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STEROIDS OF THE SPIROSTAN AND FUROSTAN SERIES FROM PLANTS OF THE GENUS Allium.

XXV. STRUCTURE OF ANZUROGENIN B FROM Allium suvorovii AND Allium Stipitatum

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A new genin of the spirostan series – anzurogenin B having the structure of 2α,5α-epoxy-(25R)-spirostan-3β,6β-diol – has been isolated form the collective fruit of the cocultivated Allium suvorovii Rgl. and Allium stipitatum Rgl. (family Liliaceae).

In a preceding communication [1] we showed that the collective fruit of the cocultivated Allium survorovii Rgl. and Allium stipitatum Rgl. (family Liliaceae) – local name anzur – was the source of five aglycons of the spirostan series. Two of them proved to be new. They were called anzurogenins A [1] and B. The present publication is devoted to a proof of the structure of anzurogenin B (I).

The characteristic color reaction with vanillin–phosphoric acid [2, 3] and a distinctive series of bands in the 800-1000 cm^{-1} region of the IR spectrum [3-5] permitted genin (I) to be assigned to derivatives of the (25R)-spirostan series.

The correctness of this assignment was also shown by the elementary composition of the molecular ion M^+ 446 ($C_{27}H_{42}O_5$), the nature of its mass-spectrometric fragmentation [6], and its ^{13}C and ^1H NMR spectra (Tables 1 and 2, respectively) [3, 7].

In the ^{13}C NMR spectrum of anzurogenin B taken with retention of carbon-proton interaction (GD spectrum), there are six signals in the region of resonance of the carbon atoms linked to one oxygen atom. One of them has triplet splitting and a chemical shift (CS) of 66.96 ppm, which are characteristic for C-26 of genins of the spirostan series. A signal with a CS of 90.42 ppm is a singlet and the other four are doublets. Three signals are worthy of note: the above-mentioned singlet at 90.42 ppm, and two doublets at 81.26 ppm and 80.38 ppm. While one of the doublets is naturally assigned to the C-16 resonance, the CSs of the second doublet and of the singlet must be regarded as unusual for carbon atoms with unsubstituted secondary and tertiary OH groups, respectively.

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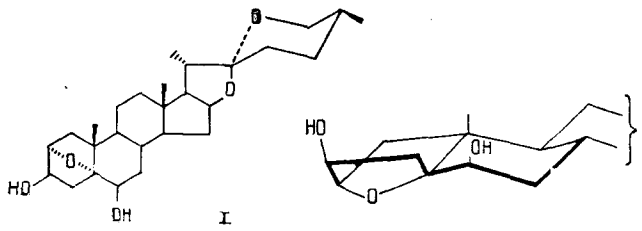
TABLE 1. Chemical Shifts of the Carbon Atoms of Anzurogenin B (C_5D_5N, δ, ppm, 0 – TMS)

Carbon atom	Chemical shift	Carbon atom	Chemical shift
1	41,45; t	15	32,25; t
2	80,38; d	16	81,26; d
3	72,12; d	17	63,19; t
4	38,26; t	18	16,69; q
5	90,42; s	19	17,21; q
6	70,90; d	20	42,13; d
7	37,31; t	21	15,05; q
8	30,19; d	22	109,30; s
9	50,23; d	23	31,90; t
10	45,66; s	24	29,34; t
11	21,67; t	25	30,67; d
12	40,61; t	26	66,96; t
13	41,2 ; s	27	17,21; q
14	56,57; d		

TABLE 2. Chemical Shifts (C_5D_5N, δ, ppm, 0 – TMS) and SSCCs (J, Hz) of the Protons of Anzurogenin B

