one 3,16-Diacetate (4) from (2). A solution of 100 mg of the diacetate (2) in 100 ml of acetone was cooled to −9°C, and then 0.1 ml of the Jones reagent [6] was added and the mixture was stirred at the same temperature for 30 min. The reaction was stopped by the addition of 2 ml of methanol. The reaction mixture was evaporated to a volume of 50 ml, diluted with 150 ml of water, and extracted with chloroform. The chloroform extract was washed with water and evaporated to dryness. The residue was chromatographed on a column, with elution by system 2. This gave 64 mg of the keto derivative (4), $C_{34}H_{52}O_7$, mp 94-95°C (from methanol), $[\alpha]_D^{26}$ + 44.8 ± 2° (c 0.58; method). ν_{max}^{KBr}, cm^{-1}: 3480 (OH), 3050 (CH_2 of a cyclopropane ring), 1735, 1250 (ester group), 1710 (C=O at C-12), CD (c 0.1; ethanol) $\Delta\varepsilon$ = −2.64 (286 nm). Mass spectrum, *m/z* (%): M^+ 572 (2.4), 557 (10.0), 514 (24.0), 497 (4.7), 479 (5.2), 471 (100), 454 (36.0), 435 (12.0), 429 (9.3), 411 (48.0), 393 (40.0), 351 (60.0), 331 (60.0), 271 (20.0), 270 (24.0), 143 (28.0).

Cycloalpigenin A (3) and 20R,24S-Epoxycycloartan-12-one 3-Monoacetate (5) from (4). The ketone diacetate (4) (30 mg) was hydrolyzed with 5 ml of a 0.1% methanolic solution of sodium hydroxie at room temperature. Then the reaction mixture was poured into water and extracted with chloroform. After the usual working up and evaporaion of the chloroform extract, the residue was chromatographed on a column, with elution by system 1. This gave 5 mg of the monoacetate (5), $C_{32}H_{50}O_6$, mp 269-272°C (from methanol), $[\alpha]_D^{24}$ −15.3 ± 2° (c 0.39; pyridine). ν_{max}^{KBr}, cm^{-1}: 3480 (OH), 1735, 1250 (ester grop), 1710 (C=O at C-12). Mass spectrum, *m/z* (%): M^+ 530 (3.5), 515 (11.3), 512 (3.7), 502 (1.9), 497 (6.1), 487 (3.0), 479 (6.5), 472 (34.8), 454 (37.0), 435 (19.6), 429 (100), 411 (82.6), 393 (65.2), 375 (37.0), 369 (65.2), 351 (87.0), 330 (41.3), 143 965.2).

By continuing the elution of the column with ethyl acetate, we isolated 12 mg of product (3), $C_{30}H_{48}O_5$, mp 222-224°C (from methanol) $[\alpha]_D^{24}$ – 26.3 ± 2° (*c* 0.37; methanol). Compound (3) was also identified as cycloalpigenin A by direct comparison with an authentic specimen on TLC and from the indices of its PMR and mass spectra [5].

Cycloalpioside B (6) – substance *6* [1], $C_{35}H_{48}O_9$, mp 253-254°C (from methanol). $[\alpha]_D^{23}$ – 26.3 ± 2° (c 0.53; methanol). ν_{max}^{KBr}, cm^{-1}: 3600-3400 (OH), 3040 (CH_2 of a cyclopropane ring). FMR spectrum (C_5D_5N): 0.34 and 0.47 (2H-19, d, ^2J = 4 Hz), 1.03; 1.25; 1.30; 1.35; 1.51; 1.51; 1.51; 1.78 (7xCH_3, s), 3.18 (H-17, d, ^3J = 8 Hz), 3.45 (H-3, d.d, $^3J_1$ = 11.6 Hz, $^3J_2$ = 4 Hz), 4.82 (H-1 of *D*-xylose, d, ^3J = 7.3 Hz), 4.91 (H-16, q, $^3J_1$ = $^3J_2$ = $^3J_3$ = 8 Hz).

Cycloalpigenin B (1) from (6). Cycloalpioside B (50 mg) has hydrolyzed with 10 ml of a 0.5% methanolic solution of sulfuric acid at 40°C for 4 h. The reaction mixture 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 system 3. This gave 18 mg of the genin (1), mp 210-211°C (from acetone) $[\alpha]_D^{26}$ + 18 ± 2° (*c*; methanol), identified as cycloalpigenin B

It was shown by the GLC method [9] that glycoside (6) contained one *D*-xylose residue.

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