New Ophiuroid-Type Steroids from the Starfish *Pteraster tesselatus*

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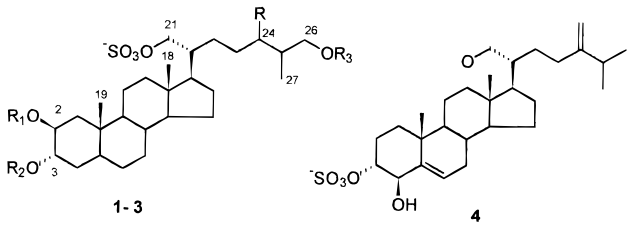
Two new steroid sulfates have been isolated and a new minor steroid sulfate identified from the starfish *Pteraster tesselatus*. The structures of the new steroids were established on the basis of spectroscopic data as the 3,21-disulfate of (20*R*,25*R*)-24-methyl-5α-cholest-24(28)-ene-2*β*,3α,21,26-tetraol (**1**) and the 2,21-disulfate of (20*R*,25*R*,*S*)-cholest-5-ene-2*β*,3α,21,26-tetraol (**2**). The new minor steroid proved to be the 3,21-disulfate of (20*R*,25*R*)-5α-cholestane-2*β*,3α,21,26-tetraol (**3**). All the compounds have structural features characteristic for ophiuroid-type steroids.

Steroid sulfates from starfish and ophiuroids have characteristic structural features and differ from each other. For instance, the sulfates from ophiuroids are sulfated diols, triols, and rarely tetraols with oxidized 3α- and 21-positions. In the sulfates from starfish, hydroxyl or sulfoxy groups occupy five or more positions such as 3*β*, 6α(or *β*), 8*β*, 15α(or *β*), 16*β*, and some others.[1] Recently, we were surprised to find that two species of starfish belonging to the family Pterasteriidae, namely *Pteraster tesselatus* and *Pteraster* sp., contain several ophiuroid-type steroid sulfates. We have previously identified two of these steroids as the 2,21- and 3,21-disulfates of cholest-5-ene-2*β*,3α,21-triol.[2]

In continuation of the studies on steroid metabolites from the starfish *P. tesselatus*, we report here the isolation of two new (**1**, **2**) and one known steroid sulfate (**4**), as well as the identification of a new minor sulfated steroid (**3**). Fresh tissues of the starfish collected from a depth of about 200 m in the Okhotsk Sea were extracted with MeOH, the solvent was evaporated under reduced pressure, and the resulting aqueous suspension was then extracted with *n*-BuOH. Separation and isolation of the sulfated steroids from the *n*-BuOH extract were achieved by chromatography on Amberlite XAD-2, Sephadex LH-60, and Si gel, followed by reversed-phase HPLC. Individual steroids **1**, **2**, and **4** and an inseparable mixture of **1** and **3** were obtained.



