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

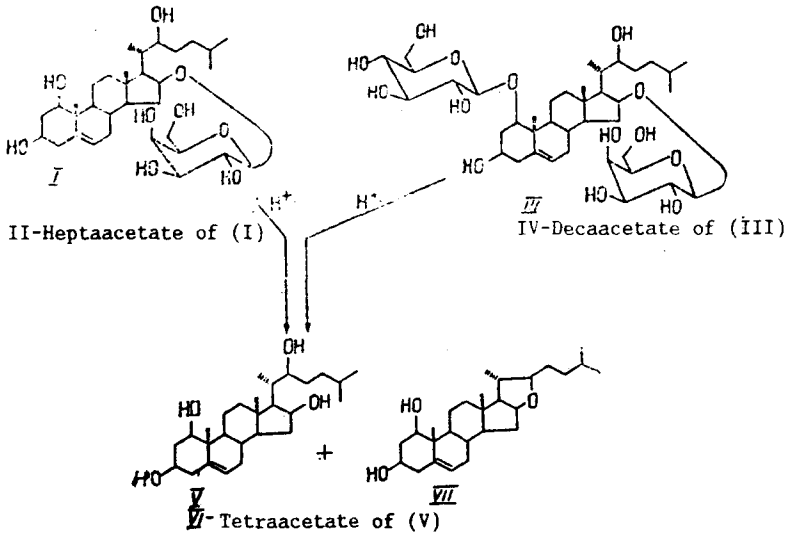
XXVII. ALLIOSTEROL AND ALLOSIDES A AND B FROM Allium suvorovii AND Allium stipitatum – STRUCTURAL ANALOGS OF FUROSTANOLS

Yu. S. Vollerner, S. D. Kravets,
A. S. Shashkov, B. Tashkhodzhaev,
M. B. Gorovits, M. R. Yagudaev,
and N. K. Abubakirov

UDC 547.918:547.926

Two new glycosides of the cholestane series (allosides A and B) have been isolated from the fruit of the cocultivated Allium suvorovii Rgl. and Allium stipitatum Rgl. (family Liliaceae, local name "anzur"). The acid hydrolysis of both compounds gave a sterol not previously described, which has been called alliosterol and has the structure of (22S)-cholest-5-ene-1β,3β,16β,22-tetraol, and the product of its dehydration, which is (16S, 22S)-furost-5-ene-1β,3β-diol. Alloside A is the 16-O-β-D-galactopyranoside, and alloside B the 16-O-β-D-galactopyranoside 1-O-β-D-glucopyranoside of alliosterol.

In preceding communications we have described the identification and proof of the structure of six genins and one glycoside of the spirostan series isolated from the fruit of the cocultivated onions Allium surovii Rgl. and Allium stipitatum Rgl. (family Liliaceae, local name "anzur") [1]. As a result of the rechromatography of the more polar fractions collected in the process of isolating these steroids, two new glycosides have been isolated which have been called allosides A (I) and B (III)



Analysis by the GLC method of the products of the methanolysis of compounds (I) and (III) showed the presence in the allioside A molecule of one D-galactose residue. The carbohydrate moiety of allioside B consisted of D-glucose and D-galactose residues in a ratio of 1:1.

After the acid hydrolysis both of the monoside (I) and of the bioside (II), products (V) and (VII) were obtained (scheme). The aglycon (V), like the initial glycosides, was re-

Institute of Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 231-241, March-April, 1991. Original article submitted May 14, 1990.

 0009-3130/91/2702-0198$12.50

TABLE 1. Chemical Shifts* of the Carbon Atoms of Allosides A (I) and B (III) and of Alliosterol (V) and the Product of Its Dehydration (VII) (C_5H_5N, δ, ppm, 0 – TMS)

Carbon atom	Compound I	III	V	VII	Carbon atom	Compound I	III	V	VII
1	78,28	82,87	78.31	78,21	22	73,40	73,26	75,70	90,57
2	44,04	37,61	44 00	44,01	23	36 82	36,80	36,83	31,89
3	68,29	68 09	68.27	68 19	24	37,47	37,45	37,50	36,45
4	43,68	43,82	43,62	43 60	25	28 99	28,03	28,53	28,46
5	140,46	139,58	140 53	140,39	26	23,15	23.13 *	22,85**	22,78**
6	124,63	124,91	124.59	124 48	27	23,15	23.18 *	23,03*	22,92**
7	32.28	31,93	32,35	32,39		β-*D*-Galactopyranose			
8	33.25	33,24	33,36	33 24	1	107,56	107.61		
9	51,60	50,39	51 63	51.50	2	73,19	73,11		
10	42,31	42,80	43,62	43,60	3	75,59	75 56		
11	24,33	23,93	24,28	24,13	4	70,22	70,15		
12	40,93	40,62	41 32	40,41	5	76,86	76,83		
13	42,31	42,24	42,54	40,69	6	62,33	62,23		
14	55,59	55,31	55,37	57,44		β-*D*-Glucopyranose			
15	33,75	33,86	32.09	33,01	1		101,53		
16	82,64	82,68	71,59	83,42	2		75,47		
17	58,31	58,16	58 50	65 96	3		78,71		
18	13,84	13,88	13,80	16,93	4		72,45		
19	13,97	14,90	14,00	14,00	5		78 21		
20	36.02	36,03	36,21	38,42	6		63,67		
21	12,62	12,64	15,32	19,29					

*The unambiguous assignments were made with the use of APT and HETCOSY (^1H and ^{13}C) (for alliosterol).
**The assignments within the corresponding columns may be interchanged.

