STEROID COMPOUNDS FROM OPHIUROIDS .

I. NEW STEROID SULFATE FROM Ophiura sarsi

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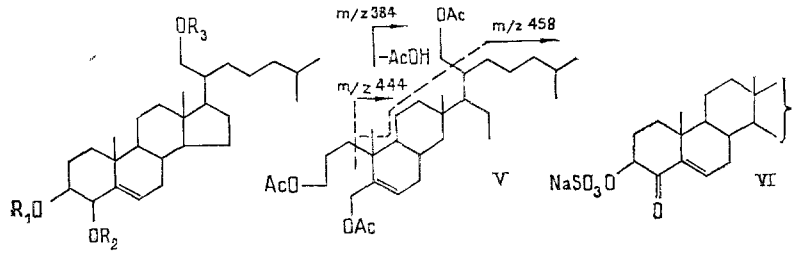
The sulfate of a steroid polyol has been isolated from the Far Eastern ophiuroid Ophiura sarsi, and its structure has been established by chemical and spectral methods as cholest-5-ene-3α,4β,21-diol 3-(sodium sulfate). No steroid compounds of such structure from ophiuroids have been described previously.

Continuing an investigation of physiologically active compounds from marine invertebrates [1], we have begun a chemical study of one of the commonest species of echinoderms of the Far Eastern seas — Ophiura sarsi.

A fraction of sulfated polyhydroxy steroids was obtained from an ethanolic extract of the ophiuroid collected on the shores of Paramushir island (Kurile islands), by chromatography on Polikhrom-1. Such compounds are widely disributed in starfish [2] and have recently been detected by Italian chemists in the ophiuroid Ophioderma longicaudum [3].

The main component of the fraction isolated — the sulfate (I) — amounted to 0.043% of the dry weight of the extract.



I. $R_1 = SO_3Na$, $R_2 = R_3 = H$
II. $R_1 = R_2 = R_3 = H$
III. $R_1 = SO_3Na$, $R_2 = R_3 = Ac$
IV. $R_1 = H$, $R_2 = R_3 = Ac$

The empirical formula for (I), $C_{27}H_{45}O_6SNa$, was determined from the results of elementary analysis and the ^{13}C NMR spectrum (Table 1). The presence of a sulfate group in it was also confirmed by its IR spectrum (1250 cm^{-1}), and that of a sodium ion by atomic absorption analysis.

After the solvolysis of (I) the desulfated derivative (II) was obtained, and its mass spectrum (15 eV) showed the peak of a molecular ions with m/z 418, corresponding to a C_{27}-steroid triol. The ^{13}C NMR spectrum of (II) (see Table 1) showed that of the three hydroxy groups one was primary and two were secondary. The acetylation of (I) gave the monosulfated diacetate (III), and the acetylation of the triol (II) led to the triacetate (IV).

The ^1H NMR spectra of steroids (I)-(IV) had in each case, in place of the five signals methyl groups that are characteristic for the spectra of cholestane derivatives, only four such signals (Table 2). The absence of the doublet of a methyl group and the presence of signals at 4.05 and 3.90 ppm (both doublets of doublets) showed that in (I) and its derivatives the primary hydroxy group was present in the side chain and most probably occupied the C-21 position. In a comparison of the ^{13}C NMR spectra of (I) and (II) and of the model compounds

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TABLE 1. ^{13}C NMR Spectra of the Sulfated Steroid (I) and Its Derivatives (II) and (III), C_5D_5N, δ, TMS = 0

Atom	I	II	II* calc	III	Atom	I	II	II* calc	III
C-1	33,5	33,1	32 7	31,9	C-17	51,7	51,2	51,4	51,3
C-2	23,3	25,3	24,6	23,6	C-18	12,7	12,7	12,2	12,3
C-3	78,2	71,0	71,3	74,4	C-19	21,7	21,6	21,5	21,5
C-4	76,3	78,9	80,1	77,2	C-20	43,5	43,2	42,7	40,0
C-5	143,0	144,0	146,4	137,6	C-21	63,0	62,4	63,4	65,4
C-6	127,5	127,5	123,9	132,2	C-22	30,8	30,4	32,4	30,8
C-7	32,6	32,3[a]	32,2	32,2[a]	C-23	24,8[a]	24,5	24,0	24,4
C-8	32,6	32,6[a]	32,2	32,6[a]	C-24	40,3	40,0	39,6	40,3
C-9	50,6	50,8	51,0	50,0	C-25	28,5	28,2	28,0	28,2
C-10	37,1	37,2	37,2	36,4	C-26	23,1[b]	22,9[b]	22,8	22,9[b]
C-11	21,0	20,8	20,9	21,0	C-27	23,0[b]	22,6[b]	22,5	22,8[b]
C-12	39,8	39,6	39,0	39,2	OAc				171,1
C-13	42,8	42,6	42,6	42,4					169,5
C-14	57,4	57,2	56,6	56,8					21,8
C-15	24,9[a]	24,5	24,2	24,4					20,7
C-16	28,0	27,8	28,5	27,9					