1 R=CH_2, R_1=R_3=H, R_2=SO_3Na, $\Delta^{24(28)}$

1a R=CH_2, R_1=R_3=Ac, R_2=SO_3Na, $\Delta^{24(28)}$

1b R=CH_2, R_1=R_2=R_3=H, $\Delta^{24(28)}$

2 R=R_2=R_3=H, R_1=SO_3Na, Δ^5 (mixture of 25R- and 25S-epimers)

3 R=R_1=R_3=H, R_2=SO_3Na

3a R=H, R_1=R_3=Ac, R_2=SO_3Na

The structures of the new compounds **1** and **2** were established by interpretation of spectral data (NMR and FABMS) as well as by comparison of their spectra with those of related compounds. (20*R*,25*R*)-24-Methyl-5α-cholest-24(28)-ene-2*β*,3α,21,26-tetraol 3,21-disulfate (**1**) showed peaks in FABMS (negative ion mode) at *m*/*z* 645 [$M(SO_3K)(SO_3^-)$] (16), 630 [$M(SO_3Na)(SO_3H)$] (38), 629 [$M(SO_3Na)(SO_3^-)$] (100), and 525 [$M - NaHSO_3 - Na$] (13) in accordance with a disulfated tetrahydroxycholestene structure. These data, along with the ^{13}C NMR spectrum, established a molecular formula of $C_{28}H_{46}O_4(SO_3M)_2$ (M = K/Na). The NMR data (Table 1) were indicative of a 21-*O*-sulfoxy fragment, a hydroxyl group at C-26, and a methylene group at C-24 in the side chain. In fact, proton signals at δ_H 4.18 (1H, dd, J = 10.0, 3.7 Hz) and 3.95 (1H, dd, J = 10.0, 6.5 Hz) and a carbon signal at δ 69.6 ppm are characteristic for compounds having a sulfoxy group at C-21.[2] The signals of an ABX system at δ_H 3.58 (dd, J = 6.1, 11.0 Hz), 3.33 (dd, J = 7.6, 11.0 Hz), and 2.30 ppm (m) in the NMR spectrum, as well as the loss of the resonance of one methyl group in the side chain, supported the presence of a hydroxyl group at C-26. Indeed, irradiation of the multiplet at 2.30 ppm collapsed the doublet at δ_H 1.06 ppm (H_3-27) into a singlet and sharpened both broad exomethylene proton singlets at δ_H 4.81 and 4.75 ppm (H-24) and the signals of the hydroxymethylene protons at δ_H 3.58 and 3.33 ppm (H_2-26).

The shifts of the C-26 and H_3-27 signals (δ_C 67.5 and δ_H 1.06 ppm) in comparison with those of known 26-hydroxylated steroids (δ_C 68.4−68.6 ppm and δ_H 0.93−0.94 ppm[3,4]) may be explained by the *γ*-effect of a 24(28) double bond.[5] The chemical shifts of C-24−C-28 and H-24−H-28 in the NMR spectra of **1** are coincident with those of the previously described (20*R*)-24-methyl-5α-cholest-24(28)-ene-3*β*,6*β*,15α,16*β*,26-pentaol from the starfish *Sphaerodiscus placenta*.[6] On this basis we suggested the same 25*R* stereochemistry for **1** as in the pentaol.

There was also a coincidence among the C-6−C-16, C-18, C-19 resonances in the spectrum of **1** and the corresponding signals in the spectrum of the 2,3,21-trisulfate of 5α-cholestane-2*β*,3α,26-triol from the ophiuroid *Ophiarachna incrassata*.[7] Moreover, all the carbon and proton signals of the tetracyclic moiety in the NMR spectra of **1** were practically identical with those of the recently described (20*R*)-5α-cholest-24-ene-2*β*,3α,21-triol 3,21-disulfate from the Antarctic ophiuroid *Astrotoma agassizii*.[8]

The positions of the hydroxyl group at C-2 and the sulfate group at C-3 were confirmed by the coupling of the broad quartets at δ_H 4.07 (H-2α, $\Delta W_{1/2}$ = 6 Hz) and δ_H 4.39 ppm (H-3*β*, $\Delta W_{1/2}$ = 5 Hz) with each other ($J_{2,3}$ = 2.0 Hz) as well as their transformation into a triplet (J = 2.5 Hz)

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Table 1. NMR Spectral Data for Compounds (**1**−**3**) (250 MHz, δ, CD_3OD)

	compounds							
	1		**1a**		**2**		**3a**	
atom	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	40.6	1e: 1.75 m; 1a: 1.33 m	38.4		39.0	1e: 2.12 dd; 1a: 1.59 m	38.4	
2	70.0	4.07 br q ($W_{1/2}$ = 6.0)	72.7	5.13 br q	78.4	4.44 m ($W_{1/2}$ = 7.5)	72.7	5.13 br q
3	78.5	4.39 br q ($W_{1/2}$ = 5.0)	75.4	4.39 br q	69.7	4.02 br d (3.0)	75.4	4.39 br q
4	30.4		30.9		35.9	4a: 2.80 br d (14.7); 4e:1.97 m	30.9	
5	41.1		40.0		139.6		40.0	
6	29.3		29.1		123.3	5.35 m	29.1	
7	33.2		33.1		32.9		33.1	
8	36.4		36.4		32.9		36.4	
9	56.7		56.5		52.1		56.5	
10	37.0		36.3		37.8		36.3	
11	22.0		21.9		21.9		21.9	
12	41.2		41.1		40.2		41.1	
13	43.7		43.7		43.4		43.7	
14	57.7		57.7		58.0		57.7	
15	25.1		25.1		25.2		25.1	
16	29.0		28.5		28.5		28.5	
17	52.1		52.1		51.8		52.2	
18	12.8	0.71 s	12.8	0.72 s	12.6	0.74 s	12.8	0.72 s
19	14.6	0.99 s	14.4	0.93 s	22.1	1.16 s	14.4	0.93 s
20	40.7		40.5		41.3		40.3	
21	69.6	4.18 dd (10.0, 3.7); 3.95 dd (10.0, 6.5)	69.6	4.24 dd (10.0, 3.7); 3.98 dd (10.0, 6.5)	69.8	4.19 dd (10.0, 3.8); 3.97 dd (10.0, 6.5)	69.8	4.18 m; 3.94 m
22	30.6		30.6		31.1 (31.0)		30.9	
23	28.5		32.2		28.5		24.1	
24	153.8	4.75 br s	153.0		34.9 (35.0)		35.0	
25	43.3	2.30 m	40.3	2.48 m	36.8 (36.9)		33.8	1.77 m
26	67.5	3.58 dd (6.1; 11.0); 3.33 dd (7.6; 11.0)	69.8	4.08 dd (6.1, 11.0); 3.95 dd (7.0, 11.0)	68.5 (69.6)	3.54 dd (10.5, 5.5); 3.45 dd (10.5, 5.5)[a]	70.6	3.92 dd (3.7; 11.0); 3.86 dd (7.0; 11.0)
27	17.2	1.06 d	17.3	1.08 d	17.2 (17.3)	0.93 d	17.3	0.93 d
28	109.4	4.81 br s	109.9	under solvent signal				
OAc			171.5	2.03, 2.02 s			171.5	2.03, 2.02 s

