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

TRITERPENE GLYCOSIDES AND THEIR GENINS FROM *Astragalus*. LXIX. ORBIGENIN, THE FIRST LANOSTANOID FROM *Astragalus* PLANTS

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

UDC 547.918:547.926

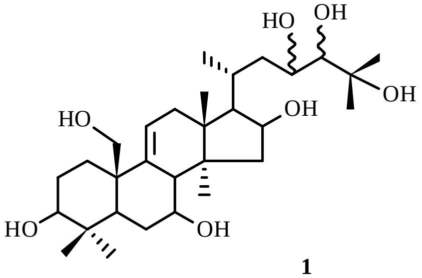
The new lanostane methylsteroid orbigenin, the structure of which is 23ξ,24ξ-lanost-9(11)-en-3β,7β,16β,19,23,24,25-heptaol, was isolated from Astragalus orbiculatus *Ledeb. (Leguminosae). The structure of the new triterpenoid, the first lanostanoid from plants of the* Astragalus *genus, was determined using electron-impact mass spectrometry and PMR and ^{13}C NMR spectroscopies interpreted using J-modulation, DEPT, and the 2D NMR methods: $^1H—^1H$ COSY, HSQC, and HMBC.*

Key words: lanostanoids, triterpenoids, orbigenin, *Astragalus*, Leguminosae, PMR and ^{13}C NMR spectra, DEPT, J-modulation, 2D NMR: $^1H—^1H$ COSY, HSQC, HMBC.

In continuation of research on isoprenoids of plants from the *Astragalus* genus [1], we established the structure of the new lanostane triterpenoid orbigenin (**1**), which was isolated from the aerial part of *Astragalus orbiculatus* Ledeb. [2]. We present proof of the structure of the new lanostanoid.

The mass spectrum of **1** does not contain a peak for the molecular ion. The peak of highest mass with *m/z* 506 corresponds to a daughter ion arising from the molecular ion by elimination of one water molecule. This fact and the PMR and ^{13}C NMR (Table 1) define the molecular formula of **1** as $C_{30}H_{52}O_7$.

According to the elemental composition, **1** is a triterpenoid with five degrees of unsaturation. The PMR and ^{13}C NMR of **1** contain signals for one olefinic proton at δ 5.64 as a doublet of triplets with spin—spin coupling constant (SSCC) $^3J_1 = 6.3$ Hz and $^3J_2 = {}^4J = 2$ Hz and two C atoms of a triply substituted olefin at δ 121.57 and 142.01. The PMR exhibits signals for seven exchangeable protons at δ 6.85, 6.65, 6.42, 5.99, 5.95, 5.76, and 4.89 (Fig. 1 and note to Table 1). These disappear upon adding trifluoroacetic acid. Therefore, all O atoms of **1** are hydroxyls. Thus, the ^{13}C NMR spectrum clearly exhibits signals for one primary alcohol C atom at δ 60.54, one tertiary alcohol C atom at δ 74.24, and five secondary alcohol C atoms at δ 71.84, 72.63, 73.21, 78.20, and 79.12. Considering that **1** has one double bond, the elemental composition $C_{30}H_{52}O_7$ indicates that **1** is a tetracyclic triterpenoid. The presence in **1** of seven methyls and one hydroxymethyl (Table 1 and Fig. 1) and the fact that cycloartane triterpenes have been isolated from *A. orbiculatus* [2] enabled us to identify the new compound as a lanostane tetracyclic triterpenoid that is biogenetically related to the cycloartane triterpenes.



1

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. 388-391, September-October, 2003. Original article submitted September 22, 2003.

TABLE 1. Chemical Shifts of C and H Atoms in Orbigenin (**1**), Experimental DEPT Data and 2D NMR Spectral Parameters: ^{1}H—^{1}H COSY, HSQC, HMBC (δ, ppm, J/Hz, C_5D_5N, 0 = TMS)

