STEROIDS OF THE SPIROSTAN AND FUROSTAN SERIES FROM PLANTS OF THE GENUS Allium. STRUCTURE OF ANZUROGENIN A FROM Allium suvorovii AND A. stipitatum

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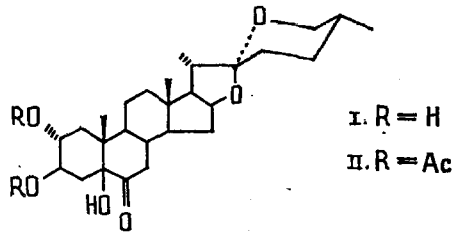
The collective fruit of the cocultivated Allium suvorovii Rgl. and A. stipitatum Rgl. (family Liliaceae) has yielded a new genin of the spirostan series – anzurogenin A, which is 2α,3β,5β-trihydroxy-(25R)-spirostan-6-one.

Continuing a study of the steroids of the spirostan and furostan series from plants of the genus Allium [1], we selected as the next object of chemical investigation the collective fruit of the onion anzur. Anzur is a local name for several species of Central Asian onions. At the present time, it is predominantly two species of allium that are cultivated under this name: A. suvorovii Rgl. and A. stipitatum Rgl. (family Liliaceae).

After the preliminary treatment of the total extractive substances, five genins of the spirostan series were isolated from various fractions of them. Three of them were native and the other two were the products of the acid hydrolysis of the purified combined glycoside. Known substances were identified among the aglycons isolated: diosgenin and yuccagenin (among the products of acid hydrolysis), and also alliogenin. Two of the native compounds proved not to have been described previously, and they have been called anzurogenins A and B. The present publication is devoted to a proof of the structure of anzurogenin A (I).



The characteristic color reaction with vanillin/phosphoric acid [2, 3] and the specific series of bands in the 800-1000 cm^{-1} region of the IR spectrum [3-5] enabled the aglycon (I) to be assigned to derivatives of the (25R)-spirostan series. In addition to the band characterizing the spiroketal grouping, the IR spectrum of anzurogenin A contained a narrow intense band at 1712 cm^{-1}. In the ^{13}C NMR spectrum of genin (I) taken under the conditions of the retention of carbon–proton interaction (GD spectrum) there was a singlet at 212.30 ppm. Consequently, the molecule of the aglycon (I) contains a keto group present in a six-membered ring.

Analysis of the CD curve of anzurogenin A ($[\theta]_{286} = -15{,}325°$, $\Delta\varepsilon = -4.64$) permitted the assumption that with the cis-linkage of rings A/B (5β), the keto grouping was located at C-6 [6].

The nature of the mass-spectrometric fragmentation of genin (I) (the presence of the peaks of ions with m/z 403, 393, 390, 348, 139), in its turn, indicated that anzurogenin A belonged to the spirostan aglycons [7]. The elementary composition of the molecular ion M^+ 462 ($C_{27}H_{42}O_6$) showed that, in addition to the keto function, the molecule of compound (I) contained three hydroxy groups.

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TABLE 1. Chemical Shifts (δ, ppm, 0 − TMS) and SSCCs (J, Hz) of the Protons of Anzurogenin A [(I), C_5D_5N] and Its Diacetate [(II), $CDCl_3$]

Proton	Compound I	Compound II
CH_3-18	0,80; s	0,77; s
CH_3-19	0,96; s	0,79; s
CH_3-21	1,09; d, $J_{21,20}$ = 7,0	0,98; d, $J_{21,20}$=6,5
CH_3-27	0,65; d $J_{27,25}$ = 5,5	0,79; d, $J_{27,25}$=6,1
2H-1	2,10; d $J_{1,2e}$ = 3,0	1,92; d, $J_{1,2e}$ = 3,4
H-2e	4,46; m $J_{2e,3e}$ = 3,0 $J_{2e,4e}$=1,2	5,05; m, $J_{2e,3e}$=3,0 $J_{2e,4e}$=1,2
H-3e	4,33; m, $J_{3e,4a}$ =3,6 $J_{3e,4e}$ =3,0	4,88; m, $J_{3e,4a}$=4,6 $J_{3e,4e}$=2,6
H-4a	3,01; dd, $J_{4a,4e}$ =14,0	2,46; dd, $J_{4a,4e}$=15,1
H-4e	1,90; ddd	1,86; ddd
H-7a	2,36; dd, $J_{7a,8a}$ =11,5 $J_{7a,7e}$ =13,6	2,31; dd, $J_{7a,8a}$=12,4 $J_{7a,7e}$=13,8
H-7e	2,44; dd, $J_{7e,8a}$ =5,9	2,48; dd, $J_{7e,8a}$=4,5
H-8a	1,91; ddd, $J_{8a,9a}$ =11,0	
H-9a	2,78; dt, $J_{9a,11a}$=11,0 $J_{9a,11e}$=4,1	
H-16	4,55; ddd, $J_{16,17}$=8,2 $J_{16,15}$=7,9 $J_{16,15'}$=5,6	4,44; ddd, $J_{16,17}$=8,5 $J_{16,15}$=8,0 $J_{16,15'}$=6,1
H-17	1,78; dd, $J_{17,20}$=6,5	1,81; dd, $J_{17,20}$=6,6
H-26a	3,45; t, $J_{26a,25a}$=10,5 $J_{26a,26e}$=10,5	3,36; t, $J_{26a,25a}$=11,0 $J_{26a,26e}$=11,0
H-26e	3,55; dd, $J_{26e,25a}$=4,0	3,48; ddd, $J_{26e,25a}$=4,6 $J_{26e,24e}$=1,9
OH-2	6,58; d, $J_{OH-2,2e}$ =3,6	
OH-3	5,76; d, $J_{OH-3,3e}$ = 7,9	
OH-5	6,89; s	
2×CH_3COO		2,09; s, 2,07; s

