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

XXVI. STRUCTURE OF ANZUROGENIN AND ANZUROSIDE FROM THE COLLECTIVE FRUITS OF Allium suvorovii AND Allium stipitatum

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A new steroid glycoside of the spirostan series, anzuroside, has been isolated from the collective fruits of the cocultivated Allium suvorovii Rgl. and A. stipitatum Rgl. (family Liliaceae, local name "anzur"). Enzymatic cleavage of the glycoside has given the previously undescribed steroid sapogenin anzurogenin C, which has the structure of (24S, 25S)-2α,3β,5,24-tetrahydroxy-5β-spirostan-6-one. Anzuroside is the 24-O-β-D-glucopyranoside of anzurogenin C.

Continuing a study of the steroids of the spirostan and furostan series of the collective fruits of the cocultivated Allium suvorovii Rgl. and A. stipitatum Rgl. (family Liliaceae, local name "anzur") [1, 2], we have isolated a new glycoside and have called it anzuroside (I).

The results of the analysis of the products of the methanolysis of compound (I) by the GLC method show that it is a monoglucoside.

Glycoside (I) formed a hexaacetate (II) (M^+ 892) in the IR spectrum of which there was a bond of hydroxyl absorption at 3485 cm^{-1}.

Under the conditions of methanolysis and Smith degradation [3], the aglycon of anzuroside underwent degradation. We succeeded in obtaining the native genin – which has been called anzurogenin C (III) – by enzymatic cleavage of the monoside (I). The IR spectra of aglycon (III) contained bands of hydroxyl (3300-3500 cm^{-1}) and carbonyl (1715 cm^{-1}) absorption.

The mass-spectrometric fragmentation of anzurogenin C showed that it belongs to derivatives of the spirostan series with a substituted ring F [4, 5]. This is witnessed by the peaks of ions with m/z 478 (M^+, $C_{27}H_{42}O_7$), 419, 393, 390, 348, 333, 319, 155, 131. A similar breakdown under the action of electron impact is undergone by karatavigenin C, which is (24S, 25S)-spirost-5-ene-2α,3β,24-triol [6] and by cepagenin, which has the structure of (24S, 25R)-spirost-5-ene-1β,3β,24-triol [7]. It follows from the facts given that in the molecule of aglycon (III), in addition to a keto group, there are four hydroxy groups, one of which is most probably located at C-24.

The CD curve of genin (III) was characterized by molecular ellipticity $[\theta]_{288}$ = 15,000° and by the differential dichronic absorption $\Delta\varepsilon = -4.54$, which indicates the presence in the molecule of a 6-keto fragment with cis-linkage of rings A/B [8].

The acetylation of anzurogenin C gave a triacetate (IV) (M^+ 604), the IR spectrum of which contained bands of hydroxyl absorption at 3470 and 3500 cm^{-1}.

It has been shown previously that 24-hydroxy derivatives of yuccagenin [6] and of (25S)-ruscogenin [7] are found in onions. We are therefore justified in assuming that anzurogenin C is the 24-hydroxy derivative of anzurogenin A – (25R)-2α,3β,5-trihydroxy-5β-

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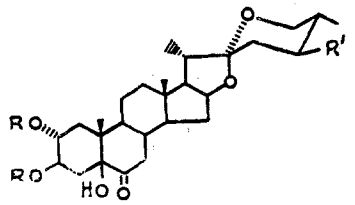
TABLE 1. Chemical Shifts of the Carbon Atoms of Anzuroside (I) and of Anzurogenins C (III) and A (V) (C_5D_5N, δ, ppm, 0 – TMS)