[a] High-field part of ABX system and the proton signals of H_2-26 of an epimer are under solvent.

and a broad triplet (J = 3.5), respectively, under double resonance conditions. The pyridine-induced shift of the signal H_3-19 from δ_H 0.99 to 1.28 ppm (C_5D_5N−CD_3OD, 7:1) also corresponded to a C-2 position of a hydroxyl group.[9] The stereochemistry at C-20 was assigned as 20*R*. This was established by comparison of the chemical shifts of the hydroxymethylene protons at C-21 in the desulfated derivative **1b** with those of (20*R*)- and (20*S*)-cholest-5-ene-3β,21-diols.[10] The (20*R*)-isomer was reported to exhibit a broad singlet at δ_H 3.70 (s), while the (20*S*)-isomer showed multiplet at δ_H 3.62 ppm. Therefore, our value of 3.72 ppm (br s) demonstrated (*R*)-configuration of C-20. On the basis of all the above data, the structure of **1** was established as the 3,21-disulfate of (20*R*,25*R*)-24-methyl-5α-cholest-24-(28)-ene-2β,3α,21,26-tetraol.

A mixture of acetates **1a** and **3a** (1:0.8) was isolated by HPLC after acetylation of a mixture of **1** and **3**. FABMS of the mixture gave peaks at *m*/*z* 714 [M_{1a} + H − Na] (15), 713 [M_{1a} − Na] (100), 691 [M_{1a} + H − 2Na] (7), and 653 [M_{1a} − Na−AcOH] (11) and another series of peaks differing by 12 amu and belonging to **3a**: *m*/*z* 702 [M_{3a} + H − Na] (10), 701 [M_{3a} − Na] (52), 679 [M_{3a} + H − 2Na] (4), and 641 [M_{3a} − Na−AcOH] (12). All the proton and carbon signals of the tetracyclic moieties in NMR spectra of the both acetates were essentially identical. The downfield shift of the H-2 signal from δ_H 4.07 to 5.13 ppm and the upfield shift of the H_3-19 signal from δ_H 0.99 to 0.93 ppm confirmed the acetylation of an axial hydroxyl at H-2 located in the 1,3 position to a methyl group at C-10. The signals of the side chains in spectra of **1a** and **3a** differed. Compound **1a** showed a doublet for H_3-27, multiplet signals of H_2-26, and a multiplet for H-25 at δ_H 1.08, 4.08, 3.95, and 2.48 ppm, respectively, while compound **3a** showed a doublet for H_3-27 at δ_H 0.93 ppm and signals for H_2-26 at δ_H 3.92 and 3.86 and a multiplet for H-25 at δ_H 1.77 ppm. The chemical shifts of C-23−C-27 as those of H_2-26 and H_3-27 in the spectra of **3a** were practically identical with those of the acetylated mosesins, shark repellents from defense secretion of the moses sole *Pardachirius marmoratus*.[11] This identity showed that the configuration at C-25 in **3a** is the same as in mosesin-1, namely, 25*R*. As the basis for this conclusion, appreciable chemical shift differences have been observed for the H_3-27 and H_2-26 signals between the spectra of 25*R*- and 25*S*-epimers of 26-hydroxy-5β-cholestan-3-one as well as between the corresponding signals in the spectra of their acetates.[12] The presence of a 26-*O*-acetylated side chain in **3a** was confirmed by decoupling with irradiation of signals at δ 3.86 and 0.93 ppm, revealing the same multiplet at 1.77 ppm (H-25). On this basis we have identified **3a** as the 2,26-diacetate of the 3,21-disulfate of (20*R*,25*R*)-5α-cholestane-2β,3α,21,26-tetraol.