*The values of the chemical shifts obtained by calculation: a; b — assignment of the signals ambiguous.

24-methylenecholest-5-ene-3β,21-diol [2] and of 5α-cholestane-3β,6α,7α,8β,15α,16β,27-heptaol [4]; the position of the primary hydroxy group at C-21 was confirmed and its position at C-26(27) was excluded.

On the basis of spectral characteristics, we assumed that the remaining two secondary hydroxy groups and the double bond were present in the polycyclic moiety of molecule (I). The signals of the methine protons of these groups were represented by a multiplet in the form of a singlet at 5.47 ppm with a half-width of 7.5 Hz and a doublet at 4.94 ppm with a splitting of 2.4 Hz and a half-width of 5.5 Hz. The olefinic proton gave a broadened doublet at 5.84 ppm with a splitting of 3.6 Hz.

Homodecoupling on 5.47 ppm converted the doublet at 4.94 ppm into a singlet with no change in the doublet at 5.84 ppm, while homodecoupling on 4.96 ppm converted the multiplet at 5.47 ppm into a triplet-like singlet (J ~ 2 hz), leaving the signal of the olefinic proton unchanged. The widths of the signals and the spin-spin coupling constants of the methine protons (see Table 2) showed their equatorial configuration and corresponed to the 3α,4β-position of the hydroxy groups in the nucleus. The Δ^5 position of the double bond was the most likely, since it had been shown previously that in this case an equatorial H-4 proton has the constants J(4e, 3e) = 2.7 Hz and J(4e, 2e) = 0.9 Hz and the decoupling of H-4e leaves the signal of the H-6 olefinic proton unchanged [5].

After the acetylation of (I) and (II), a characteristic upfield shift of the CH_3-19 signal caused by a decrease in the descreening of the axial hydroxy group, replaced by an acetoxy group, was observed. At the same time, the C-5 signal in (III) was shifted upfield and the C-6 signal downfield (see Table 1), which showed the closeness of these hydroxy groups and the double bond [7].

These effects agree well with the localization of the sulfate group at C-3; on desulfation, the H-3 signal shifted to 4.57 ppm, in agreement with literature statements [3].

To prove the presence in steroid (II) of an α-diol grouping, we carried out its periodate oxidation followed by reduction with sodium tetrahydroborate and acetylation. The fragmentation under the action of electron impact of the triacetate so obtained corresponded to the formula (V) proposed for it. The origin of the strongest peaks in its mass spectrum is connected with allyl cleavages and the splitting out of the substituent from C-10.

The oxidation of (I) with the Sarett reagent confirmed that the sulfated hydroxyl was located at C-3 and there was a secondary hydroxy group in the α-position to the double bond. The product of such oxidation had the structural fragment (VI), since its UV spectrum exhibited a strong absorption maximum at 258 nm. The bathochromic shift of this maximum in comparison with the absorption calculated for cholest-5-en-4-one (241 nm) is obviously due to the influence of the O-sulfate group located at C-3. The structure proposed for (I) was definitively confirmed by calculating the chemical shifts of the signals of the C-atoms of

TABLE 2. ^1H NMR Spectra of the Sulfated Steroid (I) and Its Derivatives (II)—(IV), C_5D_5N, δ, TMS = 0; ppm, Hz

Compound	CH-3	CH-4	CH-6	CH_3-18	CH_3-19	Compound	CH′-21	CH″-21	CH_3-26, 27	OAc
I	5,47 m	4,94 d (2,0)	5,84 d (3,6)	0,75 s	1,51 s	I	3,90 br. d (11,0)	4,05 br. d (11,0)	0,88 d (6,7)	
II	4,57 m	4,62 d (2,0)	5,85 d (3,5)	0,78 s	1,60 s	II	3,90 dd (11,0; 5,0)	4,07 dd (11,0; 2,0)	0,88 d (6,7)	
III	5,20 br. s*	6,03 br. s	5,95 d (3,5)	0,80 s	1,28 s	III	4,18 dd (11,0; 6,0)	4,43 dd (11,0; 3,0)	0,98 d (6,7)	2,10 2,26
IV	4,30 m	5,71 br. s	6,20 d (3,0)	0,73 s	1,30 s	IV	4,19 dd (11,0; 5,5)	4,43 dd (11,0; 3,0)	0,90 d (6,8)	2,15 2,10

*br. s — broadened singlet; br. d — broadened doublet.

