TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS.

XX. CYCLOORBIGENIN FROM Astragalus orbiculatus

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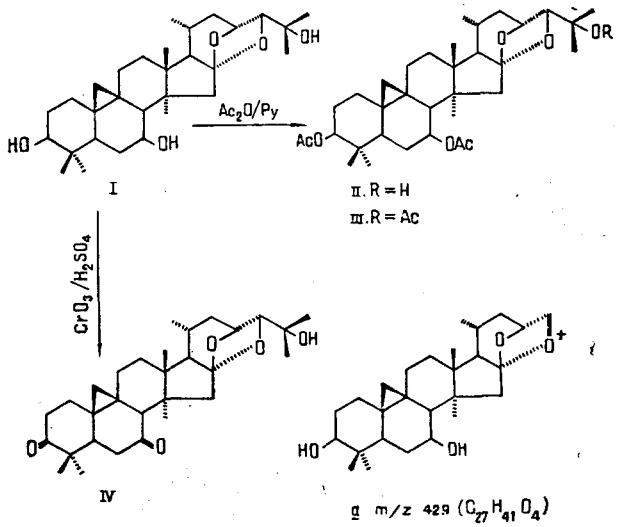
UDC 547.918:547.926

A new genin — cycloorbigenin (I), $C_{30}H_{48}O_5$, mp 217-219°C, $[\alpha]_D^{20}$ +28.3° (c 1.19; ethanol) has been obtained from a glycoside isolated from the epigeal parts of the plant Astragalus orbiculatus (Leguminosae), and on the basis of chemical transformation and spectral characteristics its structure has been established as 16β,23:16α,24-diepoxy-23(R),24(S)cycloartane-3β,7β,25-triol. The acetylation of (I) with acetic anhydride in pyridine yielded its diacetate (II), $C_{34}H_{52}O_7$, mp 148-150°C, $[\alpha]_D^{20}$ +32.6° (c 0.92; methanol) and its triacetate (III), $C_{36}H_{54}O_8$, mp 137-139°C, $[\alpha]_D^{20}$ +75° (c 0.4; methanol). The Jones oxidation of (I) led to a diketone (IV), $C_{30}H_{44}O_5$, mp 155-158°C, $[\alpha]_D^{20}$ −73° (c 0.63; methanol). Details of the PMR, IR, and mass spectra are given for all the compounds.

We have continued our investigation of the methylsteroids of plants of the genus Astragalus (Leguminosae). From the epigeal part of the Astragalus orbiculatus Ledeb. we have isolated the quantitatively main glycoside. Acid hydrolysis of this glycoside led to a new genin, which we have called cycloorbigenin (I). The present paper is devoted to a proof of its structure.

In the PMR spectrum of the genin (I) at 0.23 and 0.65 ppm (Table 1) one-proton doublets of a AB stystem characteristic of a 1,1,2,2-tetrasubstituted cyclopropane are observed, and also the signals of seven methyl groups. The presence of a cyclopropane ring is also shown by an absorption band at 3040 cm^{-1} in the IR spectrum of compound (I) [1]. The facts given indicate that compound (I) belongs to the triterpenoids of the cycloartane series [2].



The IR spectrum of the genin (I) also has a broad absorption band of hydroxy groups. It follows from the elementary composition of cycloorbigenin (I), $C_{30}H_{48}O_5$, in the light of the features of the PMR and IR spectra, that three of the oxygen atoms are present in hydroxy groups and the other two must form epoxide rings.

Institute of The Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 455-460, July-August, 1986. Original article submitted March 14, 1986.

0009-3130/86/2204-0425$12.50

TABLE 1. Chemical Shifts of the Protons of Cycloorbigenin (I) and its Derivatives (δ, ppm, 0 – HMDS)