Protons	CS; SSCC	Protons	CS; SSCC
CH_3-18	0,89; s	H-4e	2,26; dd
CH_3-19	1,58; s	H-6e	4,36; dt, $J_{6e, 6\text{-OH}} = 4,0$; $J_{6e, 7a} = 2,8$; $J_{6e, 7e} = 2,8$
CH_3-21	1,12; d, $J_{21, 20} = 7,0$		
CH_3-27	0,65; d, $J_{27, 25} = 6,0$		
H-1a	1,74; dd, $J_{1a, 1e} = 12,1$; $J_{1a, 2e} = 5,5$	H-7a	1,72; dt, $J_{7a, 7e} = 13,1$; $J_{7a, 8a} = 13,1$
H-1e	2,44; d	H-7e	1,99; dt, $J_{7e, 8a} = 2,8$
H-2e	4,54; t, $J_{2e, 3e} = 5,5$	H-16	4,53; ddd, $J_{16, 17} = 8,2$; $J_{16, 15} = 7,8$; $J_{16, 15'} = 6,3$
H-3e	4,72; m, $J_{3e, 3\text{-OH}} = 3,5$; $J_{3e, 4e} = 10,0$; $J_{3e, 4a} = 3,4$	H-26a	3,45; t, $J_{26a, 25a} = 10,5$; $J_{26a, 26e} = 10,5$
H-4a	2,57; dd, $J_{4a, 4e} = 13,1$	H-26e	3,55; d,d, $J_{26e, 25a} = 4,5$
		OH-3	6,61; d
		OH-6	6,16; d

In the PMR spectrum of aglycon (I) there are six signals in the region of resonance of protons at carbon atoms linked to one oxygen atom. Two of them (a triplet with a CS of 3.45 ppm and a doublet of doublets with a CS of 3.55 ppm) form the AB part of the ABX system that is typical for the protons at C-26 of spirostans of the (25R)-series. Judging from its CS and multiplicity, a doublet of doublets at 4.53 ppm relates to a proton at C-16. To the remaining three signals with CSs of 4.36, 4.54 and 4.72 ppm correspond only two doublets of the protons of hydroxy groups. Protons resonating in the form of a doublet at 6.16 ppm and of a doublet of triplets at 4.36 ppm are linked by a spin–spin coupling constant (SSCC). Protons resonating in the form of a doublet with a CS of 6.61 ppm and a multiplet at 4.72 ppm also have a common SSCC.



I

An experiment on selective double homonuclear resonance showed that the protons having CSs of 4.72 and 4.54 ppm were linked by an SSCC of 5.5 Hz, i.e., they are vicinal. Irradiation of the proton with a CS of 4.54 ppm led to the appearance in the double-resonance difference spectrum of a doublet at 2.44 ppm and a doublet of doublets with a CS of 1.74 ppm. In each of the last-mentioned signals there was a large SSCC of 12.1 Hz, which is

characteristic for the interaction of geminal protons. Consequently, the protons under discussion form a methylene group present in the α-position to the carbon atom bearing the proton with a CS of 4.54 ppm. Irradiation of the proton resonating at 4.72 ppm permitted the revelation in the double-resonance difference spectrum of two doublets of doublets with CSs of 2.26 and 2.57 ppm. These signals correspond to the protons of another CH_2 group (each of them having an SSCC of 13.1 Hz), which is present in the α-position to the carbon atom bearing the proton with the CS of 4.72 ppm.

The combination of facts presented permits the statement that the protons at 4.72 and 4.54 ppm belong to two neighboring carbon atoms, one of which bears a hydroxy group and the other is linked to oxygen not forming part of an OH group. Both the carbon atoms mentioned can be present only in ring A of anzurogenin B (C-2 and C-3 positions). There are methylene protons at C-1 and C-4, and there is no proton at C-5.

The proton resonating at 4.36 ppm is characterized not only by an SSCC with the proton of the geminal hydroxy group but also by two other SSCCs (2.8 Hz each) with protons having CSs of 1.72 and 1.99 ppm (doublet of triplets in the double-resonance difference spectrum). The latter are linked with one another by a geminal SSCC of 13.1 Hz, i.e., they belong to a methylene group. The preirradiation of the proton with the CS of 4.36 ppm led to the appearance in the difference spectrum, on the observation of nuclear Overhauser effects (NOEs), of signals at 1.72, 1.99, and 2.26 ppm. As was shown above the proton with a CS of 2.26 ppm is present in ring A of genin (I). Consequently, the resonance at 4.36 ppm was that of a proton linked to C-6. Judging from the nature of the splitting of its signal and the values of the SSCCs with the protons of the neighboring CH_2 group, H-6 is oriented equatorially. In addition, this confirms the absence of a proton at C-5.