(II) starting form the ^{13}C NMR spectra of some model compounds [2, 8] with allowance for the influence of a 3α,4β-diol system on the signals of the atoms of rings A and B. The calculated values agreed well with the experimental figures (see Table 1).

On the basis of the results obtained, we ascribed to the isolated compound the structure of cholest-5-ene-3α,4β,21-triol 3-(sodium sulfate) (I).

EXPERIMENTAL

The ophiuroid was collected at depths of 100—130 m in the littoral of the island of Paramushir (Kurile islands) during the second expeditionary cruise of the Scientific Research Ship Akademik Oparin in August, 1986.

The determination of the physical constants, the GLC analysis the chromato-mass-spectrometric study, and the recording of the ^1H and ^{13}C NMR spectra were carried out as described previously [1]. Metals were determined on an AA-780 atomic absorption spectrometer.

Isolation of Cholest-5-ene-3α,4β,21-diol 3-(sodium sulfate) (I). The ophiuroid was comminuted and extracted with ethanol, and the extract after evaporation to dryness (118 g) was chromatographed on a column of Polikhrom-1. On elution with aqueous ethanol (40%), 1.6 g of a mixture of sulfated polyols was obtained. Subsequent repeated column chromatography on silica gel (chloroform—ethanol—water) and on Sephadex H-20 (chloroform—methanol) gave 0.043 g (0.036% on the dry weight of the extract) of the individual compound (I) — $C_{27}H_{45}O_6 \cdot SNa$, mp 120—122°C, $[\alpha]_D^{20}$ — 12.37° (c 10, 15; methanol).

Cholest-5-ene-3α, 4β, 21-triol (II) was obtained by the solvolysis of 43 mg of (I) by the usual method [3]. Column chromatography on silica gel in the chloroform—ethanol (95:5) system yielded 31 mg of the individual compound $C_{27}H_{46}O_3$, mp 103—105°C, $[\alpha]_D^{20}$ — 15° (c 6.1; chloroform). Mass spectrum (m/z): 418 (M^+); 403 (M^+ −15); 372 (M^+ − 15 − 31).

Cholest-5-ene-3α,4β,21-dil 4,21-diacetate 3-(sodium sulfate) (III), $C_{31}H_{49}O_8SNa$, white amorphous substance, $[\alpha]_D^{20}$ — 33.6° (c 12.2; chloroform), was obtained on the acetylation of (I) by the usual method.

Cholest-5-ene-3α,4β,21-tril 3,21-diacetate (IV), $C_{31}H_{50}O_5$, white substance, mp 71—73°C, $[\alpha]_D^{20}$ — 26.3° (c 7.7; methanol), was isolated by the solvolysis of (III) followed by column chromatography on LH-20. Mass spectrum (m/z): 484 (M^+ − 18); 442 (M^+ − AcOH); 382 (M^+ − 2 × AcOH); 367 (M^+ − 2 × AcOH − 15).

Periodate Oxidation of the Steroid (II). To 15 mg of (II) in 2 ml of methanol was added 16 mg of sodium periodate in water (0.1 M solution), and the mixture was left at room temperature for two days. The residual $NaJO_4$ was decomposed with ethylene glycol, and the products were chromatographed on Al_2O_3 with elution by chloroform. The dry residue was reduced with $NaBH_4$ in ethanol, chromatographed on silica gel, and acetylated in the usual way. The resulting triacetate (6 mg) formed a white amorphous substance (V), $C_{33}H_{54}O_6$, $[\alpha]_D^{20}$ — 6.76° (c 3.4; chloroform). Mass spectrum (m/z): 546 (M^+); 444 (M^+ − 102); 458 (M^+ − 15 − 73); 384 (M^+ − 60 − 102).