Compound	Positions of the protons							
	H-3	H-7	H-15 / H-8	2H-19	H-23	H-24	CH_3 groups	OAc
I	3,38 q J=12; 5 Hz	3,70 td ^3J=10; 10; 3 Hz	2,46; 2,70 d ^2J=15 Hz	0,23; 0,65 d ^2J=4 Hz	4,62 q ^3J=9; 2 Hz	3,58 s	0,76 d (^3J=6 Hz); 0,99; 1,11; 1,13; 1,26; 1,35 (2×CH_3)	—
II	4,64 m*	4,80 td ^3J=10; 10; 3 Hz	1,91; 2,26 d ^2J=14 Hz	0,18; 0,58 d ^2J=4 Hz	4,58 q* ^3J=9; 2 Hz	3,54 s	0,71; 0,75 d (^3J=6Hz); 0,81; 1,03; 1,10; 1,27; 1,33	1,87; 1,92
	[4,50 m]	[4,74 m]		[0,30; 0,74 d ^2J=5 Hz]	[4,22 q ^3J=8; 2 Hz]	[3,30 s]	[0,80; 0,82 d; 0,83; 1,04 (2×CH_3); 1,05; 1,08]	[1,94; 1,99]
III	[4,51 q ^3J=11; 4 Hz]	[4,75 td ^3J=10,6; 10,6; 4 Hz]		[0,30; 0,74 d ^2J=4 Hz]	[4,24 q ^3J=9; 2 Hz]	[3,70 s]	[0,79; 0,81 d (^3J=6 Hz); 0,82; 1,02; 1,19; 1,25; 1,36]	[1,92; 1,94; 2,00]
IV	—	—	2,80 s	0,20 d ^2J=5 Hz	4,58 q ^3J=9; 2 Hz	3,48 s	0,71 d (^3J=6Hz); 0,91; 0,94; 0,98 (2×CH_3); 1,22 1,32	—
	—	—	[2,83 s]	[0,38; 0,76 d ^2J=5 Hz]	[4,24 q ^3J=9; 2 Hz]	[3,27 s]	[0,81 d (^3J=6Hz); 0,94; 0,99 (2×CH_3); 1,04 (2×CH_3); 1,06]	—

*The spectra were taken in $CDCl_3$ or C_5D_5N. The indices given in the square brackets were obtained with the use of $CDCl_3$. The signals in the horizontal lines marked with asterisks are superposed upon one another. The signals of the methyl groups have a singlet nature with the exception of the CH_3 at C-20, which has a doublet nature; d – doublet; q – quartet; td – triplet of doublets; m – multiplet.

The acetylation of cycloorbigenin (I) gave a diacetate (II) and a triacetate (III). In the PMR spectra of the acetates (II) and (III), the signals of only two protons geminal to acetoxy groups had undergone appreciable paramagnetic shifts. The formation of the triacetate (III) indicates the tertiary nature of one of the hydroxy groups.

The appearance in the mass spectrum of cycloorbigenin (I) of the 100% peak of an ion *a* with m/z 429 ($C_{27}H_{41}O_4$) arising on the cleavage of the C-24—C-25 bond unambiguously determines the position of the tertiary hydroxy group at C-25. This conclusion is also in harmony with the features of the PMR spectrum of the genin (I), in which the signals of six methyl groups have a singlet nature and that of one (CH_3-21) a doublet nature.

As was to be expected, the Jones oxidation [3] of the genin (I) led to a diketone (IV) (M^+ 484). The IR spectrum of compound (IV) had an absorption band at 1710-1695 cm^{-1}, which is characteristic for a 6-membered cyclic ketone. Consequently, the two secondary hydroxy groups may be located in rings A, B, and C, in positions 2, 3, 6, 7, or 12. If a keto function were present in position 1 or 11, the carbonyl group absorption band would be shifted in the direction of low frequencies because of conjugation with the cyclopropane ring [4, 5].

In the PMR spectrum of the genin (I), a proton geminal to one of the secondary hydroxy groups gives a resonance signal at 3.38 ppm in the form of a quartet with the spin—spin coupling constants (SSCCs) $^3J_1$ = 12 Hz and $^3J_2$ = 5 Hz. In the spectra of the acetate (II) and (III) ($CDCl_3$), the signal of this proton has undergone paramagnetic shift and appears at 4.50 and 4.51 ppm ($^3J_1$ = 11 Hz; $^3J_2$ = 4 Hz, respectively). The parameters given agree well with those for the 3α-H atoms in 4,4-dimethyltriterpenoids [6-8]. Consequently, the hydroxy group under consideration is located at C-3 and has the β orientation.

Cycloorbigenin (I) was not oxidized by sodium periodate. This fact, showing the absence of an α-glycol function, excludes the presence of an unidentified hydroxy function at C-2.

The signal of the proton geminal to the hydroxy group that is being sought in the spectrum of genin (I) is split in the form of a triplet of doublets with the SSCCs $^3J_1$ = $^3J_2$ = 10 and $^3J_3$ = 3 Hz, and is observed at 3.70 ppm. It follows from the multiplicity of the signal under discussion that the corresponding proton is not located at C-12, and the SSCC shows its axial orientation.

