Chemistry of Natural Compounds, Vol. 39, No. 5, 2003

TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS. LXVIII. CYCLOORBIGENIN C, A NEW CYCLOARTANE GENIN

R. P. Mamedova, M. A. Agzamova, and M. I. Isaev

UDC 547.918:547.926

The new triterpenoid cycloartane cycloorbigenin C, the structure of which is 23ξ,24ξ-cycloartan-3β,6α,16β,23,24,25-hexaol, was obtained from the aerial part of Astragalus orbiculatus *Ledeb. (Leguminosae). The structure of cycloorbigenin C was proved using chemical transformations, IR spectroscopy, electron-impact mass spectrometry, and PMR and ^{13}C NMR spectra interpreted using J-modulation and the 2D NMR spectroscopies: ^{1}H—^{1}H COSY, TOCSY, ROESY, HSQC, and HMBC.*

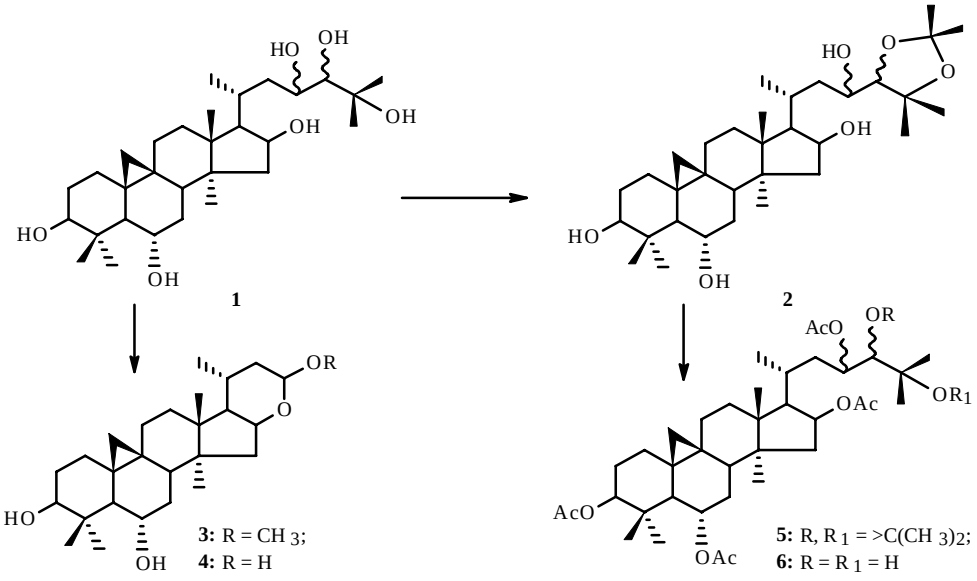
Key words: triterpenoids, cycloartanes, cycloorbigenin C, *Astragalus*, Leguminosae, PMR and ^{13}C NMR, J-modulation, 2D NMR: ^{1}H—^{1}H COSY, TOCSY, ROESY, HSQC, HMBC.

In continuation of research on cycloartane triterpenoids of plants from the genus *Astragalus* (Leguminosae) [1], we determined the structure of the new cycloartane methylsteroid cycloorbigenin C (**1**), which was obtained from the aerial part of *Astragalus orbiculatus* Ledeb. [2].

The molecular formula of **1**, $C_{30}H_{52}O_6$, and the PMR and ^{13}C NMR spectra, which exhibit signals of seven methyls and two 1H doublets of an AX system at high field at δ 0.33 and 0.61 (Table 1), indicate that **1** is a cycloartane triterpenoid [3, 4].

The elemental composition of **1** is consistent with an acyclic structure for the side chain. Therefore, all O atoms in **1** are hydroxyls. The observation of signals for five protons geminal to the hydroxyls in the PMR of **1** indicates that the sixth hydroxyl is tertiary. The ^{13}C NMR spectrum of **1** is also consistent with this. It contains signals for five secondary carbinol C atoms at δ 68.29, 72.20, 73.17, 78.41, and 79.19 and for one tertiary carbinol C atom at δ 74.37.

Since only one methyl (CH_3-21) resonates as a doublet, the only possible position for the tertiary hydroxyl is C-25.