C atom	δ_C	DEPT	δ_H (J)	HMBC
1	30.99	CH_2	α 1.36 td (13.4; 3.5)	
			β 2.65 dt (13.4; 3.5)	2, 3
2	28.66	CH_2	2.03; 2.10	
3	78.20	CH	3.59 dd (11; 4.6)	
4	39.71	C	-	
5	50.34	CH	1.40 dd (13; 2.3)	4, 6, 7, 10, 19
6	32.22	CH_2	2.06; 2.32	4, 5, 7, 8, 10
7	71.84	CH	4.12 td (10.5; 5)	
8	50.87	CH	2.88 dm (^{3}J = 10.5; ^{4}J = 2; Σ^5J = 5.3)	7, 9, 10, 11, 13, 14, 28
9	142.01	C	-	
10	45.30	C	-	
11	121.57	CH	5.64 dt (6.3; 2)	8, 10, 12, 13
12	37.54	CH_2	2.20; 2.30	9, 11, 13, 14, 18
13	45.37	C	-	
14	45.38	C	-	
15	49.56	CH_2	2.47 dd (14; 5.3)	8, 13, 14, 16, 28
			3.02 dd (14; 8)	13, 14, 17, 28
16	72.63	CH	4.84 ddd (8; 7.3; 5.2)	13, 14
17	55.50	CH	1.95 dd (11.3; 7.3)	12, 13, 14, 16, 18, 20, 21, 22
18	15.72	CH_3	1.44 s	12, 13, 14, 17
19	60.54	CH_2	4.12 d (10.5)	1
			4.26 d (10.5)	9
20	27.42	CH	2.65	13, 17, 21, 22, 23
21	20.47	CH_3	1.24 d (6.4)	17, 20, 22
22	43.03	CH_2	2.22; 2.22	20, 21, 23
23	73.21	CH	4.32 td (8.5; 3.4)	20, 24
24	79.13	CH	3.78 d (8.5)	22, 23, 25, 26, 27
25	74.24	C	-	
26	24.69	CH_3	1.73 s	24, 25, 27
27	28.90	CH_3	1.69 s	24, 25, 26
28	19.28	CH_3	1.15 s	8, 13, 14, 15
29	29.18	CH_3	1.29 s	3, 4, 5, 30
30	17.70	CH_3	1.14 s	3, 4, 5, 29

Parameters of the PMR spectrum taken with added trifluoroacetic acid (TFA) are given. The PMR spectrum without added TFA contains an additional 8 signals for hydroxyl protons and absorbed water at δ 6.85 (br.s, 23-OH), 6.65 (d, ^{3}J = 5 Hz, 24-OH), 6.42 (br.s, 25-OH), 5.99 (d, ^{3}J = 4.5 Hz, 3-OH), 5.95 (br.s, 16-OH), 5.76 (d, ^{3}J = 6.5 Hz, 7-OH), 5.05 (br.s, H_2O), 4.89 (t, $^3J_1$ = $^3J_2$ = 5.6 Hz, 19-OH), which were assigned based on ^{1}H—^{1}H COSY data. Chemical shifts of protons without multiplicities and SSCC were established using 2D spectra.

The signal for the olefinic proton in the HMBC spectrum of **1** correlates with signals for C-8, C-10, C-12, and C-13. Therefore, the double bond is located on ring C of the lanostane skeleton at C-9(11).

The mass spectrum of **1** also reveals the presence of a hydroxymethyl. The base peak with *m*/*z* 457 $[M - 2H_2O - CH_2OH]^+$ is a fragment ion arising from the molecular ion by successive elimination of two water molecules and a hydroxymethyl radical.

Protons geminal to the primary hydroxyl resonate in the PMR at δ 4.12 and 4.26 as 1H doublets of an AX system with SSCC ^{2}J = 10.5 Hz. These signals exhibit correlation peaks in the HMBC spectrum with C-1 and C-9, indicating that the primary hydroxyl is located on C-19.