TABLE 2. Chemical Shifts (δ, ppm) and Spin-Spin Coupling Constants* (J, Hz) of the Protons of Alliosterol (V) and the Product of Its Dehydration (VII) (C_5D_5N, 0 – TMS)

Proton	Compound V	VII	Proton	Compound V	VII
1a	3,81; dd	3,80; dd	15′	1,56 dt	1.45; dt
	$J_{1a,2a}$ =12,0	$J_{1a,2a}$ =12,7		$J_{15',16}$=4,5	$J_{15',16}$=5,0
	$J_{1a,2e}$ =4,5	$J_{1a,2e}$ =4,0		$J_{15',14}$=4,5	$J_{15',14}$=5,0
2a	2,24; q	2,22, q	16	4,76; dt	4,33; ddd
	$J_{2a,2e}$ =12,0	$J_{2a,2e}$ =12,0		$J_{16,17}$=8,0	$J_{16,17}$=8,0
	$J_{2a,3a}$ =12,0	$J_{2a,3a}$ =12,0	17	1,64; dd	1,54 dd
2e	2,58; dd	2,60; dd		$J_{17,20}$=11,5	$J_{17,20}$=5,0
	$J_{2e,3a}$ =6,5	$J_{2e,3a}$ =5,5	CH_3-18	1,24; s	0,94; s
3a	3,95; m	3,95; m	CH_3-19	1,36; s	1,32; s
	$J_{3a,4a}$ =12,0	$J_{3a,4a}$ =10,5	20	2 59; ddq	1,75; ddq
	$J_{3a,4e}$ =6,5	$J_{3a,4e}$ =5,5		$J_{20,22}$=3,0	$J_{20,22}$=6,1
4a	2,70; br.t	2,68; br.t	CH_3-21	1,17; d	0,95; d
	$J_{4a,4e}$ =12,0	$J_{4a,4e}$ =13,0		$J_{21,20}$=7,5	$J_{21,20}$=7,5
4e	2,62; m	2,60: m	22	4,14; m	3,34; dt
	$J_{4e,2e}$ =2,0			$J_{22,23}$=8,5	$J_{22,23}$=6,1
6	5,62; br.d	5,60; br.d		$J_{22,23}$=4,5	$J_{22,23}$=8,4
	$J_{6,7}$=6,0	$J_{6,7}$=6,0	23	1,72	1,65
7	2,00	1.93	23′	1,33	1,56
7′	1,63	1,56	24	2,34	
8	1,59	1,61	24′	1,58	
9	1 42	1,49	25	1,60	1.52
11	1,82	1,75**	CH_3-26	0,86; d	0,84; d***
11′	2,88	2,91		$J_{26,25}$=7,0	$J_{26,25}$=6,0
12	2.01	1,75**	CH_3-27	0,86; d	0,86 d***
12′	1,33	1,21		$J_{27,52}$=7,0	$J_{27,25}$=6,0
14	1,06	1,11	OH-1	6,09; d	
15	2,33; dt	2,04; ddd		$J_{OH-1,1a}$ =6,5	3,81; br.s
	$J_{15,11'}$=12,5	$J_{15,15'}$=12,5	OH-3	6,33; d	(two protons)
	$J_{15,14}$=8,0	$J_{15,16}$=6,5		$J_{OH-3,3a}$=4,0	
	$J_{15,16}$=8,0	$J_{15,16}$=7,5	OH-16	5,93; d	
				$J_{OH-16,16}$ =3,5	
			OH-22	6,40; d	
				$J_{OH-22,22}$ =5,5	

*The protons for which no SSCCs are given were assigned on the basis of an interpretation of the COSY spectra.
**Center of the H11 and H12 multiplet.
***The assignments may be interchanged.

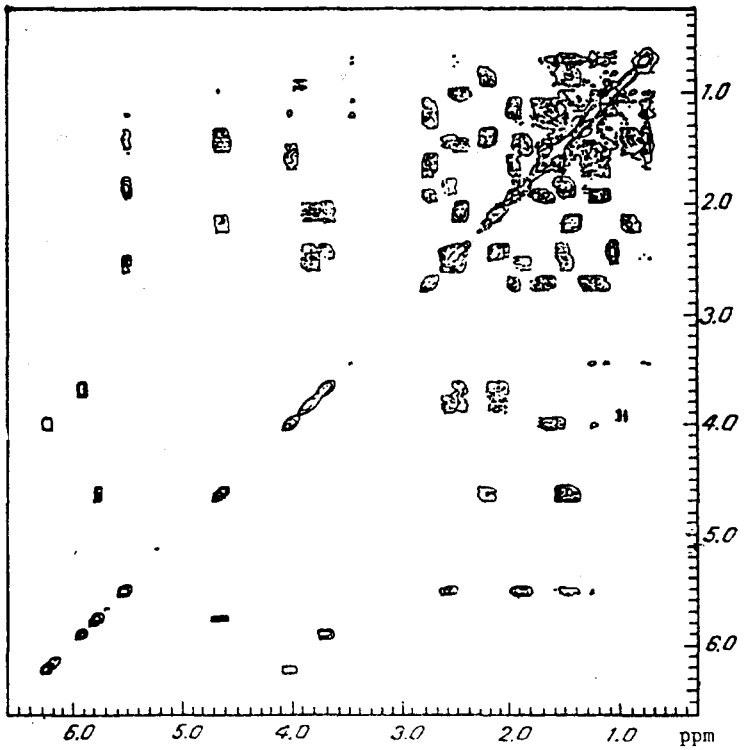


Fig. 1. COSY spectrum of alliosterol (V).