TABLE 2. Chemical Shifts of the Carbon Atoms of Anzurogenin A (C_5D_5N, δ, ppm, 0 − TMS)

Carbon atom	Chemical shift	Carbon atom	Chemical shift
1	32,53; t	15	33,61; t
2	71,70; d	16	81,06; d
3	71,70; d	17	62,90; d
4	42,66; t	18	16,64; q
5	83,46; s	19	18,15; q
6	212,30; s	20	42,13; d
7	32,04; t	21	15,10; q
8	37,26; d	22	109,40; s
9	45,65; d	23	32,04; t
10	45,24; s	24	29,37; t
11	22,77; t	25	30,71; d
12	39,85; t	26	67,06; t
13	41,34; s	27	17,43; q
14	56,72; d		

The acetylation of aglycon (I) gave a diacetate (II) (M^+ 546), the IR spectrum of which showed at 3490 cm^{-1} a narrow band characterizing the absorption of a hydroxy group. In the ^{13}C NMR GD spectrum of anzurogenin A, one of the signals of the carbinol atoms appeared in the form of a singlet at 83.46 ppm. It followed from this that one of the OH groups in the molecule of genin (I) was tertiary.

Compound (I) was cleaved with sodium periodate. The reaction took place fairly slowly and was complete after 48 h, which permits the assumption of the presence of a trans-α-diol grouping in the aglycon (I).

The PMR spectrum of anzurogenin A (I) contained all the signals that are reference signals for assignment to the genins of the spirostan series (Table 1) [3].

The chemical shifts (CSs) of the CH_3-27 doublet (0.65 ppm), and also of the H-26a triplet (3.45 ppm) and of the H-26e doublets of doublets (3.55 ppm) unambiguously showed the equatorial orientation of CH_3-27. This confirmed the assignment of genin (I) to the spirostans of the (25R)-series.

In the region of resonance of OH groups in the weak-field part of the PMR spectrum of anzurogenin A there were a one-proton singlet (at 6.89 ppm) and two one-proton doublets (at 6.58 and 5.76 ppm). It was shown by the double-resonance method that two multiplets lying in the region of resonance of protons attached to carbon linked to oxygen (at 4.46 and 4.33 ppm) corresponded to protons geminal to hydroxy groups. It was also shown that these protons had a common spin-spin constant (SSCC) of 3.0 Hz. This fact confirmed the presence of an α-diol grouping in the aglycon (I).

In the PMR spectrum of the diacetate (II), these signals were shifted downfield and were located at 5.05 and 4.88 ppm. A double-resonance experiment in the ordinary and the difference variants showed that the proton to which the multiplet with a CS of 5.05 ppm corresponded interacted with the protons giving a multiplet at 4.88 ppm (2.6 Hz) and a two-proton doublet at 1.92 ppm (3.4 Hz) and had a small SSCC (1.2 Hz) with the doublet of doublets at 1.86 ppm. It was also shown that the signal with a CS of 4.88 ppm had an SSCC with the multiplet at 5.05 ppm, with the doublet of doublets at 2.46 ppm (4.6 Hz) and with the doublet of doublets at 1.86 ppm (2.6 Hz).