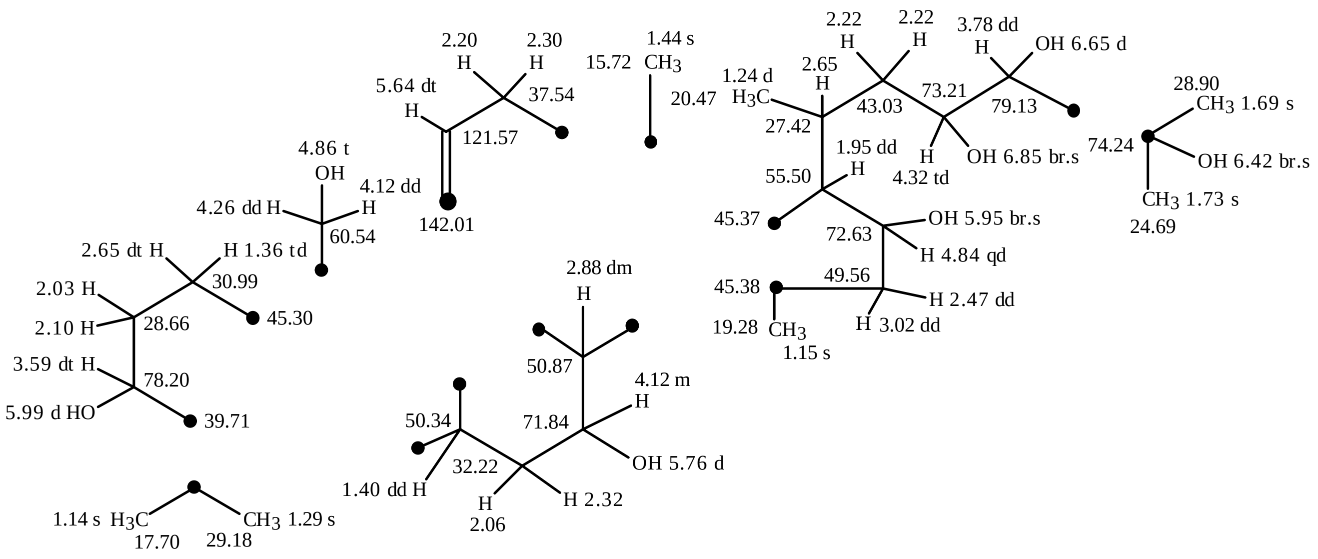


Fig. 1. Structural fragments of **1** based on PMR and ^{13}C NMR data, DEPT experiments, and 2D NMR spectra: ^{1}H—^{1}H COSY, HSQC, HMBC. Data taken from spectra recorded without added TFA are given. Through-space couplings are present for H-8 and H-11 and for H-8 and 2H-12.

The PMR spectrum of **1** shows only one methyl (CH_3-21) that resonates as a doublet with SSCC $^3J = 6.4$ Hz at δ 1.24. Therefore, the tertiary hydroxyl should be located on C-25. DEPT experiments showed that C-25 resonates at δ 74.24.

The resonances for the proton geminal to one of the secondary hydroxyls were observed in the PMR spectrum at δ 3.78 as a doublet with SSCC $^3J = 8.5$ Hz. This signal exhibits in the HMBC spectrum correlation peaks with C-22, C-23, C-25, C-26, and C-27. Therefore, this H atom and the secondary hydroxyl geminal to it are located on C-24. The HSQC spectrum shows the signal for C-24 at δ 79.13.

The fact that H-24 is a doublet indicates that another hydroxyl should be vicinal to it. In fact, the signal for H-24 in the ^{1}H—^{1}H COSY spectrum couples with that for a proton geminal to a secondary hydroxyl that is observed at δ 4.32 as a triplet of doublets with SSCC $^3J_1 = {}^3J_2 = 8.5$ Hz and $^3J_3 = 3.4$ Hz. The HSQC spectrum indicates that this proton is bound to the C atom resonating at δ 73.21, which correlates in the HMBC spectrum with H-24. Therefore, another secondary hydroxyl of the side chain is located on C-23.

Thus, of the five secondary hydroxyls, two are located in the side chain; three unidentified ones, in the polycyclic part.

The signal for H-17 of **1** appears in the PMR spectrum at δ 1.95 as a doublet of doublets with SSCC $^3J_1 = 11.3$ Hz and $^3J_2 = 7.3$ Hz. The multiplicity of this signal indicates that C-16 contains a hydroxyl. As expected, H-17 correlates in the ^{1}H—^{1}H COSY spectrum with a proton geminal to a hydroxyl that is observed at δ 4.84 as a doublet of doublets of doublets (ddd) with SSCC $^3J_1 = 8$ Hz, $^3J_2 = 7.3$ Hz, $^3J_3 = 5.2$ Hz, which is assigned as H-16. The HSQC spectrum indicates that C-16 resonates at δ 72.63. These parameters are consistent with the α-orientation for H-16 and, therefore, the β-configuration for the hydroxyl on C-16 [1].

Two protons geminal to unidentified secondary hydroxyls resonate in the PMR spectrum as a doublet of doublets with SSCC $^3J_1 = 11$ Hz, $^3J_2 = 4.6$ Hz at δ 3.59 and as a triplet of doublets with SSCC $^3J_1 = {}^3J_2 = 10.5$ Hz and $^3J_3 = 5$ Hz at δ 4.12. The multiplicities of these signals indicate that these protons and, consequently, the corresponding hydroxyls belong to rings A and B. Only one hydroxyl can be located in each ring. This means that the triplet of doublets at δ 4.12 can belong either to 6β-H or 7α-H. A selection in favor of the 7α-H position was made as follows. The signal of the 4α-methyl (CH_3-29) is observed in the PMR spectrum at δ 1.29. This precludes the presence of a 6α-hydroxyl in **1** [3, 4]. Therefore, the signal of α-axial H-5 observed at δ 1.40 is a doublet of doublets with SSCC $^3J_1 = 13$ Hz and $^3J_2 = 2.3$ Hz. As a result, the secondary hydroxyl in ring B is located on C-7 and has the β-configuration. In fact, the HSQC spectrum shows that C-7 resonates at δ 71.84. The chemical shift of C-7 confirms the choice of the 7β-hydroxyl in **1**. The presence of a correlation in the ^{1}H—^{1}H COSY spectrum between H-7 and H-8, which is observed at δ 2.88, additionally confirms this choice.