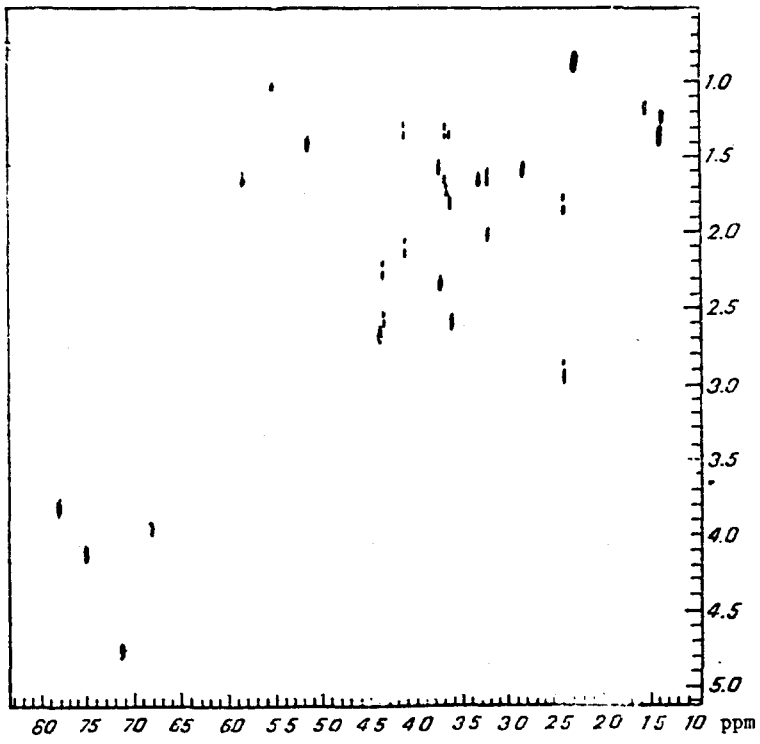


Fig. 2. HETCOSY ($^{1}H-^{13}C$) spectrum of alliosterol (V).

vealed with vanillin/phosphoric acid in the form of a blue-violet spot (TLC) [2]. Substance (VII) was not colored by this reagent.

The mass spectrum (M^+ 434, $C_{27}H_{46}O_4$) and the PMR and ^{13}C NMR spectra (Tables 1 and 2) showed that compound (V) was a derivative of the cholestane series. This conclusion followed from the presence in the alliosterol molecule of 27 carbon atoms, 5 of which were present in methyl groups (carbon resonances with the chemical shifts (CSs, ppm) 13.80, 14.00, 15.32, 22.85, and 23.03; the fact that they belonged to the HC groups was confirmed by APT – the at-

tached proton test). Two of them were tertiary (the corresponding protons resonated in the form of singlets with CSs of 1.24 and 1.36). Three methyl groups were secondary (CS 0.86; d, ^3J = 7.0 Hz – six protons; 1.17, d, ^3J = 7.5 Hz).

Judging from the spectral characteristics given in Tables 1 and 2, it was possible to conclude that the alliosterol molecule contained one trisubstituted double bond (CS >C= 140.53; –CH = 124.59; –CH= 5.62, br.d., ^3J = 6.0 Hz)and four secondary hydroxy groups (CSs of th C atoms: 68.27, 71.59, 75.70, and 78.31; CSs of the geminal protons: 3.81 dd, 3.95 m, 4.14 m, 4.76 dt). On comparing the values of the Css in the ^{13}C NMR spectrum of sterol (V) (Table 1) with literature figures [3, 4] it became obvious that the double bond was localized between C5 and C6, and two of the four hydroxy groups were present at C1 and C3. Their β-orientation was also confirmed by the values of the spin-spin coupling constants (SSCCs) of thei corresponding geminal protons (Table 2). Furthermore, it may be assumed that the third and fourth OH groups were located at C16 and C22.

The conclusions drawn above found confirmation in the results of a series of experiments on double homonuclear resonance (in the ordinary and difference variants) and the nuclear Overhauser effect (NOE), and also homonuclear (COSY) and heteronuclear (^1H–^{13}C) two-dimensional NMR spectroscopy (Tables 1 and 2 and Figs. 1 and 2). With the aid of these methods it was possible unambiguously to assign all the signals in the ^1H and ^{13}C NMR spectra of alliosterol (V). They also showed the spatial propinquity of H16 to H22 (increase in the intensity of the signal by 900% in the difference NOE spectrum), to H15 (5.0%), and to H17 (5.6%) in the molecule of alliosterol tetraacetate (VI) for the PMR spectrum, see the Experimental part). If it is assumed that in compound (V), as in the overwhelming majority of natural steroids, the proton at C17 has the α-orientation, it must be concluded that the hydroxy group at C16 has the β-orientation.

To elucidate the absolute configuration of C22, we carried out an x-ray structural investigation of alliosterol (V).

The spatial structure of the sterol (V) molecule in a projection on plane is shown in Fig. 3. Ring A has the chair conformation. The C1, C3, C4, and C10 atoms are coplanar to within an accuracy of 0.01 Å; the C2 and C5 atoms deviate from the plane mentioned by 0.71 and –0.65 Å, respectively. In ring B, the C5, C6, C7, and C10 atoms lie in one plane (0.01 Å), while the C8 and C9 atoms deviate from it on opposite sides by 0.60 and –0.15 Å. This permits the half-chair conformation to be ascribed to ring B. Ring C is present in the chair conformation with deviations of C11 (0.60 Å) and C14 (–0.71 Å) from the plane in which the C8, C9, C12, and C13 atoms lie (0.01 Å). The five-membered ring D has a half-chair conformation (with the departure of the atoms C13 (0.57 Å) and C14 (–0.21 Å) from the plane passing through C15, C16, and C17).

