STEROID COMPOUNDS FROM OPHIUROIDS

II. SULFATED STEROIDS FROM Ophiura sarsi AND Ophiura leptoctenia

É. V. Levina, S. N. Fedorov, V. A. Stonik,
P. V. Andriyashchenko, A. I. Kalinovskii,
and V. V. Isakov

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The previously described cholest-5-ene-3α,4β,21-triol 3-(sodium sulfate) has been obtained from extracts of the Far Eastern ophiuroid Ophiura leptoctenia. Two new sulfated steroid polyols have been isolated from the ophiuroid O. sarsi; cholest-5-ene-3α,4β,21-triol 3,21-di(sodium sulfate) and cholest-5-ene-2β,3α,21-triol tri(sodium sulfate). The new polyol 4β-acetoxycholest-5-ene-3α,21-diol has been identified among the products of the desulfation of this ophiuroid.

Studying the steroid sulfates from the Far Eastern ophiuroid Ophiura leptoctenia, collected on the shores of the island of Paramushia, we have isolated cholest-5-ene-3α,4β,21-triol 3-(sodium sulfate) (I), which we have described previously for Ophiura sarsi [1].

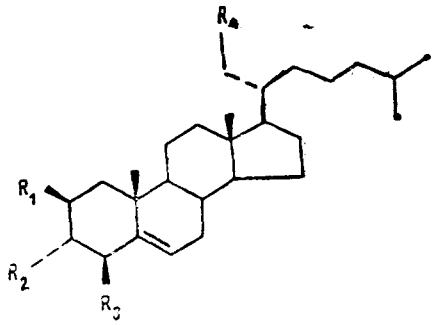
Continuing an investigation of extracts from O. sarsi, by column chromatography on Sephadex H-20, Amberlite XAD-2, and silica gel, from the total fraction we have isolated new sulfated steroid polyols (II), (III), and (IV). In view of the fact that (IV) could not be purified to the state of an individual compound, the product of its desulfation (V) was studied structurally. In addition, from O. sarsi we also isolated and identified cholesterol sulfate (0.07% of the dry weight of the extract).

Compound (II), which contained a sulfo group (IR: 1250 cm^{-1}) and Na ions (atomic absorption analysis) showed almost complete agreement of its spectral characteristics with the corresponding characteristics for (I), but the shift of the signals of the CH_2-21 protons (+0.5 ppm) and of the signals of carbon atoms C-20 and C-21 (−2.8, +5.5 ppm) upfield and downfield, respectively, could be explained by the effect of the sulfation of the hydroxyl at C-21 in compound (II) [2].

Consequently, the sulfate (II) differed from (I) by the presence of a sulfo group in the side chain (Tables 1 and 2), as was shown by the production of an identical solvolysis product (I^a) from (I) and (II).

On the basis of what has been said above, we assigned to sulfate (II) the structure of cholest-5-ene-3α,4β,21-triol 3,21-di(sodium sulfate).

Compound (III), containing sulfate groups and Na ions like (II), remained unchanged after attempted acetylation, and the product of its solvolysis (VI) gave the peak of a molecular ion with m/z 418 in its mass spectrum.



I. R_1=H, R_2=OSO_3Na, R_3=R_4=OH
I^a. R_1=H, R_2=R_3=R_4=OH
II. R_1=H, R_2=R_4=OSO_3Na, R_3=OH
III. R_1=R_2=R_4=OSO_3Na, R_3=H
V. R_1=H, R_2=R_4=OH, R_3=OAc
VI. R_1=R_2=R_4=OH, R_3=H
VII. R_1=R_2=R_4=OAc, R_3=H
VIII. R_1=H, R_2=R_3=R_4=OAc

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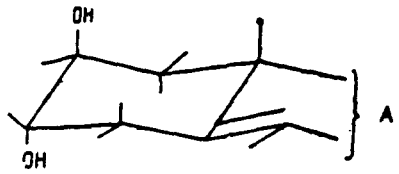
TABLE 1. ^{1}H NMR Spectra of Sulfated Polyols from Ophiuroids and Their Derivatives (C_5D_5N, δ, TMS = 0, ppm; J, Hz)

Steroid	CH-2	CH-3	CH-4	CH-6	CH_3-18
I		5,47 m	4,94 d (2,0)	5,84 d (3,6)	0,75 s
II		5,49 m	4,94 d (2,5)	5,69 d (3,5)	0,70 s
III	5,60 m	5,63 m		5,38 m	0,7 s
V		4,30 q	5,70 dd	6,03 m	0,80 s
V($CDCl_3$)		3,90 (2,8)	5,10 dd (1,2;2,7)	5,88 dd (2,2;4,9)	0,72 s
VI	4,45 m	4,51 m		5,6 m	0,80 s
VII	5,17 m	5,25 m		5,44 m	0,72 s
VIII		5,20 m	5,59 m	5,98	0,72 s