The parameters of H-3 and C-3 in the PMR and ^{13}C NMR (Table 1) unambiguously indicate that **1** contains a 3*β*-hydroxyl.

Thus, the experimental data lead to the conclusion that **1** is 23*ξ*,24*ξ*-lanost-9(11)-en-3*β*,6*β*,16*β*,19,23,24,25-heptaol. Orbigenin is the first lanostane triterpenoid found in plants of the *Astragalus* genus. We reported previously about this [2, 5]. Certain lanostane derivatives were obtained as artifacts owing to isomerization of the corresponding cycloartanoids [6-8].

EXPERIMENTAL

General comments have been reported [9]. The following solvent system was used: $CHCl_3$:CH_3OH:H_2O (1, 70:12:1).

PMR and ^{13}C NMR spectra were recorded on UNITY 400plus and Bruker WP-250 spectrometers in C_5D_5N (δ, ppm, 0 = TMS); ^{13}C NMR spectra had complete C–H decoupling, J-modulation, and DEPT. 2D NMR spectra were recorded using standard Varian programs.

Isolation and Separation of Triterpenoids from *A. orbiculatus* Ledeb. Fractions with compound **3** that accumulated during isolation of cycloorbicosides A [10, 11], B [12, 13], C [14, 15], D [1, 2], and G [16] were rechromatographed using system 1 to isolate genin **3** (64 mg) that we called orbigenin.

Orbigenin (1), $C_{30}H_{52}O_7$, mp 298-301°C (CH_3OH). Mass spectrum (*m/z*, %): 506 (0.9) $[M - H_2O]^+$, 488 (10.7) $[M - 2H_2O]^+$, 476 (7.1) $[M - H_2O - 2CH_3]^+$, 457 (100) $[M - 2H_2O - CH_2OH]^+$, 439 (10.7) $[M - 3H_20 - CH_2OH]^+$, 399 (8.9), 385 (10.7), 381 (17.9), 367 (28.6), 351 (17.9), 349 (21.4), 325 (16.1), 323 (12.5), 315 (14.3), 297 (35.4), 281 (12.5), 279 (17.9), 199 (19.6), 189 (17.9), 187 (23.2), 185 (32.1), 183 (23.2), 173 (32.1), 171 (25), 169 (25), 143 (33.9).

For PMR and ^{13}C NMR, see Table 1 and Fig. 1.

REFERENCES

1. R. P. Mamedova, M. A. Agzamova, and M. I. Isaev, *Khim. Prir. Soedin.*, 384 (2003).
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, in: Proceedings of the 5th Int. Symp. on the Chemistry of Natural Compounds, May 20-23, 2003, Tashkent, Uzbekistan, p. 27.
6. A. N. Svechnikova, R. U. Umarova, M. B. Gorovits, K. L. Seitanidi, Ya. V. Rashkes, M. R. Yagudaev, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 67 (1981).
7. I. Kitagawa, H. K. Wang, A. Takagi, M. Fuchida, I. Miura, and M. Yoshikawa, *Chem. Pharm. Bull.*, **31**, 689 (1983).
8. Yu. M. Fadeev, M. I. Isaev, Yu. A. Akimov, P. K. Kintya, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 817 (1987).
9. R. P. Mamedova, M. A. Agzamova, and M. I. Isaev, *Khim. Prir. Soedin.*, 453 (2001).
10. M. A. Agzamova, M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 455 (1986).
11. M. A. Agzamova, M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 719 (1986).
12. M. A. Agzamova, M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 809 (1989).
13. M. A. Agzamova, M. I. Isaev, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 699 (1990).
14. M. A. Agzamova and M. I. Isaev, *Khim. Prir. Soedin.*, 519 (1998).
15. R. P. Mamedova, M. A. Agzamova, and M. I. Isaev, *Khim. Prir. Soedin.*, 453 (2002).
16. M. A. Agzamova, M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 837 (1987).