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Novel Marine Steroid Sulfates from Pacific Ophiuroids

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New sulfated polyhydroxy steroids have been isolated from three species of Pacific ophiuroids. The major compound in all three species was shown to be 5β-cholestane-3α,4α,11β,21-tetrol 3,21-disulfate (1). Two minor compounds possessed the same nuclei and differed in the side chain (2, 3) and one was shown to be the 11-keto derivative of the major 1. *Ophiarachna incrassata* also contained a new group of sulfated 5α-H steroids possessing the unusual 2β,3α-disulfoxy substituents.

Recently, we reported the structure of two new steroidal glycoside sulfates[1] and of a group of cytotoxic disulfated 3α,21-dihydroxy steroids along with the moderately cytotoxic 5β-cholestane-3α,4α,11β,12β,21-pentol 3,21-disulfate from the mediterranean ophiuroid *Ophioderma longicaudum*.[2,3] These finding and our continuing interest on active metabolites from echinoderms[4] prompted us to examine some further ophiuroids for their polar steroids content.

In this paper we report on new sulfated polyhydroxy steroids isolated from three species collected off Nouméa (New Caledonia), *Ophiocoma dentata*, *Ophiarthrum elegans*, and *Ophiarachna incrassata*.

The brittle stars were extracted fresh with methanol and the extract was then partitioned between hexane and water. The aqueous phase was then extracted with 1-butanol and the butanol fractions were purified by chromatographic separation on Sephadex LH-20 and by HPLC (see Experimental Section).

***Ophiocoma dentata*.** The major polar steroid component has been characterized as **5β-cholestane-3α,4α,11β,21-tetrol 3,21-disulfate (1)**, $[\alpha]_D$ +41.3°, mp 195–196 °C. Negative ion fast atom bombardment (FAB) mass spectrum exhibited molecular ion species at m/z 631 (very small) and 617 (major), corresponding to the monopotassium and monosodium salt of the dianion, respectively. Next to the molecular ion species the spectrum displayed intense fragments with m/z 515 and 497, which correspond to the loss of $NaSO_3$ (+H) and $NaHSO_4$ from m/z 617. The most valuable structural information came from high-resolution NMR investigations. The ^{13}C NMR spectrum measured in CD_3OD at 62.9 MHz was consistent with the presence of 27 carbon atoms (Table I) and DEPT measurements revealed the presence of four methyl groups, ten methylene, seven methine, two quaternary carbons, three –OCH<, and one $-OCH_2-$. Taken together these data indicated a disulfated tetrahydroxycholestane structure with one of the five methyl groups typical of a sterol oxidized to hydroxymethylene. The ^1H NMR spectrum (Table II) confirmed the presence of four methyl groups, two tertiary and two secondary, and one oxygen-bearing methylene group with signals at δ 3.99 (1 H, dd, J = 10, 6.3 Hz) to 4.21 (dd, J = 10, 3.5 Hz, emerging from a 4 H broad multiplet). The chemical shifts and shape of the methylene proton signals were reminiscent of those observed in the spectra of 21-sulfoxy steroids.[2,5] Oxidation of CH_3-26 or CH_3-27 could be ruled out on the basis of the presence in the ^{13}C NMR spectrum of typical signals for C-26 and C-27 at 23.0 and 23.1 ppm.[6] In the spectrum of 1 three >CHO signals overlap at δ 4.22, but when we measured the spectrum of the tetrol **1a**, derived from 1 upon solvolysis, the resonances of the protons on the hydroxy-bearing carbons gave rise to five isolated signals (Table II). The resonance frequencies of the hydroxymethylene protons moved upfield as the AB part of an ABX system to δ 3.69 and 3.74 and those of the hydroxymethine protons were observed at δ 4.22 (apparent q, J = 2.5 Hz, 11-H), 3.90 (apparent t, J = 4.0 Hz, 4-H) and