The interatomic distances in the molecule of the aglycon (V) (Fig. 3) agree well with the lengths of the corresponding bonds in related compounds [5]. The error in the determination of bond lengths does not exceed 0.011 Å. However, in the alicyclic fragment the bonds are lengthened to 1.59 Å and the error amounts to 0.016 Å, which is due to the thermal fluctuations of this section. The error in the determination of the valence angles (Fig. 3) does not exceed 0.6° (in the C22–C27 chain it is up to 1.0°). The short O3···O4 intramolecular contact (2.61 Å) most probably arises because of the formation of a hydrogen bond which, apparently, plays an important role in the stabilization of the conformation of the molecule of genin (V). In the crystal lattice the molecules are linked with one another by a system of hydrogen bonds in which all the hydroxy groups participate. It can be seen from Fig. 3 that all the substituents of the steroid skeleton have the β orientation, while C22 has the S configuration. Thus, alliosterol (V) is (22S)-cholest-5-ene-1β,3β,16β,22-tetraol.

As already indicated, the acid hydrolysis of allosides A and B formed a product (VII) together with alliosterol (scheme). The values of the CSs of the carbon atoms and the protons and the SSCCs of the latter in the NMR spectra of compounds (V) and (VII) (Tables 1 and 2) showed that these substances were close in nature. The greatest deviations were shown by the CSs of the C16 and C22 carbon atoms and the corresponding protons (in the assignment of the signals in the NMR spectra of compound (VII) we used the methods mentioned above: the COSY spectrum is shown in Fig. 4). In the light of the mass spectra of alliosterol (M^+ 434, $C_{27}H_{46}O_4$) and the aglycon (VII) (M^+ 426, $C_{27}H_{44}O_3$), we came to the conclusion that compound (VII) was formed as a result of the dehydration of sterol (V). The splitting out of one molecule of water from C16 and C22 led to the appearance of an additional tetrahydrofuran ring, as was confirmed by the presence in the molecule of the product of the dehydration of (VII)

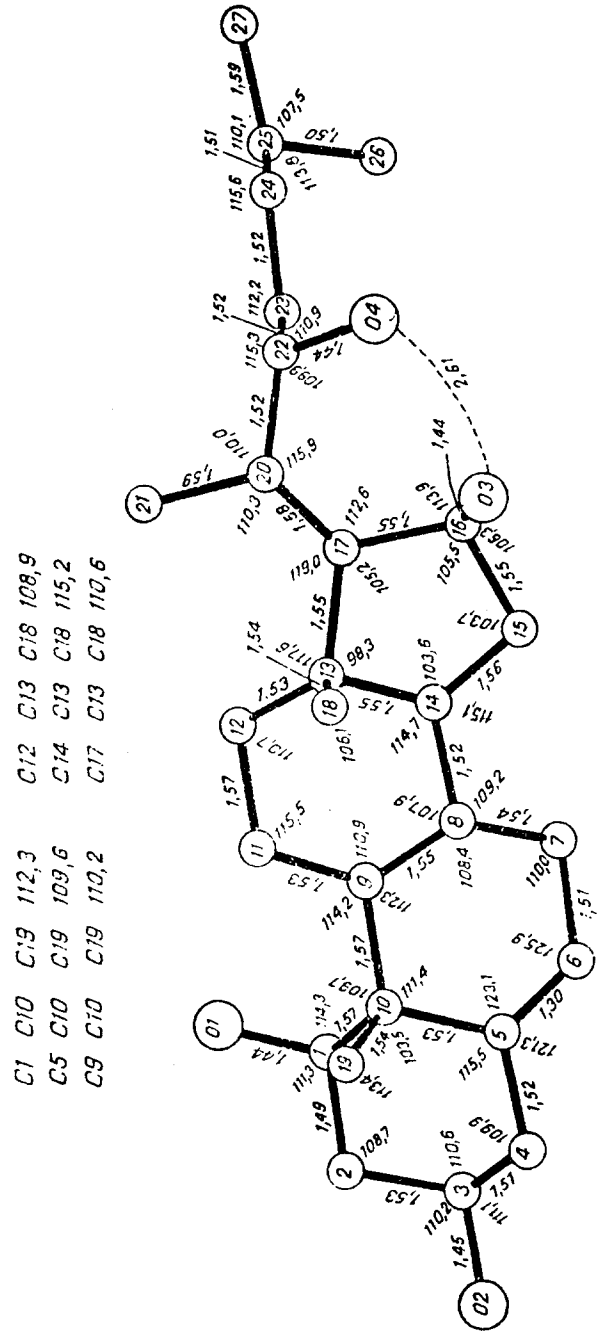


Fig. 3. Spatial structure of alliosterol (V).

of only one double bond (between C5 and C6). To answer the question as to whether dehydration was accompanied by a reversal of the C16 and C22 configurations, we recorded the difference NOE spectra of the genin (VII). According to the results obtained, H16 was close to H22 (7.5%), to H15 (6.1%), and to H17 (6.2%), while H22 was close to H16 (3.0%), H20 (1.8%), 2 H23 (7.0%), and $CH_3$21 (5.8%). Consequently, just as in alliosterol, C16 and C22 in the product of the dehydration of (VII) had the S configuration, which permits the assumption for the latter of the structure of (16S, 22S)-furost-5-ene-1β-3β-diol.

To establish the position of attachment of the carbohydrate residues in the molecules of allosides A (I) and B (III), we made a comparative characterization of the ^{13}C NMR spectra of the monoside (I), the bioside (III), and alliosterol (V) (Table 1), and also of the PMR spectra of these glycosides and their peracetates (II) and (IV) (Table 3).

