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STEROID COMPOUNDS OF MARINE SPONGES.

XI. STEROIDS OF THE AUSTRALIAN SPONGE *Trachyopsis sp.*

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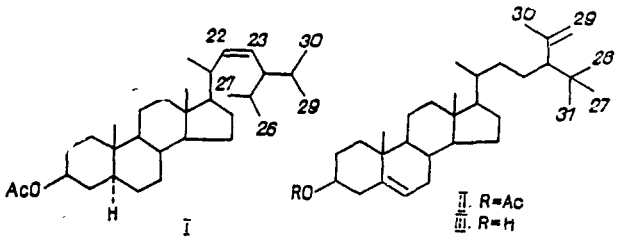
The compositions of two steroid fractions from the Australian sponge *Trachyopsis sp.* have been investigated. The fractions of steroid sulfates consisted of the trisulfates of halistanol (76%) and of 24-isopropyl-5α-cholest-22-ene-2β,3α,6α-triol (20%). In the sterol fraction, axinyssasterol (64%) and 24-isopropyl-5β-cholest-22Z-en-3 -ol (30%) were identified.

Continuing a study of physiologically active substances from sponges of the family *Halichondridae* [1], we have established the steroid composition of fractions of sulfated polyols and free sterols from the Australian sponge *Trachyopsis sp.* Both the fractions mentioned were isolated by methods that we have described previously [2].

These steroid sulfates were hydrolyzed, giving the previously known halistanol (76%) and 24-isopropyl-5α-cholest-22-ene-2β,3α,6α-triol (20%) which were identified in the form of the triacetates (GLC-MS) [2]. Analysis of the free sterols, performed with the aid of GLC-MS, showed the presence in this fraction of two main components (64 and 30% of the weight of the fraction). The two substances differed from the usual steroids of the majority of invertebrates and had 31 and 30 carbon atoms, respectively. The sterols were isolated, after acetylation, by chromatography on silica gel impregnated with silver nitrate.

Although these compounds were known previously as minor components of the sponges *Halichondria sp.* and *Pseudoaxinyssa sp.* [3, 4], their spectral properties have remained little studied.



I

II. R=Ac
III. R=H

The acetate of sterol (I) proved to be identical with the acetate of 24-isopropyl-5α-cholest-22Z-en-3β-ol from *Halichondria sp.* [3]. The assignment of the signals of the carbon atoms in its ^{13}C NMR spectrum was made with the aid of J-modulation, using INDOR and by comparing the spectrum with the spectra of model cholestanols [5, 6] (Table 1).

The acetate of sterol (II) was identical in structure with the acetate of axinyssasterol from *Pseudoaxinyssa sp.* In actual fact, the deacetylation of (II) gave this sterol (III), which was shown by a comparison of its ^{13}C NMR spectrum (in C_6D_6) with that described in the literature [5].

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TABLE 1. ^{13}C NMR of the Sterol Acetates (I) and (II) (δ, ppm, $CDCl_3$, $\delta_{TMS} = 0$)

C-atom	I	II	C-atom	I	II	C-atom	I	II
1	36,9	37,1	11	21,4	21,2	21	21,4	18,7
2	27,6	27,9	12	28,4	39,9	22	138,0	34,6
3	73,8	74,0	13	42,8	42,5	23	125,8	24,5
4	34,2	38,2	14	56,6	56,8	24	49,7	57,3
5	44,8	139,7	15	24,3	24,4	25	34,2	33,2
6	28,8	122,6	16	40,9	28,3	26	19,0	28,7
7	32,1	32,0	17	57,1	56,3	27	21,9	23,7
8	35,7	32,0	18	12,3	12,0	28	29,7	146,8
9	54,5	50,2	19	12,5	19,4	29	19,6	112,9
10	35,7	36,7	20	40,9	35,6	30	22,0	21,4
						31		28,7

We have succeeded in determining previously unknown physical constants of this compound and in analyzing the ^{13}C NMR spectrum of its acetate (II) (see Table 1).

It is interesting that all the steroids that we have found in the sponge Trachyopsis sp. have an unusual side-chain structure. This is also characteristic for other sponges from the family Halichondridae accumulating cytotoxic steroid polysulfates of the type of halistanol sulfate [1, 2, 7].

EXPERIMENTAL

The sponge was collected in August, 1988, off the north-eastern coast of Australia at a depth of 10-20 m in the seventh voyage of the Scientific Research Vessel Akademik Oparin.

GLC analysis was performed on a Perkin-Elmer Sigma 2000 chromatograph with a Shimadzu Chromatopak C-R 3A data-processing system using a capillary column 0.25 mm × 25 m with the immobilized phase OV-101, the carrier gas being helium, the flow split 1:60, and the temperature of separation 280°C.

A chromato-mass spectrometric study was conducted on a LKB-2091 spectrometer coupled with a Packard 438 A chromatograph, an injector of the "falling needle" type, a quartz capillary column 25 m long with the phase SE-54, the temperature of analysis being from 220 to 260°C, the carrier gas helium, and the ionizing 70 energy eV.

For chromatography we used type L silica gel (Czechoslovakia), 5/40 μm, with the addition as fixing agent of 5% of gypsum (TLC); and also the 40/100 μm variety (for column chromatography).

The ^1H and ^{13}C NMR spectra were obtained on a Bruker WH-250 instrment in deuterochloroform and deuterobenzene. The signals in the NMR spectra are given on the δ-scale; tetramethylsilane was used as internal standard.