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(1) Riccio, R.; D'Auria, M. V.; Minale, L. *J. Org. Chem.* **1986**, *51*, 533.
(2) Riccio, R.; D'Auria, M. V.; Minale, L. *Tetrahedron* **1985**, *41*, 6041.
(3) Andersson, L.; Bohlin, L.; Iorizzi, M.; Minale, L.; Riccio, R., manuscript on the biological activities of these compounds in preparation.
(4) Minale, L.; Riccio, R.; Pizza, C.; Zollo, F. In *Natural Products and Biological Activities*, a Naito Foundation Symposium; Imura, H., Goto, T., Murachi, T., Nakajima, T., Eds.; University of Tokyo Press: Tokyo 1986; pp 59–73.
(5) D'Auria, M. V.; Finamore, E.; Minale, L.; Pizza, C.; Riccio, R.; Zollo, F.; Pusset, M.; Tirard, P. *J. Chem. Soc., Perkin Trans 1* **1984**, 2277.
(6) Blunt, J. W.; Stothers, J. B. *Org. Magn. Reson.* **1977**, *9*, 439.

0022-3263/87/1952-3947$01.50/0

Table I. ^{13}C NMR Spectral Data[a]

C	1a	1[b]	2	3	4	5[c]	6[c]
1	36.1	3.61	36.1	36.1	35.5	39.2	39.2
2	25.8	23.5 (−2.3)	23.5	23.5	23.4	76.1	76.1
3	74.1	82.5 (+8.4)	82.5	82.5	82.1	76.2	76.5
4	77.2	75.3 (−1.9)	75.3	75.3	75.5	30.6	32.7
5	48.1	48.8 (+0.7)	48.8	48.8	48.8	40.2	139.0
6	27.0	27.0	27.0	27.0	27.3	29.2	123.4
7	28.2	28.5	28.5	28.8	28.6	33.2	32.9
8	31.9	31.8	31.9	31.9	37.7	36.4	32.7
9	46.0	45.9	46.0	46.0	54.6	56.2	52.0
10	36.4	36.4	36.4	36.4	35.6	36.4	37.0
11	69.1	69.0	69.0	68.9	215.6	22.0	21.9
12	49.8	49.8	49.6	49.6	58.7	41.4	41.2
13	42.8	42.7	42.6	42.7	*d*	43.8	43.5
14	59.4	59.3	59.3	59.4	56.9	57.9	58.2
15	25.1	25.1	25.1	25.1	25.0	25.1	25.2
16	30.0	29.8	29.9	29.9	29.7	29.2	29.2
17	52.7	52.8	52.8	52.8	51.1	57.8	57.7
18	15.2	15.0	15.1	15.1	13.6	12.5	12.3
19	27.4	27.3	27.3	27.3	24.1	14.2	22.1
20	43.8	41.5 (−2.3)	41.4	46.5	41.0	37.00 (37.10)	37.00 (37.10)
21	63.4	69.8 (+6.4)	69.7	71.6	69.2	19.2	19.2
22	30.6	31.0 (+0.4)	31.9	131.0	31.0	37.40 (37.30)	37.40 (37.30)
23	24.9	24.7	30.0	134.0	24.6	24.51 (24.45)	24.51 (24.45)
24	40.8	40.7	158.1	43.2	40.6	34.77 (34.88)	34.71 (34.88)
25	29.1	29.0	34.9	29.6	29.0	34.36 (34.40)	34.36 (34.41)
26	23.1	23.1	22.4	22.7	23.1	74.08 (73.96)	74.08 (73.96)
27	23.0	23.0	22.3	22.7	23.0	17.04 (17.25)	17.04 (17.25)
28			106.9				

[a] At 62.9 MHz; values relative to CD_3OD = 49.00 ppm (central peak); assignments aided by DEPT technique. [b] In parentheses are the sulfation shifts. [c] Mixture of 25*R* and 25*S* isomers; in parentheses are the shifts of the minor isomer. [d] Signal under methanol signal.