S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75, e-mail: m_isaev@rambler.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 384-387, September-October, 2003. Original article submitted September 15, 2003.

 0009-3130/03/3905-0470$25.00

TABLE 1. Chemical Shifts of C and H in Cycloorbigenin C (**1**) and Parameters of Its 2D NMR Spectra: ^{1}H—^{1}H COSY, TOCSY, HSQC, HMBC, ROESY (δ, ppm, J / Hz, C_5D_5N, 0 = TMS)

C atom	δ_C	δ_H (J)	HMBC (C atoms)	ROESY (H atoms)
1	32.85	1.28; 1.65		
2	31.49	1.95; 2.05		
3	78.41	3.67 dd (11.5; 4.5)	30	
4	42.51	-		5
5	54.01	1.75 d (9.2)		
6	68.29	3.81 td (9.3; 3.4)		
7	38.62	1.75; 2.05		19 (δ 0.61), 30
8	47.19	1.97		
9	21.30	-		
10	29.64	-		
11	26.36	1.25; 1.95		
12	33.05	1.65; 1.65		
13	46.18	-		
14	46.90	-		
15	47.78	1.80; 2.15		
16	72.20	4.72 td (7.2; 5.2)	13	15 (δ 2.15), 17
17	57.51	1.86		
18	18.94	1.40 s	12, 13, 17	
19	30.11	0.33; 0.61 d (4)	1, 11; 8	
20	27.41	2.61 m		18, 21
21	20.36	1.21 d (7)	17, 20, 22	
22	42.97	2.15; 2.20		
23	73.17	4.33 t (8.5)	20, 24	20, 21, 22, 26
24	79.19	3.77 d (8.2)	22, 25, 26, 27	22, 27
25	74.37	-		
26	24.70	1.72 s	24, 25, 27	
27	28.98	1.68 s	24, 25, 26	
28	20.25	1.03 s	13	12, 17
29	29.41	1.89 s	3, 4, 5, 30	
30	16.20	1.37 s	3, 4, 5, 29	

Chemical shifts of protons, given without multiplicities and SSCC, were determined using 2D spectra. Lines of the H-23 triplet are broadened.

Cycloorbigenin C forms isopropylidene derivative **2**. The mass spectrum of **2** contains a peak for an ion with *m*/*z* 329, which arises through cleavage of the C-17–C-20 bond and elimination of a water molecule. The formation of an ion with *m*/*z* 329 indicates that the polycyclic part of the molecule and side chain each contain three hydroxyls whereas the isopropylidene moiety is located in the side chain.

Acetylation of **2** with acetic anhydride in pyridine gave the tetraacetate of the acetonide **5**, work up of which with acetic acid produces the tetraacetate **6**. The signals of four protons geminal to the acetoxy groups underwent a low-field shift in the PMR of **6** (Table 2). Therefore, the tertiary hydroxyl participated in the formation of the isopropylidene derivative and **2** is the 24,25-acetonide of **1**. As expected, the magnitude of the chemical shift of H-24 in the PMR spectrum of **6** is similar to those in the spectra of **1** and **2** and has δ 3.82. The doublet for H-24 in the PMR spectra of these compounds leads to the conclusion that the tertiary hydroxyl of the side chain is located vicinal to the hydroxyl on C-24, i.e., all hydroxyls on C-23, C-24, and C-25 form an α,β-triol system. Thus, H-24 in the HMBC spectrum of **1** (Table 1) has correlation peaks with C-22, C-25, C-26, and C-27; H-23, with C-20 and C-24.