Steroid	CH_3-19	CH'-21	CH''-21	CH_3-26, 27	OAc
I	1,51 s	3,90 br.d (11,0)	4,05 br.d (11,0)	0,88 d (6,7)	
II	1,50 s	4,43 m (11,0)	4,64 m (11,0)	0,85 d (6,8)	
III	1,32 s	4,47 m	4,69 m	0,85 d (6,8)	
V	1,30 s	4,07 A m	3,92 B dd	0,90 d (6,8)	2,10
V ($CDCl_3$)	1,13 s	3,70 m	3,70 m	0,87 d (6,8)	2,05
VI	1,60 s	3,09 m	4,06 m	0,90 d (6,8)	
VII	1,18 s	4,20 dd (10,5;5,5)	4,42 dd (10,5;3,2)	0,89 d (6,8)	2,03 2,09 2,15
VIII	1,20 s	4,20 dd	4,42 dd	0,90 d	2,05 2,1 2,17

The ^{13}C NMR spectrum (Table 2) contained the signals of three carbon atoms linked to oxygen, and of those at a double bond. The acetylation of (VI) led to the triacetate (VII), which differed from the triacetate of cholest-5-ene-3α,4β,21-triol (VIII) (Table 1).

A comparison of the ^{13}C NMR spectra of the polyols (VI) and (I^a) and also of the ^{1}H spectra of their acetates (Tables 1 and 2) showed almost complete agreement of the C_{12}–C_{27} signals and the small difference of the signals in rings A and B. It was concluded that the side chains of the triols (VI) and (I^a) were identical. On the basis of spectral characteristics, we assume that the two secondary hydroxy groups and the double bond were present in the polycyclic moiety of the molecule and formed fragment A:



The CH-2 and CH-3 methine proton signals consisted of multiplets at 4.45 and 4.51 ppm, while the olefinic proton gave a broadened doublet at 5.44 ppm. The signals of the protons H-4a (3.43 ppm, dm, J = 14.2 Hz) and H-4e (4.42 ppm, ddd, J = 14.2, 0.9, 2.7 Hz) were revealed by difference decoupling. Irradiation on H-4e did not change the signal of the vinyl proton, while irradiation on H-4a caused a change in the signal. These facts, and also the widths of the signals of the methine protons (Table 1) showed their equatorial configuration and the 2β,3α-position of hydroxy groups.

Analogous, but not identical, steroid sulfates with the same arrangement and configuration of the functions in ring A and B have been isolated previously from the sponge Halichondria sp. [3] and the ophiuroid Ophiarachna incrassata [4].

A comparison of the spectral characteristics of the sulfate (III) and the polyol (VI) and its acetate (VII) (Tables 1 and 2) with literature information [3, 4] showed good agreement, confirming that (III) had the structure of cholest-5-ene-2β,3α,21-triol tri(sodium sulfate).

Since the minor sulfate (IV) could not be isolated in the individual state, we studied the product of its desulfation (V). The ^{13}C NMR spectrum of the steroid polyol (V) contained the signals of a double bond and of three CH–OH groups, one of which was acetylated.

TABLE 2. ^{13}C NMR Spectra of Sulfated Steroids and Their Derivatives [C_5D_5N (or CD_3OD, for (III)), δ, TMS = 0]

Atom	I	Ia	II	III	VI	VII	V
C-1	33,5	33,1	33,6	40 2	41,2	38,3	31 7 g
C-2	23,3	25,3	23,2g	76,4	71,9g	70,9 g	24,7 g
C-3	78,2	71,0	78,1	76,6 g	71,3 g	70,2 g	78,7
C-4	76,3	78,9	76,3	32,9	36,2	32,9	68,0
C-5	143,0	144,0	143,2	139,6	140,6	137,1	137,0
C-6	127,5	127,0	127,4	122,9	121,7	a	132,9
C-7	32 6	32,3 g	32,5	33,1	31,8 g	31,9 g	31,8 g
C-8	32,6	32,6 g	32,5	33,1	32,3 g	32,3 g	32,2 g
C-9	50,6	50,8	50 6	52,0	50 9	50,5	50,4
C-10	37,1	37,2	37,0	36,9	37,4	36,7	36,5
C-11	21,0	20,8	20,9	21,8	21,3	21,0	20,4
C-12	39,8	39,6	39,5	40 7	39,6	39,1	39,3
C-13	42,8	42,6	42,8	43,6	42,6	42,4	42,2
C-14	57,4	57,2	57,4	58,1	57,1	56,7	56,9
C-15	24,9 g	24,5	24,6	25,1	24,5	24,2 g	24,4 g
C-16	28,0	27,8	28,0	29,5	27 7	27,9	27,6
C-17	51,7	51,2	51,3	51,5	51,3	51,3	50,7
C-18	12,7	12,7	12,6	12,7	12,4	12 2	12,2
C-19	21,7	21,6	21,8	21,8	22,7g	21,6	20,6
C-20	43,5	43,3	40 7	41,3	43,2	39,9 g	42,6
C-21	63,0	62,4	68,5	69,6	62,5	65,3	63,1
C-22	30,8	30,4	30,8	30,9	30,4	30,8	29,9
C-23	24,8 g	24,5	24,6	24 6	24,5	24,3 g	24,2g
C-24	40,3	40,0	40,1	40,7	39,9	39,8 g	39 6
C-25	28,5	28,2	28,4	29,0	28,2	28,2	28,1
C-26	23,1 g	22,9 g	23,4g	23,1 g	22,8g	22,7 g	22,8 g
C-27	23,0 g	22,6 g	23,2g	23,0 g	22,6g	22,6 g	22,6 g
OAc						170,6;20 8 169,5;20,9 169,6;21,0	169,4;21 5

a – The signal is masked by the signal of the solvent;
g – Assignment of the signals ambiguous.