Carbon atom	Compound			Carbon atom	Compound		
	I	III	V		I	III	V
1	32,53	32,50	32,53	18	16,59	16,54	16,64
2	71,68	71,69	71,70	19	18,18	18,12	18,15
3	71,68	71 69	71,70	20	42,26	42,34	42,13
4	42,83	42,79	42,66	21	14,96	15,01	15,10
5	83,47	83,43	83,46	22	111,81	111,90	109,40
6	212,35	212,25	212,30	23	40,95	41,87	32,04
7	31,92	31,99	32,04	24	81,50	70,67	29,37
8	37,20	37,21	37,26	25	38,27	39,95	30,71
9	45,60	45,61	45,65	26	65,35	65,46	67,06
10	45,24	45,18	45,24	27	13,58	13,67	17,43
11	22,73	22,72	22,77		β-D-Glucopyranose		
12	39,75	39,75	39,85	1	106,36		
13	41,31	41,30	41,34	2	75,68		
14	56,67	56,70	56,72	3	78,06		
15	33,59	33,59	33,61	4	71,92		
16	81,50	81,37	81,06	5	78,64		
17	62,44	62,51	62,90	6	63,04		

spirostan-6-one (V) – which we isolated from the collective fruits of the onion anzur [1].



I. R = H, R′ = β-D-Glc$_p$
II. R=Ac, R′=β-D-Glc$_p$ — tetraacetate
III. R=H, R′=OH
IV. R=Ac, R′=OAc
V. R = R′ = H

A comparison of the chemical shifts (CSs) of the carbon atoms of these aglycons (Table 1) shows that they are practically identical for the C-1–C-21 atoms. The difference in the values of the CSs of the carbon atoms forming ring F can be explained only by the presence of a hydroxy group at C-24 of compound (III).

A comparative analysis of the PMR spectra of anzurogenins A (V) [1] and C (Table 2) showed the presence in the latter of a doublet of triplets at 3.98 ppm, absent from the former, and a shift of the doublet corresponding to the CH_3-27 resonance by 0.40 ppm. The chemical shifts and spin-spin coupling constants of the other protons differ extremely slightly. Thus, the NMR spectra indicate the identity of the steroid moieties of the aglycons being compared.

A doublet of triplets with the CS 3.98 ppm in the spectrum of compound (III) must be assigned to the resonance of the proton geminal to the hydroxy group at C-24. The SSCC of this proton shows its axial orientation. Consequently, C-24 has the S-configuration.

In a consideration of the signals due to the resonance of the protons at C-26, it becomes obvious, that H-25 is oriented axially [9]. Thus, C-25 also has the S-configuration.

The downfield shift of the CH_3-27 doublet in the spectrum of genin (III) is due to the influence of the equatorial hydroxy group at C-24.

On the basis of what has been said above, anzurogenin C must be assigned the structure of (24S, 25S)-2α,3β,5,24-tetrahydroxy-5β-spirostan-6-one.

Analysis of the PMR spectra of anzuroside and its hexaacetate (II) (see Table 2) confirmed the presence of one β-D-glucopyranose residue in the molecule of the glycoside (I) [10]. It can be seen from the figures given in Table 2 that on passing from the triacetate (IV) to the hexaacetate (II) the signal corresponding to the H-24 underwent a pronounced upfield shift. Thus, in the glycoside (I) the sugar is attached to the aglycon by the hydroxy group at C-24. A comparison of the CSs of the carbon atoms in the ^{13}C NMR spectra of the genin (III) and the monoside (I) (see Table 1) confirmed this conclusion.

The result of the glycosylation of the OH group at C-24 of anzurogenin C was a downfield shift of the C-24 resonance signal in the spectrum of the glycoside (I) by 10.83 ppm. In addition, there were upfield shifts of the C-23 and C-25 signals by 0.93 and 1.68 ppm, respectively [11, 12]. The chemical shifts of the other carbon atoms scarcely differed.

Consequently, anzuroside is anzurogenin C 24-O-β-D-glucopyranoside.

EXPERIMENTAL

For the sections "General Observations" and "Preparative Treatment of the Total Extractive Substances" see [1]. For the GLC conditions, see [7].

Anzuroside (I). As the result of column chromatography on silica gel, 600 g of the "sum III" (in 200-g batches) in system 2 [1] fractions containing the desired glycoside were collected. Compound (I) was revealed on Silufol plates by vanillin/phosphoric acid [13] in the form of gray-green spots. After repeated rechromatography of the combined fractions in the same system, 1.6 g of anzuroside was obtained: $C_{32}H_{52}O_{12}$, mp 242-244°C (from methanol–ethyl acetate), $[\alpha]_D^{20}$ −62±2° (*c* 0.96; ethanol), ν_{max}^{KBr}, cm^{-1}: 820, 850, 910, 1715 (>C=O), 3300—3500 (OH). M+ 640. The yield calculated on the weight of the air-dry raw material was 0.002%.