Due to the nuclear Overhauser effect (NOE), the preirradiation of the CH_3-19 protons in the PMR spectrum of anzurogenin A (I) led to an increase in the intensity of the two-proton doublet with a CS of 2.10 ppm. Consequently, this signal belonged to the protons at C-1 (coincidence of the H-1a and H-1e CSs). Therefore, the two-proton doublet at 1.92 ppm in the PMR spectrum of the diacetate (II) was likewise assigned to the protons at C-1. In this case there is no doubt that the vicinal protons with CSs of 5.05 and 4.88 ppm were located at C-2 and C-3, respectively. This means that the signal with a CS of 2.46 ppm belonged to H-4a, and the doublet of doublets at 1.86 ppm to H-4e. The values of the SSCCs of the multiplets with CSs of 5.05 and 4.88 ppm indicated the equatorial orientation of the corresponding protons. Thus, the α-diol grouping in the molecule of the genin (I) is trans-diazial. It must be mentioned that the small SSCC between H-2e and H-4e mentioned above is due to a long-range spin-spin interaction (^4J) caused by the W-conformation of the corresponding section of ring A. The multiplicity of the signals of the protons at C-4 excludes the presence of a proton at C-5. It is obvious that the tertiary OH group is located at C-5 of the aglycon (I).

A series of experiments on double resonance and on the NOE in the difference variant enabled the type of linkage of rings A/B and the assignment of the protons of ring A to be confirmed and also permitted the assignment of the signals of the protons at C-7 (and thereby the localization of the keto group at C-6), C-8, C-9, and C-17 in the PMR spectrum of compound (I) to be unambiguously determined (see Table 1).

The preirradiation of the proton of the OH group at C-5 of the genin (I) led to a clear NOE signal for the CH_3-19 protons. This unambiguously determined the cis-linkage of rings A/B.

The signals in the ^{13}C NMR spectrum of the aglycon (I) (Table 2) were assigned partially with the aid of selective double heteronuclear resonance and partially by comparison with literature figures [8]. The multiplicities of the lines in the GD spectrum were also taken into consideration.

Thus, anzurogenin A has the structure of 2α,3β,5β-trihydroxy-(25R)-spirostan-6-one.

EXPERIMENTAL

General Observations. Thin-layer chromatography (TLC) was performed on Silufol plates. For column chromatography we used alumina, and also silica gel of types KSKG and L (particle dimensions <63 and 63-100 μm). The following solvent systems were used: 1) chloroform–methanol [a – (100:1); b – (50:1); c – (20.1); d – (10:1)]; and 2) chloroform–methanol–water (65:15:2).

The steroids were detected with vanillin/phosphoric acid.

IR spectra were taken on a UR-20 instrument in tablets with KBr or in Nujol. Mass spectra were measured on a MKh-1310 mass spectrometer. CD curves were recorded on a JASCO J-20 spectropolarimeter, and ^1H and ^{13}C NMR spectra on WM-250 and AM-300 instruments, respectively. The solvents were C_5D_5N and $CDCl_3$, and the standard was TMS.

Preliminary Treatment of the Total Extractive Substances. The collective fruit of Allium suvorovii and A. stipitatum (after the separation of the ripe seeds) was collected in June 1985, in the Varganza sovkhoz [collective farm], Kitab region, Kashkadar'ya province, Uzbek SSR. Since the two onions are cultivated together and are morphologically very close, the plant material was not separated by species.

The air-dry raw material (80 kg) was exhaustively extracted with ethanol (6 × 300 liters). The combined extracts were evaporated to a volume of 15 liters and after the addition of an equal volume of water the ethanol was distilled off. The residue was treated with hexane (6 × 2 liters). The solution purified in this way was extracted with n-butanol (6 × 2 liters). The aqueous solution remaining after the butanolic extraction was concentrated, sulfuric acid was added to give a concentration of 7.5%, and the glycosides were hydrolyzed at 100°C for 6 h. The precipitate that had deposited was separated off, washed with water, and dried. The weight of hydrolysis product was ~400 g [the sum (IV)].

The hexane extract was concentrated and the resinous residue was dried to constant weight on silica gel [the sum (I), ~600 g].

The butanolic extracts were evaporated to dryness and the residue was dissolved in portions in methanol, and each portion was precipitated with a tenfold volume of acetone. As a result, a total of ~2.4 kg of precipitate was obtained [the sum (II)]. The acetone solution was concentrated and dried on silica gel [the sum (III), ~600 g].

Diosgenin. Part of the sum (II) (10 g) was hydrolyzed at 100°C in 100 ml of 50% aqueous methanol containing 7.5% of sulfuric acid for 6 h. After the end of the reaction, 50 ml of water was added, the methanol was distilled off, and the precipitate that had deposited was separated off, washed with water, and dried. This gave 3.35 g of hydrolysis product. After chromatography on alumina (with system 1a as eluent) and recrystallization of the corresponding fractions from methanol, 75 mg of diosgenin was isolated with mp 198-199°C, $[\alpha]_D^{24}$ −124.8 ± 2° (c 1.01; chloroform). According to the literature: mp 208°C, $[\alpha]_D$ −129° [9].