TABLE 2. Proton Chemical Shifts (δ, ppm), Multiplicities, and SSCC (J/Hz) of Cycloorbigenin C (**1**) and its Derivatives (0 = HMDS)

Compound	Proton position						
	H-3	H-6	H-16	2H-19	H-23	H-24	CH_3-, OAc-groups
1	3.50 m*	3.50 m*	4.58 m	0.19; 0.48 d (4)	4.15 m	3.63 d (7.5)	0.90; 1.06 d (7.5); 1.18; 1.25; 1.52; 1.56; 1.69
2	3.50 m*	3.50 m*	4.60 m	0.18; 0.48 d (4)	3.90 m	3.88 d (8)	0.94; 1.06 d (8); 1.22; 1.34; 1.40; 1.40; 1.48; 1.74; 1.82
3	[3.26 dd (10.4)]	[3.50 td (9; 3)]	[4.20 q (7; 7; 7)]	[0.30; 0.50 d (4)]	[4.67 t (7)]	-	[0.84-0.91 (CH_3-21); 0.88; 0.91; 1.04; 1.20; 3.28 (OCH_3)]
6	4.74 m*	4.74 m*	5.40 m	0.14; 0.39 d (4)	5.78 dd (10;4)	3.82 d (4)	0.74; 0.87; 0.98; 1.00; 1.06 d (7); 1.45; 1.45; (1.94; 1.94; 1.94; 2.12)

Spectra were recorded on a Tesla BS-567A spectrometer in C_5D_5N or $CDCl_3$. Parameters given in square brackets were obtained in $CDCl_3$. Signals marked with an asterisk are mutually overlapped in horizontal rows. Signals of methyls are singlets except for CH_3-21.

Periodate oxidation of **1** was performed in order to confirm the location of the triol system. The nor-products **3** and **4** with molecular weights of 432 and 418 amu, respectively, were produced. The loss of 90 amu on going from **1** to **4** confirms that the 23,24,25-triol group is located in the side chain.

A 3H singlet for a OCH_3 group was observed in the PMR spectrum of **3** at δ 3.28. Taking this and the molecular weight (M^+ 432) of **3** into account, it can be seen that this product is an acetal that arises through cleavage of the C-23–C-24 bond and loss of a four-carbon fragment.

In fact, the PMR of **3** has a triplet with spin—spin coupling constant (SSCC) ^3J = 7 Hz at δ 4.67 that belongs to an anomeric proton. We produced previously similar nor-products from cycloartan-16β,24,25-triols [5-7], where the 16β-hydroxyl participated in the formation of internal hemiacetals, further alkylation of which at the anomeric hydroxyl gave acetals. Therefore, it can be assumed that **1** also contains a 16β-hydroxyl. The 1H multiplet at δ 4.58 in the PMR spectrum of **1** (Table 2), which is assigned to a 16α-hydrogen atom [4], confirms this. The ^{13}C NMR and high-resolution PMR (Table 1) confirm that **1** contains a 16β-hydroxyl. Atom C-16 resonates at δ 72.20 in the ^{13}C NMR of **1**. C-16 in the HSQC spectrum of **1** correlates with a triplet of doublets at δ 4.72 with SSCC $^3J_1 = {}^3J_2 = 7.2$ and $^3J_3 = 5.2$ Hz, which is assigned to H-16. The ROESY spectrum exhibits a nuclear Overhauser effect (NOE) between H-16 and H-17, indicating the α-orientation of the former and also confirming the β-orientation of the C-16 hydroxyl.

The signal for H-16 in the PMR spectrum of **3**, as expected, underwent a high-field shift and was observed at δ 4.20 as a quartet with line intensities 1:3:3:1 and SSCC $^3J_1 = {}^3J_2 = {}^3J_3 = 7$ Hz. This is consistent with the proton located in a five-membered ring. The agreement of the chemical shifts, multiplicities, and the SSCC of H-16 and H-23 in the PMR spectrum of **3** and dasyanthogenin [8] indicate that the stereochemistries of the side chains of these compounds are identical.

The signal for C-5 occurs at δ 54.01 in the ^{13}C NMR spectrum of **1**. This indicates that this compound is 6α-hydroxycycloartane [9]. The chemical shift of the 4α-methyl (CH_3-29) at δ 1.89 in the PMR spectrum of **1** is also consistent with this [3, 4]. The same PMR spectrum clearly exhibits at δ 3.81 a 1H triplet of doublets with SSCC $^3J_1 = {}^3J_2 = 9.3$ and $^3J_3 = 3.4$ Hz. The signal for this proton correlates in the HSQC spectrum with that for the C atom at δ 68.29. These parameters agree well with those for H-6β and C-6. The orientation of H-6 was also found using the ROESY spectrum, in which NOEs were observed between H-6 and CH_3-30 and between H-6 and H-19, which resonated at δ 0.61. Therefore, **1** contains a 6α-hydroxyl.