The SSCC of the H1 atom of the D-galactose residue of glycoside (I) ($J_{1,2}$ = 8.0 Hz) indicated the β-configuration of the glycosidic bond and the $^4C_1$ conformation of the carbohydrate ring. On passing from the monoside (I) to the heptaacetate (II), the CSs of the protons geminal to the oxygen-containing substituents at C1, C3, and C22 underwent downfield shifts by +0.93, 0.73, and 0.74 ppm, respectively, while Δ_{H16} amounted to −0.26 ppm. On the other hand, a comparison of the CSs of the carbon atoms of genin (V) and of glycoside (I) showed a large α-effect of glycosylation for C16 (Δ_{C16} = +11.05 ppm) and weak β-effects for C15 (+1.66 ppm) and C17 (−0.19 ppm). The CSs of the other carbon atoms scarcely differed. Consequently, alloside A was alliosterol 16-O-β-D-galactopyranoside.

In the case of alloside B, both the D-glucose and D-galactose residues ($J_{1,2}$ = 7.5 Hz for each) were linked to the aglycon by β-glycosidic bonds; the carbohydrate rings had the $^4C_1$ conformation. On passing from the bioside (III) to the decaacetate (IV) the downfield shifts of H3 and H22 amounted to +0.66 and 0.67 ppm, respectively, and there were no such shifts for H1 and H16. If the practically absolute coincidence of the CSs of C15, C16, and C17 in glycosides (I) and (III) are taken into account, no doubt remains of the substitution by the β-D-galactopyranose residue of the hydroxy group at C16 in each glycoside. Consequently, in bioside (III) the β-D-glucopyranose residue substitutes the OH at C1 (Δ_{C_1} = +4.56; Δ_{C_2} = −6.39 ppm on passing from sterol (V) to glycoside (III). Thus, alloside B has the structure of alliosterol 16-O-β-D-galactopyranoside 1-O-β-D-glucopyranoside.

Derivatives of the cholestane series bearing oxygen-containing constituents in the C16 and C22 positions form a small group of compounds numbering about 10 representatives [6 (pp. 109, 110), 7, 8]. It is not difficult to convince oneself that these substances have been isolated from plants which either produce steroids of the spirostan and furostan series or belong to plant families and genera rich in such species. It is quite possible that cholesterol derivatives oxidized in the C16 and C22 positions are intermediate compounds in the biosynthesis of steroid sapogenins [6 (p. 88)].

EXPERIMENTAL

General observations have been given in [1]. The preliminary treatment of the total extractive substances is given in the same paper. For the conditions of performing methanolysis and GLC, see [9].

For chromatography we used the following solvent systems: 1) chloroform—methanol—water (65:15:2) (a) and (65:22:4) (b); and 2) chloroform—methanol (20:1).

NMR Spectroscopy. The COSY spectra were taken on a WM-250 instrument by the standard procedure of the mathematical provision of the Bruker firm for an ASPECT-2000 computer. The size of the two-dimensional matrix was 1 × 0.5 K, the spectral window 1930 Hz, and the resolution 3.8 Hz/point. In the pulse sequence we used 90° (5.8 μs) and 45° (2.9 μs) pulses (COSY-45 variant). The relaxation delay was 2 s. Before Fourier transformation, the spectra in temporal presentation were multiplied by a square sinusoidal function with zero shift.

The two-dimensional ^1H–^{13}C spectrum was taken on an AM-300 instrument by the standard procedure of the mathematical provision of the Bruker firm as applied to an aspect 3000 computer. The size of the matrix was 2 × 0.25 K and the spectral window 5620 Hz along the ^{13}C CS axis and 1400 Hz along the ^1H CS axis, the resolution being 5.5 Hz/point in both directions. In the pulse sequence we used 90° and 180° pulses for protons (25.5 and 51 μs, respectively) and pulses for the same angular magnitudes for ^{13}C (14 and 28 μs, respectively). The relaxation delay was 1 s and the D3 and D4 delays in the standard XHCORRD program 3.33 and 1.67 μs, respectively (the optimum for an SSCC $^1J_{^{13}C-^1H}$ = 150 Hz). On treatment of the spectra in temporal presentation we used a square sinusoidal function with zero shift.

TABLE 3. Chemical Shifts (δ, ppm, 0 – TMS) and Spin-Spin Coupling Constants (J, Hz) of the Protons of Allosides A (I) (C_5D_5N) and B (III) (C_5D_5N) and Their Peracetates (II, IV) ($CDCl_3$)