By the same method, the sum (IV) (100 g) gave 1.2 g of diosgenin. The yield calculated on the weight of the air-dry raw material was 0.028%.

Yuccagenin. When the chromatography of the hydrolysis products of the sum (II) was continued (with system 1d as eluent), 360 mg of yuccagenin was isolated with mp 242-244°C (from methanol), $[\alpha]_D^{24}$ −121.8 ± 2° (c 0.94; chloroform); according to the literature: mp 248°C, $[\alpha]_D$ −122° [9].

Under the same conditions, the sum (IV) (100 g) provided 4.3 g of yuccagenin. The yield was 0.129%.

Alliogenin. Part of the sum (I) (200 g) was subjected to chromatography on silica gel with elution by system 1a and then system 2. The fractions containing the desired product were collected with the use of system 2. Recrystallization from methanol led to the production of 6.7 g of alliogenin with mp 319-320°C, $[\alpha]_D^{24}$ −73.3 ± 2° (c 0.96; pyridine). According to the literature: mp 321-325°C, $[\alpha]_D$ −71.4° [10]. Yield 0.025%.

The chromatographic behavior (TLC) and spectral characteristics (IR, mass, and PMR spectra) of the three aglycons described above were identical with the corresponding indices of authentic samples.

Anzurogenin A (I). Part of the sum (III) (200 g) was chromatographed on silica gel in system 2. Repeated rechromatography in systems 1b and 1c of the fractions that had shown the presence of the genin (I) (TLC) yielded 1.0 g of anzurogenin A, $C_{27}H_{42}O_6$, mp 235-236°C (from methanol), $[\alpha]_D^{24}$ −81.8 ± 2° (c 1.08; pyridine); ν_{max}^{KBr} (cm^{-1}): 845, 870, 905>925, 985 [spiroketal chain of the (25R) series], 1712 (C=O), 3360-3370, 3470-3490 (OH). CD (c 1.04; ethanol), $[\theta]_{286}$ −15,325°, $\Delta\varepsilon$ −4.64.

Mass spectrum, m/z (%): M^+ 462(22), 447(1), 432(2), 403(10), 393(10), 390(27), 348(55), 333(6), 319(8), 139(100), 126(7), 115(3). Yield 0.004%.

Azurogenin A Diacetate (II) from (I). A solution of 200 mg of genin (I) in 5 ml of pyridine was treated with 2 ml of acetic anhydride, and the reaction mixture was left at room temperature for 96 h. After working up by the generally adopted method and recrystallization of the product from aqueous methanol, 143 mg of the diacetate (II), $C_{31}H_{46}O_8$, was obtained with mp 183-186°C, $[\alpha]_D^{24}$ −90.5 ± 2° (c 0.96; chloroform). ν_{max}^{Nujol} (cm^{-1}): 850, 875, 910>930, 960, 970, 990 [spiroketal chain of the (25R) series]; 1240, 1260, 1730, 1745, (CH_3COO–); 1712 (C=O); 3490 (OH). Mass spectrum, m/z (%): M^+ 546(29), 487(14), 477(10), 474(31), 432(16), 417(6), 372(66), 139(100).

CONCLUSIONS

The combined fruit of the cocultivated Allium suvorovii Rgl. and A. stipitatum Rgl. has yielded a new genin of the spirostan series – anzurogenin A, which is 2α,3β,5β-trihydroxy-(25R)-spirostan-6-one.

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TRITERPENEGLYCOSIDES OF Astragalus AND THEIR GENINS.

XXV. CYCLOCANTHOSIDE D FROM Astragalus tragacantha

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The epigeal part of the plant of Astragalus tragacantha Habl. (Leguminosae) has yielded, together with cyclosieversigenin 3-O-β-D-xylopyranoside, a new glycoside of the cycloartane series – cyclocanthoside D, the structure of which has been established on the basis of chemical transformations and spectral characteristics as 24S-cycloartane-3β,6α,16β,24,25-pentaol 16-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside.

The study of cycloartane derivatives isolated from the epigeal part of the plant Astragalus tragacantha (Leguminosae) [1] has continued. Glycoside 5 has been identified as cyclosieversigenin 3-O-β-D-xylopyranoside [2-4]. In the present paper we consider the structure of glycoside 12, which we have called cyclocanthoside D [(I), scheme].

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