Table II. Selected 250-MHz Chemical Shifts of 5β-Steroids 1–4[a]

sterol	H-3β	H-4β	H-11α	H_3-18	H_3-19	H_2-21	H_3-26,27	other signals
1	4.22 m	4.22 m	4.22 m	0.95 s	1.18 s	3.99 dd (10, 6.3) 4.22 d (10, 3.5)	0.91, 6 H,d (7)	
1a	3.62 dt (12, 3.5)	3.90 t (4.0)	4.22 q (2.5)	0.91 s	1.16 s	3.69 dd (11, 4.5) 3.74 dd (11, 3)	0.87, 6 H, d (7)	H-9 2.03 dd (11.5, 2.5); H_2-12 1.53 dd (14, 2.5), 2.06 dd (14, 2.5)
2	4.22 m	4.22 m	4.22 m	0.96 s	1.17 s	4.00 dd (10, 6.5) 4.22 dd (10, 3.5)	1.06, 6 H, d (7)	H_2-28 4.72 br s, 4.74 br s; H-25 2.31 m
2a	3.62 dt (12, 3.5)	3.90 t (4.0)	4.22 q (2.5)	0.91 s	1.16 s	3.68 dd (10, 6) 3.76 dd (10, 3.5)	1.03, 6 H, d (7)	H_2-28 4.69 br s, 4.75 br s; H-25 2.27 m
3	4.22 m	4.22 m	4.22 m	0.97 s	1.18 s	3.84 t (10) 4.25 m	0.91, 3 H, d (7) 0.92, 3 H, d (7)	H-22 5.24 dd (15, 8); H-23 5.45 m
4	4.19 m	4.19 m		0.68 s	1.16 s	3.95 d (10, 5) 4.11 d (10, 3.5)	0.91, 6 H, d (7)	9-H 3.18 d (10); H_2-12 2.47 d (12.5), 2.53 d (12.5); H-1 2.68 dt (14, 2.5)

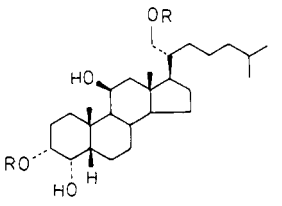
[a] The spectra of the sulfate steroids 1–4 were measured in CD_3OD, and those of steroids **1a** and **2a** in $CDCl_3$. The chemical shift values are given in ppm and were referenced to CD_3OD (3.34 ppm) and $CDCl_3$ (7.26 ppm). The coupling constants are given in hertz and are enclosed in parentheses.

3.62 (dt, J = 12, 3.5 Hz, 3-H). Irradiation at δ 3.90 (4-H) transformed the double triplet at δ 3.62 (3-H) into a double doublet with J = 12.0 and 4.0 Hz, while irradiation at δ 3.62 transformed the former into a doublet (J = 4 Hz). This experiment lead to a structural element with vicinal hydroxy groups, the former adjacent to a C bearing one proton and the other to one bearing two protons. There is only one way such a fragment can be put into a steroidal skeleton, i.e. at C-3 and C-4. Further decoupling experiments showed the sequence >HC–C(9)H–C(OH)H–C-(12)H_2–C(–) < and established the location of the fourth hydroxyl group at C-11. The configuration assigned to **1a**, and accordingly to the native **1**, rests on the following evidence. The cis A/B ring junction follows from the low-field chemical shift of the 19-methyl carbon (δ_C 27.4).[2,7] The equatorial orientation of the hydroxy substituent at C-3 follows from the large axial–axial coupling (J = 12 Hz) caused by interaction of the corresponding geminal proton with the axially oriented proton in the C-2 adjacent position. The downfield shift (δ 3.90) and the narrowing of the signal for 4-H are consistent with its equatorial orientation. Thus, assuming the 5β-cholestane skeleton for our steroid, the 3α,4α-dihydroxy moiety in **1a** could be established. Finally the downfield shift and the shape of the signal for 11-H (apparent quartet with small couplings, J = 2.5 Hz; δ 4.22) indicated an axial orientation for the hydroxy group located there. This assignment was supported from the downfield shifts of the angular methyls [CH_3-18, δ_H, 0.91, δ_C, 15.2; CH_3-19, δ_H, 1.16, δ_C, 27.4] both subjected to 1,3-diaxial interaction with 11β-OH. Thus, the 3α,4α,11β-trihydroxy-5β-cholestane feature was established. We note that the alternative 3β,4β,11β-trihydroxy-5α-cholestane structure would require inter alia the CH_3-19 resonating at δ_H 1.33 (calcd using Arnolds substituent increment parameters[8]) and δ_C 17.9 (calcd