Proton	Compound			
	I	II	III	IV
CH_3-18	0,99; s	0,83; s	0,96; s	0,88; s
CH_3-19	1,27; s	1,10; s	1,24; s	0,97; s
CH_3-21	1,10; d	0,93; d	1,10; d	0,95; d
CH_3-26/27	0,87; d 0,88; d	0,86; d	0,89; d, 0,90; d	0,84; d
1a	3,69; dd	4,62; dd	3,79; dd	3,38; dd
3a	3,85; m	4,58; m	3,93; m	4,50; m
6	5,51; br.d	5,58; br.d	5,49; br.d	5,56; br.d
16	4,48; m	4,22; m	4,30; m	4,23; m
22	4,21; m	4,95; m	4,30 m	4,97; m
β-D-Galactopyranose				
1	4,59; d, $J_{1,2}$=8,0	4,53; d, $J_{1,2}$=8,0	4,65; d, $J_{1,2}$=7,5	4,54; d $J_{1,2}$=8,0
2	4,28; dd, $J_{2,3}$=9,5	5,14; dd $J_{2,3}$=10,5	4,38; dd, $J_{2,3}$=9,5	5,15; dd, $J_{2,3}$=10,0
3	3,97; dd $J_{3,4}$=3,5	4,97; dd, $J_{3,4}$=3,5	4,05; dd, $J_{3,4}$=3,5	4,97; dd, $J_{3,4}$=3,5
4	4,45; dd, $J_{4,5}$=1,0	5,34; dd, $J_{4,5}$=1,5	4,55; br.d	5,34; dd, $J_{4,5}$=1,0
5	3,88; dt, $J_{5,6}$=6,5	3,84; dt, $J_{5,6}$=6,5	3,92 m $J_{5,6}$=7,0	3,88 dt, $J_{5,6}$=6,5
6	4,37; dd, $J_{6,6'}$=11,0	4,14 dd, $J_{6,6'}$=11,0	4,47; dd, $J_{6,6'}$=11,0	4,14 dd, $J_{6,6'}$=11,0
6'	4,28; dd, $J_{6',5}$=6,5	4,05 dd, $J_{6',5}$=6,5	4,37; dd, $J_{6',5}$=6,0	4,05 dd, $J_{6',5}$=6,5
β-D-Glucopyranose				
1			4,97; d, $J_{1,2}$=7,5	4,48; d $J_{1,2}$=8,0
2			4,02; dd, $J_{2,3}$=9,0	4,92; dd, $J_{2,3}$=9,5
3			4,21; t $J_{3,4}$=9,0	5,15; t, $J_{3,4}$=9,5
4			4,10; t $J_{4,5}$=9,0	5,06; t, $J_{4,5}$=9,5
5			3,94; m	5,62; ddd, $J_{5,6}$=4,5
6				4,20; dd, $J_{6,6'}$=12,0
6				4,09; dd, $J_{6',5}$=2,5
OAc		1,94; 1,98; 2,00 2,01; 2,04; 2,12; s		1,95; 1,98; 2,00 2,02; 2,04; 2,15; s,

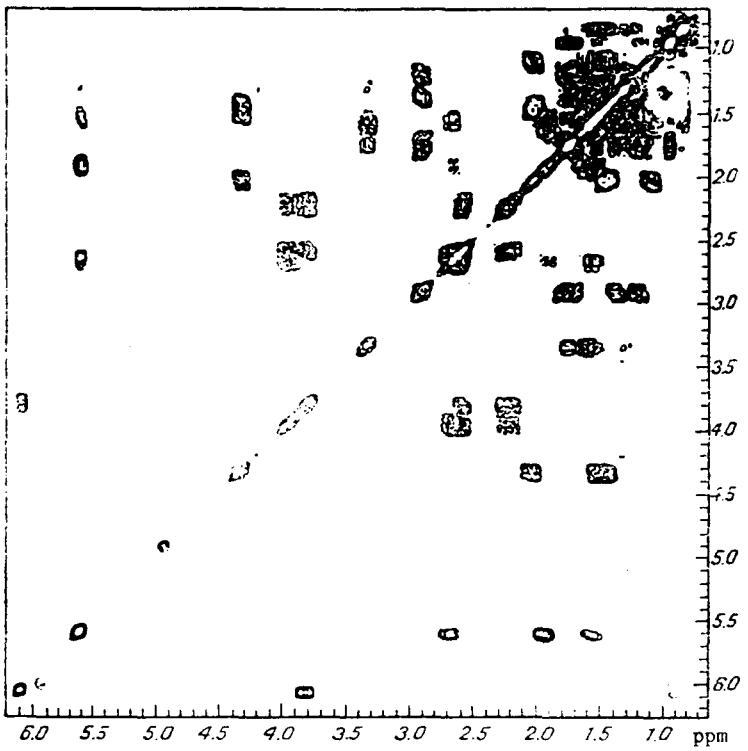


Fig. 4. COSY spectrum of the product of the dehydration of alliosterol (VII).

TABLE 4. Coordinates ($\times 10^4$) of the Nonhydrogen Atoms of the Alliosterol Molecule

Atom	x	y	z	Atom	x	y	z
C1	5897 (5)	1944 (5)	0773 (3)	C16	9054 (5)	4534 (7)	3573 (3)
C2	4833 (5)	1780 (6)	0356 (3)	C17	9780 (5)	4313 (6)	2944 (3)
C3	3762 (5)	1598 (6)	0793 (3)	C18	8601 (7)	5670 (6)	2228 (4)
C4	3592 (5)	2613 (7)	1238 (3)	C19	5682 (6)	4149 (6)	0814 (4)
C5	4716 (6)	2832 (5)	1621 (3)	C20	10943 (6)	5057 (6)	2914 (3)
C6	4723 (6)	2801 (7)	2251 (4)	C21	11631 (6)	4817 (9)	2255 (4)
C7	5780 (6)	3097 (9)	2690 (4)	C22	11775 (6)	4934 (6)	3486 (4)
C8	6711 (5)	3712 (6)	2312 (3)	C23	12095 (7)	3707 (8)	3666 (5)
C9	6959 (5)	3113 (6)	1654 (3)	C24	13170 (7)	3659 (8)	4109 (5)
C10	5825 (5)	3068 (5)	1215 (3)	C25	13565 (8)	2453 (11)	4300 (5)
C11	8632 (6)	3659 (8)	1318 (3)	C26	14697 (11)	2432 (15)	4678 (8)
C12	9166 (5)	3779 (6)	1750 (3)	C27	12684 (11)	1852 (12)	4731 (7)
C13	8876 (6)	4403 (6)	2341 (5)	O1	6066 (3)	1856 (4)	0400 (2)
C14	7850 (5)	3719 (7)	2609 (3)	O2	2717 (4)	1395 (4)	0403 (2)
C15	7754 (5)	4251 (9)	3302 (3)	O3	9063 (4)	5717 (6)	3785 (2)
				O4	11312 (4)	5545 (5)	4038 (2)

X-Ray Structural Analysis. Colorless crystals of alliosterol (V) were grown from methanol–ethyl acetate and had the form of elongated prisms. The parametes of the unit cell were determined by the photo (CuK_α radiation): a = 11.324(3), b = 11.624(3), c = 20.599(5) Å, V = 2711.5 Å^3, d_{calc} = 1.069 g/cm^3. Space group $P2_12_12_1$, Z = 4. A three-diffractometer; 1588 reflections with I > 2σ were used in the calculations.