The steroid disulfate **2** showed signals for C-1−C-22 and H-18, H-19, and H-21 practically identical with those of the previously described 2,21-disulfate of cholest-5-ene-2β,3α,21-triol from *P. tesselatus*.[2] The corresponding spectral data for C-23−C-27 coincided with those of 5α-cholestane-3β,5,6β,15α,16β,26-hexaol 3-sulfate isolated from the starfish *Luidia quinaria*.[3] The signals of the side chain carbons (especially the C-22−C-26 and C-27 signals) in the ^{13}C NMR spectra of **2** appeared as doublets with a separation between the peaks ranging from 0.04 to 0.2 ppm. This is characteristic of C-25 epimeric steroids with a hydroxyl group at C-26.[7] One epimer was present in relatively major amount, although we could not establish which epimer it is (25*R* or 25*S*). Therefore, compound **2** is a mixture of the 25*R* and 25*S* isomers of the 2,21-disulfate of (20*R*,25*R,S*)-cholest-5-ene-2β,3α,21,26-tetraol.

Besides the new steroids **1**−**3**, we have isolated the known 3,21-disulfate of (20*R*)-24-methyl-cholesta-5,24(28)-diene-3α,4β,21-triol (**4**), previously isolated by Minale et al. from the ophiuroids *Ophiotrix fragilis* and *Ophiura texturata*.[13] Identification of **4** was carried out by comparison of NMR data with those reported earlier.[13]

Prior to our recent work, sulfated steroids hydroxylated at the 3α- and 21-positions were found in ophiuroids only.[1,14] Recently we reported the isolation of ophiuroid-type steroids, namely the 2,21-disulfate of cholest-5-ene-2β,3α,21-triol and the 3,21-disulfates of cholest-5-ene-2β,3α,21-triol and cholest-5-ene-3α,4β,21-triol, from starfish of the genus *Pteraster*.[2] Earlier D'Auria et al.[15] isolated related disulfates of 3β,21-dihydroxysteroids from the starfish *Euretaster insignis*. It is of special interest that both the genera *Euretaster* and *Pteraster* belong to the same Pterasteriidae family. Steroids **1**−**3** not only have a 3α,21-dioxidized fragment similar to other ophiuroid-type steroids, but also a 26-hydroxylated side chain like those of many polyhydroxylated starfish steroids and their glycosides.[1,14] These data as well as our recent communication[2] and the paper of D'Auria et al.[15] confirm the opinion of some taxonomists[16] that ophiuroids and starfish are phylogenetically closely related.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM-250 spectrometer at 250 MHz, with TMS as internal standard. Melting points were determined on a Boethius apparatus. Optical rotations were measured using a Perkin−Elmer 141 apparatus. Preparative HPLC was carried out on a DuPont 8800 chromatograph equipped with an refractive index detector. The column LPLC was performed using Si gel L (Chemapol, the former Czechoslovakia), Amberlite XAD-2 (20−80 mesh), Sephadex LH-60 (Sigma, Chemical Co.).

Animal Material. Specimens *P. tesselatus* were collected by dredging (the f/s "Dalarik") in August 1983, from a depth of 100 m near Onekotan Island (the Okhotsk sea). Species identification was carried out by Dr. V. V. Karpenko and Prof. O. G. Kusakin (Institute of Marine Biology of the Far East Division of the Russian Academy of Science, Vladivostok). Voucher specimens (PIBC 017-173a) are kept in the collection of marine organisms of PIBC RAS.