The choice between the alternative positions 6α and 7β for the hydroxy group was made in favor of 7β in the following way.

It has been shown previously [2, 5, 7, 9-11] that in the PMR spectra of 6α-hydroxycycloartanes taken in deuterocycloroform the signal of the 4α-methyl groups shifts downfield to 1.8 ppm under the influence of a 6α-hydroxy group. As can be seen from Table 1, in the spectrum of cycloorbigenin (I) no such phenomenon was observed.

An additional confirmation of the conclusion about the position of the hydroxy group at C-7 may also be considered to be the fact that the H-8 signal in the PMR spectrum of the diketone (IV) has been converted into a singlet and is observed at 2.80 ppm (C_5D_5N) and 2.83 ppm ($CDCl_3$) [12].

Cleavage of the C-24—C-25 bond in the mass-spectrometric fragmentation of genin (I), leading to the appearance of the maximum peak of ion *a* with m/z 429 permits the assumption that one of the epoxide oxygen atoms is attached to C-24.

In the PMR spectrum of cycloorbigenin (I) a singlet signal is observed at 3.58 ppm and a quartet signal at 4.62 ppm, each of one proton unit. The signals scarcely changed their positions in the spectrum of the diacetate (II) and, as was to be expected, were retained in the spectrum of the diketone (IV). Consequently the signals under consideration belong to protons geminal to epoxy functions. The multiplicity of the gem-epoxide protons shows that they are located vicinally, i.e., the oxygen atom of the second epoxy function is attached to C-23. This means that the observed singlet signal (3.58 ppm) belongs to H-24 and the quartet signals (4.62 ppm) to H-23. The correctness of this assignment is also confirmed by the paramagnetic shift of the H-24 signal in the PMR spectrum of the triacetate (III) by 0.4 ppm in comparison with the analogous index of the spectrum of the diacetate (II). The detection of the signals of only two gem-epoxide protons permits the assumption that the epoxide rings form a ketal system.

In the PMR spectrum of genin (I), one-proton doublets of an AB system with the SSCC ^2J = 15 Hz are observed at 2.46 and 2.70 ppm. These signals must be assigned to the protons of an isolated methylene group connected to a ketal carbon atom. On this basis, the latter is C-16.

Thus, cycloorbigenin (I) has a side chain similar to the side chain of cimigenol [13, 14]. The values of the chemical shifts, the multiplicities, and the SSCCs of H-23 in the spectra of compounds (II) and (IV) practically coincide with those for cimigenol [13]. A consideration of the H-23–H-24 and 2H-22–H-23 bihedral angles and the SSCCs following from them similar to that performed in [13] permits the conclusion of the identity of the C-16, C-23, and C-24 chiral centers of cycloorbigenin and cimigenol [13, 14].

Thus, cycloorbigenin (I) has the structure of 16β,23:16α,24-diepoxy-23(R),24(S)-cycloartane-3β,7β,25-triol.

EXPERIMENTAL

General Observations. Thin-layer chromatography (TLC) was performed on Silufol plates. In TLC, the compounds were detected by spraying the plates with a 25% methanolic solution of tungstophosphoric acid followed by heating at 100-110°C for 2-5 min. Silica gels of types KSK and L (Czechoslovakia) with grain sizes of 50-100 μ, were used for column chromatography. The following solvent systems were employed: 1) chloroform–methanol–water (70:12:1); 2) benzene–ethyl acetate (5:1).

Mass spectra were obtained on a MKh-1310 instrument at an ionizing voltage of 50 V and a temperature of 130-170°C. IR spectra were recorded on a UR-20 spectrophotometer in KBr, and PMR spectra on XL-100 (Varian) and Tesla BS-567 A spectrometers in deuteropyridine or deuterochloroform (δ, ppm, 0 – HMDS).

Isolation of the Cycloartane Triterpenoids. The dried and comminuted epigeal parts of the plant Astragalus orbiculatus collected in June, 1985 (Tashkent province, Ugamskii range, environs of the village of Aktash) (7 kg) were exhaustively extracted with methanol (150 liters) at room temperature. The methanolic extract was evaporated to a viscous syrupy consistency and was dissolved in 2 liters of water and the solution was filtered. The filtrate was extracted successively with hexane, ethyl acetate, and n-butanol. The dried ethyl acetate extract (156 g) was chromatographed on a column with elution first by chloroform and then by system 1. The quantitatively main glycosides of the epigeal parts of Astragalus orbiculatus were isolated (yield 0.6% on the air-dry raw material).