The structure of alliosterol was interpreted by the direct method and was refined in the full-matrix anisotropic approximation. A difference electron-density synthesis revealed 19 H positions out of 46. The final value of the divergence factor was 0.094. The coordinates of the nonhydrogen atoms of genin (V) are given in Table 4. All the calculations were made by the Rentgen-75 program [10].

Alloside A (I). After the chromatography of 600 g of the total material (III) [1] (in 200-g portions) in system Ia and rechromatography of the appropriate fractions in the same solvent system, we obtained 4.6 g of glycoside (I), $C_{33}H_{56}O_9$, mp 185-187°C (from methanol–water)

$[\alpha]_D^{25}$ −14 ± 2°, (c 1.01; pyridine). ν_{max}^{KBr}, cm^{-1}: 3300-3500 (OH). $(M^+ - 18) = 578$.* The yield calculated on the weight of the air-dry raw material was 0.006%.

Alloside A Heptaacetate (II). A solution of 200 mg of glycoside (I) in 5 ml of pyridine was treated with 2 ml of acetic anhydride and the mixture was left for 72 h. Then it was worked up by the generally adopted procedure. After recrystallization of the product from methanol–water, 170 mg of the heptaacetate (II), $C_{47}H_{70}O_{16}$, was obtained, with mp 156-159°C, $[\alpha]_D^{25}$ −10 ± 2° (c 1.01; chloroform). ν_{max}^{Nujol}, cm^{-1}: 1240, 1750 (ester bond); no absorption in the 3000-3600 region. $(M^+ - 60) = 830$.

Alloside B (III). To isolate glycoside (III) from the above-mentioned total material (III) [1] we used system 2b [sic]. After recrystallization of the chromatographically homogeneous product from methanol–water, 10.2 g of compound (III), $C_{39}H_{66}O_{14}$, was obtained with mp 209-211°C, $[\alpha]_D^{25}$ −21 ± 2° (c 1.08; pyridine). ν_{max}^{Nujol}, cm^{-1}: 3200-3600 (OH). $(M^+ - 180) = 578$. Yield 0.013%.

Decaacetate of Alloside B (V). Glycoside (III) (200 mg) was acetylated and the reaction mixture was worked up as described above for the peracetate (II). After the product had been recrystallized from methanol, 190 mg was obtained of the decaacetate (IV), $C_{59}H_{86}O_{24}$, mp 240-242°C, $[\alpha]_D^{25}$ −19 ± 2° (c 1.01; chloroform). ν_{max}^{Nujol}, cm^{-1}: 1240, 1750 (ester bond). There was no absorption in the 3000-3600 region. $(M^+ - 60) = 1118$.

Methanolysis of Allosides A (I) and B (III). Glycosides (I) (11.00 mg) and (III) (11.35 mg) were cleaved separately and the reaction mixtures were worked up as described in [9]. It was shwon by the GLC method that compound (I) contained a D-galactose residue, while the molecule of glycoside (III) contained residues of D-glucose and D-galactose in a ratio of 1.00: 0.93. By using L-rhamnose as internal standard it was shown that alloside A was a monoside and alloside B a bioside.

Acid Hydrolysis of Allosides A (I) and B (III). Glycoside (III) (1.0 g) was dissolved in 100 ml of 50% aqueous methanol containing 5 ml of H_2SO_4 (d = 1.84), and the reaction mixture was heated at the boil for 8 h. Then 50 ml of water was added, the methanol was driven off, and the resulting aqueous suspension was extracted with butanol (2 × 25 ml). The butanolic extracts were washed with water and evaporated to dryness, and the residue was chromatographed in system 2. After recrystallization of the appropriate fractions, 200 mg of alliosterol (V) and 150 mg of the product of its dehydration (VII) were obtained.

Hydrolyzed glycoside I (50 mg) and the reaction mixture were worked up as above. At R_f in system 2 the reaction products could not be distinguished from genins V and VII.

Alliosterol (V), $C_{27}H_{46}O_4$, mp 200-202°C (from methanol–ethyl acetate), $[\alpha]_D^{25}$ −33 ± 2° (c 1.11; pyridine). ν_{max}^{KBr}, cm^{-1}: 3300-3500 (OH). M^+ 434.

Product of the dehydration of alliosterol (VII), $C_{27}H_{44}O_3$, mp 142-144°C (from benzene), $[\alpha]_D^{25}$ + 11 ± 2° (c 1.25; pyridine). ν_{max}^{KBr}, cm^{-1}: 3300-3400 (OH). M^+ 416.

Alliosterol Tetraacetate (VI). The aglycon (V) (110 mg) was acetylated and the reaction mixture was worked up as in the same way as in the production of the peracetates of glycosides (I) and (III) (see above). As a result of recrystallization of the product from aqueous methanol, 100 mg of the tetraacetate (VI), $C_{35}H_{38}O_8$, was obtained with mp 184-186°C $[\alpha]_D^{25}$ + 15 ± 2° (c 1.04; chloroform). ν_{max}^{Nujol}, cm^{-1}: 1250, 1750 (ester bond); there was no absorption in the 3000-3600 region. PMR spectrum (δ, ppm, O–TMS, $CDCl_3$): 0.86 (CH_3-18, s), 0.86; 0.87 (CH_3-26, CH_3-27, d, J = 6.5 Hz), 0.98 (CH_3-21, d, J = 7.0 Hz), 1.13 (CH_3-19, s), 1.25 (H-17, dd, J = 7.5; 11.5 Hz), 2.00; 2.01; 2.02; 2.03 (4 × CH_3COO–, s), 2.42 (H-15, ddd, J = 6.5; 8.0; 13.0 Hz), 4.64 (H-3, m), 4.65 (H-1, m), 4.79 (H-22, br.t, J 7.5 Hz), 5.18 (H-16, ddd, J = 4.5; 7.5; 8.5 Hz), 5.60 (H-6, br.d, J - 6.0 Hz), $(M^+ - 120) = 482$.