Methanolysis of Anzuroside (I). Glycoside (I) (13.05 mg) was dissolved in 3 ml of absolute methanol containing 5% of hydrogen chloride, and the solution was boiled for 14 h. Then an equal volume of water was added to the reaction mixture, the acid was neutralized with silver carbonate, and the inorganic salts and the products of the degradation of aglycon were separated off by filtration. The filtrate was evaporated to dryness. D-glucose was detected by the GLC method. For quantitative determination, glycoside (I) was subjected to methanolysis with the use of L-rhamnose as internal standard. Calculation showed that anzuroside is a monoglucoside.

Anzuroside Hexaacetate (II). Glycoside (I) (100 mg) was dissolved in 2 ml of pyridine, and then 1 ml of acetic anhydride was added and the mixture was left at room temperature for 48 h. It was then worked up in the usual way. After recrystallization of the product from methanol, 110 mg of the hexaacetate (II) was obtained: $C_{45}H_{64}O_{18}$, mp 208-211°C, $[\alpha]_D^{20}$ −61 ±2° (*c* 1.05; chloroform). ν_{max}^{Nujol}, cm^{-1} : 915, 990, 1240 (CH_3CO), 1715 (shoulder, >C=O), 1750 (CH_3CO), 3485 (OH). M^+ 892.

Anzurogenin C (III). Glycoside (I) (700 mg) was suspended in 200 ml of water, 500 mg of freeze-dried gastric juice of the snail Helix pomatia was added, and the mixture was incubated at 34-36°C with stirring for 14 days. The course of the reaction was followed by the daily analysis of the reaction mixture with the aid of TLC. After the end of enzymolysis, the suspension was filtered and the residue on the filter was washed repeatedly with a hot mixture of chloroform and methanol (1:1). The filtrate was evaporated to dryness. The desired product was isolated by the column chromatography of the residue on silica gel in system 1 in [1]. After the recrystallization from methanol of the corresponding fractions, the yield of genin (III) amounted to 370 mg. Anzurogenin C: $C_{27}H_{42}O_7$, mp 255-257°C, $[\alpha]_D^{20}$ −143±2° (c 0.96; pyridine). ν_{max}^{KBr}, cm^{-1}: 805, 845, 900, 935, 960, 985, 1000, 1715 (>C=O), 3350—3450 (OH). Mass spectrum, m/z (%): 478 (M^+, 8.6), 460(1.3), 445(0.9), 442(0.6), 433(0.7), 419(10.0), 393(8.6), 390(17.2), 375(1.6), 348(57.1), 333(10.0), 330(15.7), 319(12.9), 155(100), 131(20).

Triacetate of Anzurogenin C (IV). The genin (III) (110 mg) was dissolved in 2 ml of pyridine, 1 ml of acetic anhydride was added, and the mixture was left at room temperature for 48 h. It was then worked up in the usual way. After recrystallization of the reaction products from aqueous methanol, 130 mg of the triacetate (IV) was obtained: $C_{33}H_{48}O_{10}$, mp 186-189°C, $[\alpha]_D^{20}$ −85±2° (*c* 0.85; chloroform). ν_{max}^{Nujol}, cm^{-1}: 810, 840, 865, 890, 910, 965, 1000, 1235—1250 (CH_3CO), 1710 (>C=O), 1745 (CH_3CO), 3470, 3500 (OH). M+ 604.

TABLE 2. Chemical Shifts (δ, ppm, 0 – TMS) and Spin-Spin Coupling Constants (J, Hz) of the Protons of Anzuroside (I) (C_5D_5N), Its Hexaacetate (II) ($CDCl_3$), and Anzurogenin C (III) (C_5D_5N) and Its Triacetate (IV) ($CDCl_3$)