The location and configuration of the remaining unidentified hydroxyl was determined using a doublet of doublets at δ 3.67 with SSCC $^3J_1$ = 11.5 Hz and $^3J_2$ = 4.5 Hz in the PMR spectrum of **1**, which is characteristic of 3α-H [3, 4]. The corresponding C atom resonates at δ 78.41 in the ^{13}C NMR spectrum of **1**. Therefore, **1** also contains a 3β-hydroxyl.

Thus, the results lead to the conclusion that **3** is the 23-methyl ether of 24-nor-16β,23*S*-epoxycycloartan-3β,6α,23-triol; **4**, 24-nor-16β,23*S*-epoxycycloartan-3β,6α,23-triol; **1**, 23ξ,24ξ-cycloartan-3β,6α,23,24,25-hexaol.

EXPERIMENTAL

General comments appeared previously [10]. The following solvent systems were used: $CHCl_3$:CH_3OH:H_2O (1, 70:12:1) and $CHCl_3$:CH_3OH (2, 15:1; 3, 50:1).

PMR and ^{13}C NMR spectra were obtained on Bruker DRX-500, Bruker WM-250, and Tesla BS-567A spectrometers in C_5D_5N or $CDCl_3$. ^{13}C NMR spectra were recorded using full C—H decoupling and *J*-modulation. 2D NMR spectra were recorded using standard Bruker programs.

Isolation and Separation of *Astragalus orbiculatus* Ledeb. Triterpenoids. Intermediate fractions containing **6** that accumulated during isolation of cycloorbicosides A [11] and G [12] were rechromatographed over a column using system 1. We isolated glycoside **6** and called it cycloorbicoside D [2].

Cycloorbigenin C (1). Cycloorbicoside D (**6**, 334 mg) was treated with methanolic H_2SO_4 (21 mL, 0.25%) and heated at 70°C for 3 h. The mixture was diluted with H_2O. The CH_3OH was evaporated. The resulting precipitate was filtered off and washed with water. The dried solid was chromatographed over a column with elution by system 2 to isolate **1** (170 mg), $C_{30}H_{52}O_6$, mp 256-258°C (CH_3OH). IR spectrum (KBr, ν, cm^{-1}): 3540-3200 (OH), 3050 (CH_2 of cyclopropane ring). Mass spectrum (*m*/*z*, %): M^+ 508 (1.4), 490 (8.6), 475 (4.4), 472 (8.8), 457 (8.8), 454 (8.8), 439 (11.1), 383 (33.3), 367 (16.7), 349 (16.5), 339 (16.7), 323 (16.7), 311 (27.8), 295 (27.8), 293 (22.2), 271 (33.3), 253 (38.9), 239 (44.4), 213 (66.7), 201 (83.3), 189 (77.8), 171 (100), 163 (88.9), 141 (77.8), 115 (88.9), 99 (83.3).

For PMR and ^{13}C NMR spectra, see Tables 1 and 2.

24,25-Acetonide of 1 (2). Compound **1** (110 mg) was dissolved in acetone (40 mL) containing H_2SO_4 (0.2%). The reaction mixture was left at room temperature for 1 d, diluted with water, and treated with $CHCl_3$. The $CHCl_3$ extract was washed with water and evaporated. The solid was chromatographed over a column with elution by system 2 to isolate **2** (100 mg), $C_{33}H_{56}O_6$. Mass spectrum (*m*/*z*, %): M^+ 548 (1.4), 533 (28.6), 530 (21.4), 515 (17.9), 513 (35.7), 497 (7.1), 472 (14.3), 439 (17.9), 421 (17.9), 401 (67.9), 383 (100), 367 (42.9), 365 (50), 349 (25), 329 (32.1), 311 (42.9), 271 (28.6).

For PMR spectrum, see Table 2.