LITERATURE CITED

1. Yu. S. Vollerner S. D. Kravets, A. S. Shashkov, M. B. Gorovits, and N. K. Abubakirov Khim. Prir. Soedin., 68 (1988); 218 (1988); 505 (1989).
2. E. Stahl, Thin-Layer Chromatography (1st English ed.), Springer/Academic Press, New York (1965).
3. P. K. Agrawal, D. C. Jain, R. K. Gupta, and R. S. Thakur, Phytochemistry, 24, No. 11, 2479-2496 (1985).

*The authors thank Yu. M. Mil'grom for recording the mass spectra of compounds (I-VII).

4. J. W. Blunt and J. B. Stothers, Org. Magn. Reson., 9, No. 8, 439-464 (1977).
5. A. P. Polishchuk, M. Yu. antipin, T. V. Timofeeva, V. I. Kulishov, and Yu. T. Struckhov, Kristallografiya, 31, No. 4, 671-675 (1986).
6. A. V. Kamernitskii, N. K. Abubkirov, M. B. Gorovitz, Yu. S. Sollerner, N. E. Voishvillo, I. G. Reshetova, and V. A. Paseshnichenko, The Chemistry of the Spirostanols [in Russian], Moscow (1986), p. 176.
7. F. Giral and C. Rivera, Phytochemistry, 14, No. 3, 7893-796 (1975).
8. F. Giral, C. Rivera, and P. L. Garsia, Phytochemistry, 23, No. 9, 2089-2090 (1984).
9. S. D. Kravets, Yu. S. Vollerner, A. S. Shashkov, M. B. Goroviits, and N. K. Abubakirov, Khim. Prir. Soedin., 843 (1987).
10. V. I. Andrianov, Z. Sh. Safina, and B. L. Tarnopol'skii, Zh. Strukt. Khim., 15, 911 (1974).

PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS Silene.

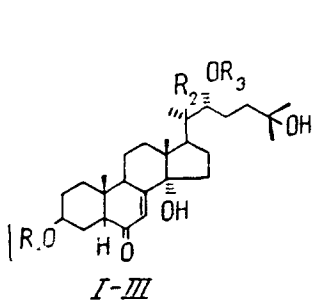
XVIII. 2-DEOXYECDYSTERONE 20,22-MONOACETONIDE FROM Silene brahuica

M. Kh. Dzhukharova, Z. Saatov,
M. B. Gorovits, and N. K. Abubakirov

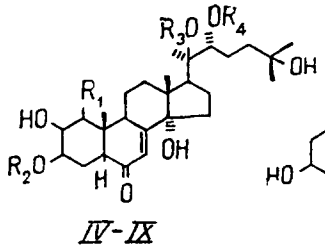
UDC 547.926

In addition to the known 2-deoxy-α-ecdysone, 2-deoxyecdysterone, 2-deoxy-α-ecdysone-22-O-acetate, ecdysterone, integristerone A, and sileneoside A, B, and C, the new ecdysteroid 2-deoxyecdysterone 20,20-monoacetonide has been isolated from the roots of the plant Silene brahuica Boiss.

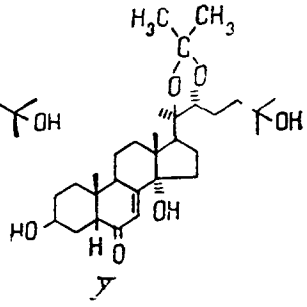
We have previousyl reported the isolation from the epigeal organs of the plant Silene brahuica Boiss., gathered in the valley of the river Chonkemin (Kungai Ala-Tau range, northern Tien-Shan) of a number of phytoecdysteroids [1]. Having studied the roots of this plant, in addition to the known 2-deoxy-α-ecdysone (I), 2-deoxyecdysterone (II), 2-deoxy-α-ecdysone-22-O-acetate (III), ecdysterone (Iv), integristerone A (V), and sileneosides A (VI), B (VII), and C (VIII), we have isolated a new phytoecdysteroid (X) with the composition $C_{30}H_{48}O_6$.



I-III



IV-IX



X

I. $R_1=R_2=R_3=H$
II. $R_1=R_3=H$; $R_2=OH$
III. $R_1=R_2=H$; $R_3=Ac$
IV. $R_1=R_2=R_3=R_4=H$
V. $R_1=OH$; $R_2=R_3=R_4=H$
VI. $R_1=R_2=R_3=H$; $R_4=\alpha$-*D*-Gal
VII. $R_1=R_2=H$; $R_3=R_4=\alpha$-*D*-Gal
VIII. $R_1=OH$; $R_2=R_3=H$; $R_4=\alpha$-*D*-Gal
IX. $R_1=R_2=H$; $R_3=R_4=(CH_3)_2C$

The mass spectrum of the ecdysteroid (X) contained the peak of the molecular ion, M^+ 504, and also the peak of an ion with m/z 347 (M − 157), and also fragments with m/z 201 and

Institute of Chemistry of Plant Substances Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 241-244, March-April, 1991. Original article submitted May 3, 1990.

0009-3130/91/2702-0207$12.50