(7) Gough, J. L.; Guthirif, J. P.; Stothers, J. B. *J. Chem. Soc., Chem. Commun.* **1977**, 670.

based on the substituent effects that have been published for hydroxy steroids[9]), which are far off from our values, δ_H 1.16; δ_C 27.4. To the best of our knowledge the presence of the C-21 hydroxy group does not affect the chemical shifts of the CH_3-19 in both ^1H and ^{13}C NMR.

The sulfate groups are assigned to C-3 and C-21 in **1** by consideration of chemical shift and coupling patterns in comparison with ^1H NMR data for the desulfated derivative **1a**, and comparison of ^{13}C NMR spectra (Tables I, II). Assignments of C signals in spectra of **1** and **1a** have



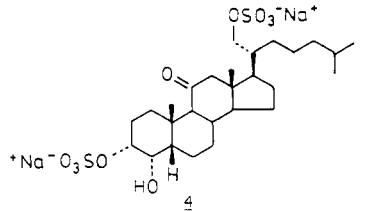
1; R = $SO_3^-Na^+$

2; R = $SO_3^-Na^+$, 24-Me, $\Delta^{24(28)}$

3; R = $SO_3^-Na^+$, Δ^{22E}

1a; R = H

2a; R = H, 24-Me, $\Delta^{24(28)}$



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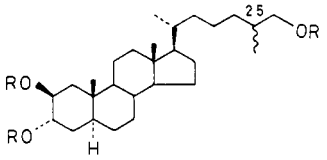

5; R = $SO_3^-Na^+$ (mixture of 25R- and 25S-epimers)

6; R = $SO_3^-Na^+$, Δ^5 (mixture of 25R- and 25S-epimers)

5a; R = H (mixture of 25R- and 25S-epimers)

been made by using 5β-cholestan-3α-ol[6] and 5β-cholestane-3α,4α,11β,12β,21-pentol[2] as reference compounds and substituent effects that have been published for hydroxy steroids.[9] The stereochemistry at C-20 is suggested to be 20*R* (natural configuration) on the basis of the chemical shift of the CH_2OH protons in **1a** (3.69 dd to 3.74 dd) as compared with those of (20*R*)- and (20*S*)-cholest-5-ene-3β,21-diol,[10] which are reported centered at δ 3.70 and 3.62, respectively.

The minor steroid sulfate components of *Ophiocoma dentata* are **2** and **3**. In the negative FAB MS spectrum of **2**, $[\alpha]_D$ +15.8°, a molecular ion species was observed at *m/z* 629, 12 mass units shifted relative to **1** (*m/z* 617). Comparison of the spectral data in Tables I and II immediately suggested that **2** is related to **1** by introduction of a exo methylene at C-24. The ^1H NMR spectrum included two one-proton olefinic signals at δ 4.72 (br s) and 4.74 (br s) and the signals for 25-H and 26- and 27-CH_3, which were downfield shifted to δ 2.31 (1 H, heptet, J = 7 Hz) and 1.06 (6 H, d, J = 7 Hz). In confirmation the ^{13}C NMR spectrum included two sp^2 carbon signals at 106.9 (CH_2) and 158.1 (C) ppm.