3,6,16,23-Tetraacetate of 2 (5). Compound **2** (76 mg) was acetylated with acetic anhydride (1 mL) in absolute pyridine (2 mL) over 15 d at room temperature. The solvents were evaporated. The solid was chromatographed over a column with elution by system 3 to afford **5** (80 mg), $C_{41}H_{64}O_{10}$. Mass spectrum (*m*/*z*, %): $[M - 15]^+$ 701 (40.7), 658 (22.2), 656 (29.6), 641 (7.4), 614 (1.9), 596 (85.2), 581 (48.1), 553 (3.7), 538 (35.2), 521 (35.2), 496 (3.7), 478 (29.6), 463 (33.3), 413 (51.9), 403 (22.2), 389 (18.5), 353 (37), 293 (100), 277 (28.8), 271 (28.8), 201 (96.3).

3,6,16,23-Tetraacetate of 1 (6) from 5. Compound **5** (73 mg) in acetic acid (3 mL) was heated at 50°C for one week. The solution was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water and evaporated. The solid was chromatographed over a column with elution by system 3 to isolate **6** (32 mg), $C_{38}H_{60}O_{10}$. Mass spectrum (*m*/*z*, %): $[M - 18]^+$ 658 (8), 616 (60), 598 (20), 556 (70), 541 (30), 538 (30), 523 (15), 498 (25), 496 (25), 484 (35), 481 (30), 424 (50), 413 (65), 339 (55), 293 (85), 201 (70), 199 (70), 185 (100).

For PMR spectrum, see Table 2.

24-Nor-16β,23*S*-epoxycycloartan-3β,6α,23-triol 23-Methyl Ether (3) and 24-Nor-16β,23*S*-epoxycycloartan-3β,6α,23-triol (4) from 1. Compound **1** (10 mg) in CH_3OH (1.5 mL) was treated with sodium periodate (20 mg) in water (0.5 mL) and left at room temperature for 2 d. The excess of oxidant was destroyed by adding ethyleneglycol (2 drops). The supernatant solution was decanted. The precipitate was washed with a small amount of CH_3OH. The methanolic solutions were combined and evaporated. The solid was chromatographed over a column with elution by system 2 to isolate **3** (6 mg), $C_{27}H_{44}O_4$. Mass spectrum (*m*/*z*, %): M^+ 432 (5.2), 414 (54.5), 399 (45.5), 396 (27.3), 381 (27.3), 355 (27.3), 232 (45.5), 201 (27.3), 161 (45.5), 121 (81.8), 107 (90.9), 85 (100).

For PMR spectrum, see Table 2.

Continued elution of the column with the same solvent system afforded **4** (1 mg), $C_{26}H_{42}O_4$. Mass spectrum (*m*/*z*, %): M^+ 418 (2.8), 403 (2.7), 400 (16), 385 (17), 382 (12), 367 (15), 357 (9), 349 (4), 341 (7), 313 (4.2), 297 (4.5), 279 (5.3), 261 (6.8), 253 (5.6), 235 (15), 232 (18), 217 (15), 203 (14), 201 (27), 199 (26), 121 (90), 109 (70), 107 (100), 95 (80).

REFERENCES

1. R. P. Mamedova, M. A. Agzamova, and M. I. Isaev, *Khim. Prir. Soedin.*, 460 (2002).
2. R. P. Mamedova, M. A. Agzamova, and M. I. Isaev, *Khim. Prir. Soedin.*, 296 (2002).
3. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 431 (1985).
4. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 156 (1989).
5. M. I. Isaev, M. B. Gorovits, N. D. Abdullaev, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 458 (1982).
6. M. I. Isaev, M. B. Gorovits, N. D. Abdullaev, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 587 (1983).
7. M. I. Isaev, M. B. Gorovits, N. D. Abdullaev, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 432 (1984).
8. R. I. Evstratova, V. I. Sheichenko, and D. A. Pakalns, *Khim. Prir. Soedin.*, 102 (1981).
9. M. A. Agzamova and M. I. Isaev, *Khim. Prir. Soedin.*, 348 (1999).
10. R. P. Mamedova, M. A. Agzamova, and M. I. Isaev, *Khim. Prir. Soedin.*, 453 (2001).
11. M. A. Agzamova, M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 455 (1986).
12. M. A. Agzamova, M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 837 (1987).