Melting points were measured on a stage of the Boëtius type, and specific rotations on a Perkin-Elmer 141 polarimeter.

Isolation of the Steroid Fractions. The frozen animals were comminuted and extracted with chloroform–ethanol (2:1). The extract was concentrated in vacuum to dryness. The residue was dissolved in aqueous ethanol, hexane was added, and substances were distributed between aqueous ethanol and hexane. The hexane layer, after chromatography on a column of silica gel and recrystallization from ethanol, yielded 660 mg (1.17% of the dry weight of the animals) of the total steroid fraction. From the aqueous ethanolic layer, after chromatography on Polychrome [2], 1.0 g (0.25% of the dry weight of the animals) of a total steroid trisulfate fraction was obtained.

The analysis of the steroid trisulfates was made on the corresponding triacetates by GLC-MS [2, 7]. The composition of the fraction was: halistanol triacetate (76%); 24-isopropyl-5α-cholest-22-ene-2β,3α,6α-triol (20%).

The analysis of the sterols was carried out after the preparation of their acetates (115 mg, acetic anhydride–pyridine (1:1), 25°C, 16 h), separation on a column of silica gel impregnated with 20% of $AgNO_3$, and elution with hexane–toluene (22:1 → 6:1) at a temperature of +10°C. The fractions obtained were analyzed by the GLC, GLC-MS, and ^1H NMR methods.

Two individual compounds were isolated (in the order of their emergence from the column): 24-isopropyl-5α-cholest-22Z-en-3β-ol acetate (I), 29 mg, RRT 1.73, mp 146-148°C (from ethyl acetate), $[\alpha]_{578}^{20}$ − 20° (c 0.1; $CHCl_3$). Mass spectrum (m/z, %): 470 (M^+, 20); 427 (M^+ −C_3H_7, 23); 367(M^+ − C_3H_7−AcOH, 100); 344 (cleavage of C_{20}−C_{22}, 14), 315 (M^+ −2 × C_3H_7 − AcOH, 43); 273(17), 257(74), 255(23), 229(23), 215(26). ^1H NMR ($CDCl_3$): 0.68 (s, CH_3-18); 0.82 (s, CH_3-19); 0.95 (d, J = 6.5; CH_3-21); 0.88 (d, J = 6.8; CH_3-26), 0.79 (d, J = 6.8; CH_3-27); 0.81 (d, J = 6.8; CH_3-29); 0.91 (d, J = 6.8; CH_3-30); 4.7 (x, H-3); 5.2 (d.d, J = 10.0; 11.5; H-22); 4.95 (d.d, J = 10.0; 11.5; H-23). For details of the ^{13}C NMR spectrum, see Table 1.

24-Isopropenyl-25-methylcholest-5-en-3β-ol acetate (II), 29.4 mg, RRT 1.98, mp 99-101°C (from ethanol), $[\alpha]_{578}^{20}$ −34° (c 0.1; $CHCl_3$). Mass spectrum (m/z, %): 422 (M^+ − AcOH, 100); 407 (M^+ − AcOH − CH_3, 10); 366 (M^+ − AcOH − C_4H_8, 42); 351 (M^+ − AcOH − C_4H_8 − CH_3, 23); 253 (33), 228 (15), 213 (30). ^1H NMR ($CDCl_3$): 0.68 (s, CH_3-18); 1.02 (s, CH_3-19), 0.91 (d, J = 6.5; CH_3-21); 0.89 (c, CH_3-26, 27, 31); 1.68 (d, J = 2.5; CH_3-30); 4.61 (m, H-3); 4.64 (d, J = 2.5; H_a-29); 4.82 (t, J = 2.5; H_0-29); 5.38 (m, H-6); for details of the ^{13}C NMR spectrum, see Table 1. In addition, 57.7 mg of a mixture of (I), (II), and minor sterols was isolated.

24-Isopropenyl-25-methylcholest-5-en-3β-ol (III) was obtained from 20.4 mg of the acetate (II) in absolute methanol after treatment with 0.5 N sodium methanolate at room temperature for 4 h, neutralization of the solution with KU-2 resin (H^+), evaporation, and crystallization from ethanol. Yield 19.2 mg, mp 109-112°C, $[\alpha]_{578}^{20}$ −24°C (c 0.8; $CHCl_3$). Mass spectrum (m/z, %): 440 (M^+, 36); 425 (M^+ −CH_3, 17); 422 (M^+ − H_2O, 10); 407 (M^+ − CH_3−H_2O, 10); 384 (M^+−C_4H_8, 521); 369 (M^+−C_4H_8−CH_3, 67); 366 (M^+−C_4H_8−H_2O, 21); 351 (M^+−C_4H_8−CH_3−H_2O, 36); 328 (10), 314(11), 299(80), 271(100), 255(82), 229(33), 215(31), 213 (60). ^1H NMR ($CDCl_3$): 0.68 (s, CH_3-18); 1.02 (s, CH_3-19); 0.91 (d, J = 6.5, CH_3-21); 1.38 (m, H-20); 0.89 (s, CH_3-26, 27, 31); 1.68 (d, J = 2.5 CH_3-30); 1.68 (m, H-24); 4.61 (m, H-3); 4.64 (d, J = 2.5, H_a-29); 4.82 (t, J = 2.5; H_b-29); 5.38 (m, H-6). The ^1H NMR spectrum in C_6D_6 was identical with that given in the literature for axinyssasterol [4].

*Relative retention time (RRT) in GLC taking the RT of cholesterol acetate as 1.0.

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