Extraction and Isolation. Fresh animals were immediately extracted with MeOH. Extracts of *P. tesselatus* (15 kg, wet wt) were concentrated under *vacuum* at 50 °C, and aqueous residue was then extracted twice with *n*-BuOH. The dark oil, obtained after the evaporation of the *n*-BuOH extract (450 g), was chromatographed on Amberlite XAD-2 column using MeOH−H_2O (0:100 → 60:40). The fractions, eluted with 40% MeOH, were combined and concentrated under vacuum to give a brownish residue (10 g). This residue, containing a crude mixture of steroid sulfates, was repeatedly chromatographed on a column (3.5 × 25 cm) with Si gel L, 40/100 μ, using $CHCl_3$−MeOH (5:1 → 3:1). Fractions were analyzed by TLC in the *n*-BuOH−EtOAc−H_2O (5:1:1) system. Subfraction 1 contained three known compounds, previously isolated from some ophiuroids.[2] Subfraction 2 contained sulfated steroids (420 mg) and was repeatedly purified on a Sephadex LH-60 column (40 × 1.5 cm) using the system $CHCl_3$−MeOH (4:1). The final purification of the subfraction was achieved by preparative reversed-phase HPLC on a Zorbax-ODS column (4.6 mm × 25 cm) with elution by 40−45% MeOH to give 310 mg of the fraction. Repeated HPLC on the same column (45% MeOH) yielded **2** (25.3 mg), a mixture of **1** and **3** (18.4 mg), **1** (7.5 mg), and **4** (13.4 mg).

(20*R*,25*R*)-24-Methyl-5α-cholest-24(28)-ene-2β,3α,21,26-tetraol 3,21-disulfate (1): mp 178−179.5 °C (MeOH); $[\alpha]^{20}_D$ +1.78° (c 11.8, MeOH); ^{13}C and ^{1}H NMR spectra, see Table 1; FABMS, see text.

Solvolysis of Compound 1. A solution of **1** (4.8 mg) in a mixture of pyridine (0.5 mL) and dioxane (0.5 mL) was heated at 110 °C for 2 h in a stoppered reaction vial. After removal of the solvents under vacuum the residue was chromatographed on Si gel column (7 × 1 cm) and **1b**, mp 123−125 °C, was eluted with $CHCl_3$ −MeOH (100:0 → 80:20); ^{1}H NMR (CD_3 OD, 250 MHz) δ 0.69 (3H, s, CH_3-18), 0.98 (3H, s, CH_3-19), 3.72 (2H, m, H-21, H-3β), 3.66 (1H, m, H-2α), 3.51 (1H, dd, H-21), 1.06 [3H, d (6.5), CH_3-27].

Mixture of 2,26-diacetates of (20*R*,25*R*)-24-methyl-5α-cholest-24(28)-ene-2β,3α,21,26-tetraol 3,21-disulfate (1a) and (20*R*,25*R*)-5α-cholestane-2β,3α,21,26-tetraol 3,21-disulfate (3a). The acetylation of the mixture of **1** and **3** treating with Ac_2O (1 mL) and pyridine (1 mL) by stirring for 12 h at room temperature afforded a mixture (19 mg) of acetates **1a** and **3a**, further purified by HPLC on a Zorbax-ODS column (4.6 mm × 25 cm) with 65% MeOH as eluent, mp 182−185 °C; $[\alpha]^{20}_D$ +9.15° (*c* 9.4, MeOH). Steroid **3** was identified as the 3,21-disulfate of (20*R*,25*R*)-5α-cholestane-2β,3α,21,26-tetraol by ^{1}H NMR spectroscopy of its acetate **3a** (see Table 1). The spectrum of **3a** was obtained by subtraction of the spectrum **1a** from the spectrum of a mixture of **1a** and **3a** and by careful studies of the mixture by difference using spin-decoupling and NOE experiments. For the NMR data for **1a** and **3a**, see Table 1; FABMS, see text.

(20*R*,25*R*)-Cholest-5-ene-2β,3α,21,26-tetraol 2,21-disulfate (2): mp 187−189 °C (MeOH − H_2O); $[\alpha]^{20}_D$ −17° (*c* 17.2; MeOH); NMR data, Table 1.

(20*R*)-24-Methylcholesta-5,24(28)-diene-3α,4β,21-triol 3,21-disulfate (4): amorphous powder; $[\alpha]^{20}_D$ −16.0° [*c* 11.2; MeOH]; ^{1}H and ^{13}C NMR data identical with those reported by D'Auria et al.[13]

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