A comparison of the ^1H spectra of (V) and of 4β,21-diacetoxycholest-5-en-3α-ol, which we have described previously [1] showed agreement of all the signals apart from those of CH_3-18 and CH_2-21 (Table 1), which indicated the absence of an acetate group at C-21. On the assumptions that the functional groups were located in (V) in the same way as in (I^a) and that the acetoxy group was localized at C-4, we confirmed this by homo-decoupling. In agreement with [3], irradiation with the frequency corresponding to the signal at 5.88 ppm (CH-6) left unchanged the signals at 3.90 and 5.10 ppm (CH-3 and CH-4), while homodecoupling at 5.10 ppm converted the multiplet at 3.90 ppm into a triplet with SSCC of 2.7 Hz. In its turn, homodecoupling with irradiation at 3.90 converted the signal at 5.1 ppm into a doublet with SSCC 1.2 Hz, without changing the signal of the olefinic proton. The width of the signals and the SSCCs of the methine protons (Table 1) showed their equatorial configuration and corresponded to the 3α,4β-position of the functional groups.

To confirm the structure of (V) it was acetylated, and the constants and characteristics of the acetates (Table 1) were compared with the triacetate (VIII) obtained from (I^a); they proved to be identical. On this basis, the polyol (V) was assigned the structure of 4β-acetoxy-cholest-5-en-3α,21-diol.

EXPERIMENTAL

The ophiuroid was collected at depths of 100-130 m off the coast of the island of Paramushia (Kurile Islands) during the second and seventh expeditionary voyages of the Scientific Research Vessel Akademik Oparin.

The determination of the physical constants and the acquisition of the ^1H and ^{13}C NMR spectra were carried out as descried previously [2]. Metals were determined on a AA-780 atomic absorption spectrometer.

Isolation of Cholest-5-ene-3α,4β,21-triol 3,21-Di(sodium sulfate) (II). The ophiuroid was comminuted and extracted with ethanol, and the extract after evaporation to dryness (300 g) was chromatographed on a column of Polykhrom-1. On elution with aqueous ethanol (20%), 4 g of a mixture of sulfated polyols was obtained. On subsequent repeated chromatography on

columns with silica gel [chloroform–ethanol–water (5:2, to saturation)], Sephadex LH-20 (chloroform–methanol), and Amberlite HAB-2 (aqueous methanol), fractions 1-3 were isolated, one of which (1) consisted of an individual compound (II) in an amount of 0.18 g (0.06% on the dry weight), $C_{27}H_{44}O_9S_2Na_2$, mp 189-190.5°C (methanol), $[\alpha]_D^{20}$ −15.64° (c 7.0; methanol).

The solvolysis of (II) under the usual conditions gave the polyol (I^a).

Cholest-5-ene-2β,3α,21-triol Tri(sodium sulfate) (III). Fraction 2 obtained in the separation of the sulfates on XAD-2 contained a substance which was additionally chromatographed on Florisil (column chromatography, 200-300 mesh) in the chloroform–methanol (5:2) system. A colorless substance (III) was obtained with mp 151-153°C (methanol), $[\alpha]_{578}^{20}$ +4.1° (c 1.0; methanol).

4β-Acetoxycholest-5-en-3α,21-diol (V). Fraction obtained when the mixture of sulfates was chromatographed on Amberlite XAD-2 showed in its ^1H NMR spectrum a signal at 2.10 ppm. Since it was impossible to purify it to the state of an individual compound, the product of its desulfation (dioxane with pyridine) was obtained, and this, after chromatography on silica gel [hexane–ethyl acetate (5:1)] gave a colorless crystalline substance $C_{29}H_{45}O_4$, mp 145-147°C, $[\alpha]_{578}^{20}$ −6° (c 6.0; chloroform).

Cholest-5-ene-2β,3α,21-triol (VI). The solvolysis of (III) under the usual conditions with subsequent chromatograph on silica gel yielded the triol (VI) with mp 158-159.5°C (methanol), $[\alpha]_D^{20}$ −16.9° (c 1.75; chloroform).

Cholest-5-ene-2β,3α,21-triol Triacetate (VII). After the acetylation of (VI) by the usual method, a colorless crystalline substance was obtained with mp 102-104°C (methanol), $[\alpha]_{578}^{20}$ +18.4° (c 0.8; chloroform).

Cholest-5-ene-3α,4β-21-triol Triacetate (VIII). The acetylation of (I^a) by the usual method gave a colorless substance with mp 80-82°C (methanol), $[\alpha]_{578}^{20}$ −30.4° (c 4.2; chloroform). An analogous acetate was obtained by the acetylation of compound (V).

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