Oxidation of the Sulfate (I) by Sarret's Complex. The Sarrett reagent (150 mg of CrO_3 in 2 ml of pyridine) was added to 82 mg of (I) in 3 ml of pyridine, and oxidation was carried out by the usual method followed by the purification of the ketone obtained on Polikhrom, silica gel, and Sephadex LH-20. This gave 11.5 mg of an amorphous yellow substance (VI). $[\alpha]_D^{20}$ + 13.12° (C 3.2; chloroform). IR spectrum, cm^{-1}: $\lambda_{max}^{CHCl_3}$ 1640(C = C), 1716(C = O). UV spectrum, nm: $\lambda_{max}^{CH_3OH}$ 258 (ε_{max} = 3390).

SUMMARY

A new sulfated polyhydroxy steroid — cholest-5-ene-3α,4β,21-triol 3-(sodium sulfate) — has been isolated from the Far Eastern ophiuroid Ophiura sarsi.

LITERATURE CITED

1. A. A. Kicha, A. I. Kalinovskii, E. V. Levina, Ya. V. Rashkes, V. A. Stonik, and G. B. Elyakov, Khim. Prir. Soedin., 356 (1985).
2. M. V. D'Auria, E. Finamore, L. Minale, C. Pizza, R. Riccio, F. Zollo, M. Pusset, and P. Tirard, J. Chem. Soc., Perkin Trans. I, 2277 (1984).
3. R. Riccio, M. V. D'Auria, and L. Minale, J. Org. Chem., 51, 533 (1986).

4. A. A. Kicha, A. I. Kalinovskii, E. V. Levina, V. A. Stonik, G. B. El'yakov, Bioorg. Khim., 9, 975 (1983).
5. T. N. Makar'eva, L. K. Shubina, A. I. Kalinovskii, and V. A. Stonik, Khim. Prir. Soedin., 272 (1985).
6. P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari, and E. Wenkert, J. Am. Chem. Soc., 90, 5480 (1968).
7. I. Whalberg, S. O. Almquist, T. Nishida, and C. R. Enzell, Acta Chem. Scand., B29, 1049 (1975).
8. C. L. Van Antwerp, H. Eggert, A. D. Meakins, J. O. Miners, and C. Djerassi, J. Org. Chem, 46, 5399 (1981).

TRITERPENE GLYCOSIDES OF Hedera taurica.

II. THE STRUCTURE OF TAUROSIDES B AND C FROM LEAVES OF CRIMEAN IVY

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Two triterpene glycosides have been isolated from the leaves of Hedera taurica Carr. (Crimean ivy), family Araliaceae — taurosides B and C, for which, on the basis of the results of chemical and physicochemical methods of investigation the following structures are proposed: 3-O-α-L-arabinopyranosylhederagenin and 3-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl]oleanolic acid.

Continuing a study of the triterpene glycosides from the leaves of Crimean ivy Hedera taurica Carr.* [1], we have isolated two weakly polar glycosides which have been called taurosides B and C, and we have established their structures.

The glycosides were isolated by the method of preparative column chromatography on silica gel followed by supplementary chromatographic purification in the form of the methyl esters at the free carboxy groups of the aglycons.

Analysis of acid hydrolysates of the glycosides with the aid of thin-layer chromatography (TLC) and paper chromatography (PC) showed that tauroside B contained hederagenin as the aglycon and tauroside C contained oleanolic acid; the carbohydrate moiety of glycoside B consisted of arabinose, and that of glycoside C of arabinose and rhamnose. The subsequent use of ^{1}H and ^{13}C NMR spectroscopy enabled the structures of the glycosides isolated to be determined completely without the use of traditional chemical methods such as methylation and periodate oxidation.

The general form of the PMR spectrum of tauroside B corresponded to the monomeric composition arabinose:hederagenin = 1:1. In a detailed analysis of the spectrum it was possible to determine the position and nature of the coupling of the majority of the skeletal protons of the monosaccharide residue (see the Experimental part). The values of the spin-spin coupling constants (SSCCs) unambiguously agreed with the α-arabino configuration of the carbohydrate moiety.

The assignment of the signals of the carbon atoms of the aglycon in the ^{13}C NMR spectrum of glycoside B (Table 1) was made by comparison with the spectrum of hederagenin taken under similar conditions [2], the downfield shift of the C-3 signal indicating the participation of the hydroxy group at this atom in the formation of a glycosidic bond. The other signals in the ^{13}C NMR spectrum belonging to the sugar residue were assigned with the aid of the method

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