Protons of the aglycon	Compound				Protons of the sugar	β-D-Glucopyranose residue in glycoside (I) and the acetate (II)
	I	II	III	IV		
CH_3-18	0,71; s	0,75; s	0,79; s	0,75; s		I
CH_3-19	0,94; s	0,77; s	0,95; s	0,78; s	H-1	4,85; d, $J_{1,2}=7,7$
CH_3-21	1,10; d	0,95; d	1,11; d, $J_{21,20}=6,8$	0,98; d, $J_{21,20}=7,0$	H-2	3,97; dd, $J_{2,3}=9,0$
CH_3-27	1,01; d	0,82; d	1,05; d, $J_{27,25}=6,5$	0,82; d, $J_{27,25}=6,7$	H-3	4,16; t, $J_{3,4}=9,0$
2H-1	2,07; d	1,90; d	2,10; d, $J_{1,2\,e}=2,5$	1,91; d, $J_{1,2e}=3,5$	H-4	4,20; t, $J_{4,5}=9,0$
H-2e	4,43; m	5,03; m	4,45; m, $J_{2e,3e}=2,5$	5,04; m, $J_{2e,3e}=3,0$	H-5	3,81; ddd, $J_{5,6}=5,0$
H-3e	4,32; m	4,85; m	4,31; m, $J_{3e,4a}=3,0$	4,87; m, $J_{3e,4a}=4,0$	H-6	4,32; dd, $J_{6,6'}=11,5$
			$J_{3e,4e}=3,0$	$J_{3e,4e}=4,0$	H-6′	4,44; dd, $J_{6',5}=2,5$
H-4a	2,98; dd	2,43; dd	3,01; dd, $J_{4a,4e}=14,0$	2,44; dd, $J_{4a,4e}=15,0$		
H-4e	1,88; dd	1,86; dd	1,89; dd	1,85; dd		II
H-16	4,51; dt	4,36; dt	4,56; dt, $J_{16,17}=8,5$	4,39; ddd, $J_{16,17}=8,8$	H-1	4,58; d, $J_{1,2}=8,0$
			$J_{16,15}=6,0$	$J_{16,15}=6.2$	H-2	4,96; dd, $J_{2,3}=10,0$
			$J_{16,15'}=6,0$	$J_{16,15'}=8,0$	H-3	5,17; t, $J_{3,4}=10,0$
H-23a	1,90; dd	1,68; dd	1,97; dd; $J_{23a,23e}=13,0$	1,57; dd, $J_{23a,23e}=12,3$	H-4	5,01; t, $J_{4,5}=10,0$
			$J_{23a,24a}=10,5$	$J_{23a,24a}=11,5$	H-5	3,69; ddd, $J_{5,6}=5,5$
H-23e	2,60; dd	2,43; dd	2,29; dd, $J_{23e,24a}=4,8$	2,01; dd, $J_{23e,24a}=5,3$	H-6	4,19; dd, $J_{6,6'}=12,0$
H-24a	3,95; dt	3,50; dt	3,98; dt, $J_{24a,25a}=10,5$	4,86; dt, $J_{24a,25a}=11,0$	H-6′	4,09; dd, $J_{6',5}=3,0$
H-25a			1,80; m	1,73; m		
H-26a	3,52; t	3,31; t	3,55; t, $J_{26a,26e}=11,0$	3,43; t, $J_{26a,26e}=11,0$		
			$J_{26a,25a}=11,0$	$J_{26a,25a}=11,0$		
H-26e	3,61; dd	3,47; dd	3,67; dd, $J_{26e,25a}=5,0$	3,53; dd, $J_{26e,25a}=5,5$		
OH-2			6,53; d, $J_{OH\text{-}2,2e}=3,5$			
OH-3			5,72; d, $J_{OH\text{-}3,3e}=8,5$			
OH-5	6,90; s	3,94; s	6,93; s	3,97; s		
OH-24			6,35; d, $J_{OH\text{-}24,24a}=5,0$			
CH_3COO		1,98; 2; 00		2,04; 2,06; 2,07; s		
		2,04; 2,06; s				

SUMMARY

A new steroid glycoside of the spirostan series – anzuroside – has been isolated from the collective fruits of the cocultivated Allium suvorovii Rgl. and A. stipitatum Rgl. Its enzymatic cleavage has given a previously undescribed steroid sapogenin – anzurogenin C – which has the structure of (24S, 25S)-2α,3β,5,24-tetrahydroxy-5β-spirostan-6-one. Anzuroside is the 24-O-β-D-glucopyranoside of anzurogenin C.

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