Again the comparison of the spectral data in Tables I and II makes it clear that compound **3**, $[\alpha]_D$ -24.5°, FAB MS (negative mode) *m/z* 615 (two mass units shifted relative to **1**, *m/z* 617), is related to the major component **1** by introduction of a Δ^{22E}-double bond in the side chain. The ^1H NMR spectrum included two well-separated olefinic protons at δ 5.24 (1 H, dd, J = 15, 8 Hz, 22-H) and 5.45 (1 H, m, 23-H). The presence of the Δ^{22} double bond also caused the expected slight downfield shift of CH_3-18 signal to δ 0.97 and an high field shift of one of the two (C-21) protons to δ 3.84 (δ 3.99 in **1**). The ^{13}C NMR spectrum (Table I) included two olefinic carbons at 131.0 (CH) and 134.0 (CH) ppm.

Ophiarthrum elegans. This organism contains the steroid sulfate **1** as the major component accompanied by smaller amounts of the Δ^{22E} derivative **3** and of a new compound **4**, $[\alpha]_D$ +25°, FAB MS spectrum (negative mode) of **4** exhibited molecular ion species at *m/z* 629 (small; monopotassium salt of dianion) and 615 (major; monosodium salt of the dianion), two mass units shifted relative to **1** (*m/z* 631, 617). The circular dichroism (CD) spectrum of **4** showed a weak positive Cotton curve, $[\theta]_{300}$ +765 (positive maximum) due to the n→π^* transition. A signal at 215.6 ppm in the ^{13}C NMR spectrum confirmed the presence of a keto group in **4**. The ^1H NMR spectrum of **4** (Table II) showed signals ascribable to an oxygen-bearing methylene group [δ 3.95 (1 H, dd, J = 10, 5 Hz) to 4.11 (1 H, dd, J = 10, 3.5 Hz; 21-H_2)], two >CHO–groups [δ 4.19 (2 H overlapping narrow multiplets; 3-H, 4-H)], one methine and one isoalted methylene group both adjacent to the carbonyl [δ 3.18 (1 H, d, J = 10 Hz; 9-H) and δ 2.47–2.53 (each 1 H d, J = 12.5 Hz; 12-H_2)], one >C*H*(H) signal strongly deshielded by the carbonyl at δ 2.68 (1 H, dt, J = 14, 2.5 Hz, 1-Heq), two tertiary methyl group [δ 0.68 (3 H, s, CH_3-18); 1.16 (3 H, s, CH_3-19)], and two secondary methyl groups [δ 0.91 (6 H, d, J = 7 Hz, CH_3-26, -27)].

Based on the above evidence, it has been concluded that **4** is the 11-keto derivative of the major **1**. Comparison of ^{13}C NMR spectrum of **4** with that of **1** (Table I) confirmed the suggested formulation. The major differences between the two spectra were observed at carbons 8, 9, 13, 14, 17, 18, and 19 and very similar effects were reported on passing from 5β-pregnan-11β-ol to 5β-pregnan-11-one.[6] The relationship between **1** and **4** was definitively established by oxidation with Sarret reagent[11] of **1** to give **4** and conversely by reduction with sodium borohydride of **4** to give **1**.

Ophiarachna incrassata. This organism also contain major amounts of the steroid sulfate **1** along with minor amounts of two new more polar steroid sulfates, **5** and **6**. The new compounds contain three sulfate groups, which was deduced by a strong IR absorption[12] at 1220 and 1240 cm^{-1} and FAB mass spectrometry (negative mode), which gave molecular ion species at *m/z* 719 [St($OSO_3^-Na^+$)($OSO_3^-K^+$)(OSO_3^-)], 703 (major peak) [St($OSO_3^-Na^+$)$_2$(OSO_3^-)], 697 [St($OSO_3^-K^+$)(OSO_3H)(OSO_3^-)], and 681 [St($OSO_3^-Na^+$)(OSO_3H)(OSO_3^-)] for compound **5** and *m/z* 701 [St($OSO_3^-Na^+$)$_2$(SO_3^-)], 695 [St($OSO_3^-K^+$)(OSO_3H)(OSO_3^-)], and 679 [St($OSO_3^-Na^+$)(OSO_3H)(OSO_3^-)] for compound **6**, where St = steroid skeleton. Next to the

(8) Arnolds, W.; Meister, W.; Englert, G. *Helv. Chim. Acta* **1974**, *57*, 1559. Zürcher, R. F. *Ibid.* **1963**, *46*, 2054.