Cycloorbigenin (I). The isolated glycoside (220 mg) was dissolved in 100 ml of methanol containing 0.5% of sulfuric acid. The reaction mixture was boiled in the water bath for 1.5 h. The reaction products were diluted with water, and the methanol was evaporated off. The precipitate that deposited was filtered off, washed with water, and dried. Recrystallization from methanol yielded 90 mg of cycloorbigenin (I), $C_{30}H_{48}O_5$, mp 217-219°C, $[\alpha]_D^{20}$ +28.3 ± 2° (c 1.19; ethanol). ν_{max}^{KBr}, cm^{-1}: 3525-3215 (OH); 3040 (CH_2 of a cyclopropane ring). Mass spectrum, m/z (%): M^+ 488 (1.6), 473 (5.0), 470 (5.3), 455 (5.3), 452 (1.6), 437 (1.9), 429 (100), 411 (18.5), 400 (2.1), 393 (4.0), 374 (5.8), 332 (7.9), 290 (8.2), 272 (10.3).

The 3,7,25-Triacetate (III) and 3,7-Diacetate (II) of Cycloorbigenin from (I). Cycloorbigenin (I) (148 mg) was acetylated with 2 ml of acetic anhydride in 4 ml of absolute pyridine at room temperature for 72 h. The residue after evaporation of the solvents was chromatographed on a column with elution by system 2. This led to the isolation of 10 mg of the triacetate (III), $C_{36}H_{54}O_8$, mp 137-139°C (from ethanol), $[\alpha]_D^{20}$ +75 ± 2° (c 0.4; methanol). ν_{max}^{KBr}, cm^{-1}: 3040 (CH_2 of a cyclopropane ring), 1740, 1245 (ester groups). Mass spectrum, m/z (%): M^+ 614 (0.25), 599 (3.8), 555 (84.6), 554 (84.6), 539 (84.6), 512 (84.6), 495 (84.6), 484 (9.0), 479 (84.6), 453 (87.1), 419 (38.5), 411 (10.3), 393 (100), 365 (41.0), 253 (66.7).

On continuing the elution of the column with the same system, 150 mg of the diacetate (II) was isolated; $C_{34}H_{52}O_7$, mp 148-150°C (from ethanol), $[\alpha]_D^{20}$ +32.6 ± 2° (c 0.92, methanol). ν_{max}^{KBr}, cm^{-1}: 3505 (OH); 3040 (CH_2 of a cyclopropane ring); 1740, 1720, 1245 (ester groups). Mass spectrum, m/z (%): M^+ 572 (0.64), 557 (6.4), 554 (2.1), 539 (0.9), 529 (0.5), 513 (84.6), 512 (100), 497 (46.1), 494 (3.4), 484 (4.4), 479 (2.2), 453 (84.6), 452 (92.3), 437 (31.9), 419 (5.5), 393 (53.8), 253 (23.0).

25-Hydroxy-16β,23:16α,24-diepoxy-23(R),24(S)-cycloartane-3,7-dione (IV) from (I). At −5°C, 0.15 ml of the Jones reagent [3] was added to 90 mg of cyclorbigenin (I) in 10 ml of acetone, and the mixture was stirred for 10 min. Then 1.5 ml of methanol was added to the reaction mixture to decompose the excess of oxidant. The reaction products were poured into water and extracted with chloroform. The residue after the usual working up and evaporation of the chloroform extract was recrystallized from methanol. This gave 50 mg of the diketone (IV), $C_{30}H_{44}O_5$, mp 155-158°C, $[\alpha]_D^{20}$ −73 ± 2° (c 0.63; methanol); ν_{max}^{KBr}, cm^{-1}: 3510-3430 (OH); 3050 (CH_2 of a cyclopropane ring); 1710-1695 (C=O at C-3 and C-7). Mass spectrum, m/z (%): M^+ 484 (1.3), 469 (3.0), 455 (3.5), 425 (100), 411 (8.5), 409 (5.7), 407 (2.1), 396 (3.7), 383 (3.3), 369 (14.3), 343 (4.2), 327 (8.6), 285 (5.0).

SUMMARY

A new methylsteroid of the cycloartane series — cycloorbigenin — has been isolated from the epigeal plant of the plant Astragalus orbiculatus (Leguminosae); it has the structure of 16β,23:16α,24-diepoxy-23(R),24(S)-cycloartane-3β,7β,25-triol.

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