Preirradiation of the proton with a CS of 2.26 ppm led to the appearance in the NOE difference spectrum of signals at 2.57, 4.72, and 4.36 ppm. Since the protons resonating at 2.26 and 2.57 ppm belonged to a methylene group, it becomes obvious that they are attached to C-4. It was shown above that both these protons have SSCCs with the proton the signal of which is located at 4.72 ppm. Consequently, the latter is present at C-3. The facts presented permit the unambiguous assignment of the other three protons of ring A of aglycon (I) (Table 2).

A consideration of molecular model shows that with any type of linkage of rings A and B the equatorial proton C-6 can be close only to the equatorial proton at C-4. Thus, an experiment with the observation of NOEs showed that the proton with a CS of 2.26 ppm must be equatorial and that with a CS of 2.57 ppm axial. However, the values of the SSCCs of the protons at C-4 with H-3 are completely inexplicable for the chair conformation of ring A. The equatorial proton has the SSCC $J_{3,4}$ = 10.0 Hz and the axial proton one of 3.4 Hz. On the other hand, such constants are completely normal for the boat conformation where the two equatorial protons at C-3 and C-4 form an eclipsed conformation (the dihedral angle between C–H bonds is close 0°). The boat conformation is energetically unsuitable and has never been detected for ring A in natural steroids of the spirostan series. However, if the facts mentioned above are taken into account (the absence of a hydroxy group at C-2 and of a proton at C-5, and the weak-field CSs of two carbon atoms linked to oxygen), an unusual conformation of ring A in anzurogenin B with the formation of an ether bond between C-2 and C-5 becomes acceptable.

To confirm this hypothesis we performed an experiment with the observation of the NOE on the preirradiation of the proton with a CS of 2.57 ppm at C-4. Together with the appearance in the difference spectrum of the expected signals with CSs of 2.26 ppm (H-4) and 4.72 ppm (H-3), a signal of the CH_3-19 protons was also clearly seen in the spectrum, and there was no H-6 signal. The increase in the intensity of the CH_3-19 singlet in the experiment described showed unambiguously that the C(2)–O and C(5)–O bonds have the α-orientation. Thus, the proton at C-4 with a CS of 2.57 ppm and a small SSCC with H-3 does in fact occupy the axial position, and the proton at C-4 with a CS of 2.26 ppm the equatorial position, which is possible only if ring A has the boat conformation.

The signals in the ^{13}C NMR spectrum of anzurogenin B (see Table 1) were assigned with the use of the method of selective double heteronuclear resonance and also by a comparison with the spectra of other genins of the (25R)-spirostan series [7]. The multiplicities in the GD spectrum were also taken into account. The weak-field position of the C-2 and C-5 signals is a confirmation of the formation of an additional cyclic system in ring A.

The above discussions permits anzurogenin B to be assigned the structure of 2α,5α-epoxy-(25R)-spirostan-3β,6β-diol.

EXPERIMENTAL

For the "General Observations" and "Preliminary working up of the total extractive substances" sections, see [1].

Anzurogenin B (I). Part (200 g) of the combined material (III) was chromatographed on silica gel in system 2. After repeated rechromatography of the fractions containing genin (I) in systems 1b and 1c, 150 mg of anzurogenin B was obtained: $C_{27}H_{42}O_5$ mp 210-212°C (from methanol), $[\alpha]_D^{24}$ −32.9 ± 2° (c 0.98; pyridine), ν_{max}^{KBr} (cm^{-1}); 850, 875, 910 > 930, 990 (spiroketal chain of the (25R)-series), 3300, 3430, 3490 (OH). Mass spectrum, m/z (%): M^+ 446(5), 431 (0.3), 416(0.5), 387(1.5), 377(2.5), 374(5), 332(13), 317(4), 314(12), 303(8), 139(100), 126(1.5), 122(8), 115(7).

The yield calculated on the weight of the air-dry raw material was 0.0006%.

SUMMARY

A new genin of the spirostan series – anzurogenin B, which is 2α,5α-epoxy-(25R)-spirostan-3β,6β-diol – has been isolated form the collective fruit of the cocultivated Allium suvorovii Rgl. and Allium stipitatum Rgl.

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