(9) Eggert, H.; Van Antwerp, C. L.; Baccha, N. S.; Djerassi, C. *J. Org. Chem.* **1976**, *41*, 21. Van Antwerp, C. L.; Eggert, H.; Meakins, G. D.; Miners, J. O.; Djerassi, C. *J. Org. Chem.* **1977**, *42*, 789.

(10) Byon, C.-y.; Büyüktür, G.; Cheay, J.; Gut, M. *J. Org. Chem.* **1977**, *42*, 19.

(11) Poos, G. I.; Arth, G. E.; Beyler, R. E.; Sarrett, L. H. *J. Am. Chem. Soc.* **1953**, *75*, 422.

(12) Turney, J. R. *Adv. Carbohdydr. Chem.* **1965**, *20*, 183.

Table III. Comparison of the 250-MHz ^{1}H NMR Spectral Data[a] of the 5α-Steroids 5, 5a, and 6

	5[b]	5a[b]	6[b,c]
H-2	4.72 m ($W_{1/2}$ = 6)	3.88 m	4.75 q (2.5)
H-3β	4.74 m ($W_{1/2}$ = 8)	3.88 m	4.81 q (2.5)
H-6			5.39 br d (5.5)
H_3-18	0.71 s	0.63 s	0.75 s
H_3-19	1.02 s	0.99 s	1.21 s
H_3-21	0.99 d (7)	0.90 d (7)	0.98 d (7)
H_2-26	3.80, 1 H, dd (10, 6.5)	3.42, 1 H, dd (10, 6.5)	3.80, 1 H, dd (10, 8.5)
	3.90 dd (10, 5) } 1 H	3.52, 1 H, m	3.90, 1 H, dd (10, 5)
	3.91 dd (10, 5) }		
H_3-27	0.98 d (6.5) } 3 H	0.91 d (6.5) } 3 H	0.98, 3 H, d (7)
	0.96 d (6.5) }	0.92 d (6.5) }	

[a] The spectra of the sulfate steroids 5 and 6 were measured in CD_3OD, and that of the steroid 5a in $CDCl_3$. The chemical shift values are given in ppm and were referred to CD_3OD (3.34 ppm) and $CDCl_3$ (7.26 ppm). The coupling constants are given in hertz and are enclosed in parentheses. [b] Mixture of 25R and 25S isomers; indistinguishable in the spectrum of 6. [c] The NMR data reported for this compound were measured at 500 MHz; other signals: H_2-1 2.22 br d (13), 1.63 dd (13.2); H_2-4 2.84 dt (15.2), 2.33 br d (15) ppm.

molecular ion species the spectra displayed intense fragments m/z 601 and 499 in the spectrum of 5 and m/z 599 and 497 in that of 6, corresponding to the sequential losses of two $NaSO_3$ and 1 H transfer from the major peak due to the disodium salt of the trianion (m/z 703 and 701 in 5 and 6, respectively). The presence of sulfate groups in the major 5 was confirmed by acid hydrolysis affording the corresponding triol 5a. The EI mass spectrum gave a molecular ion peak at m/z 420, corresponding to a trihydroxycholestane structure, and also indicated the presence of a saturated hydroxylated C_8 side chain (e.g., peak at m/z 249, ring D fission).[13] The ^{1}H NMR spectrum of the native 5 on comparison with that of 5a was consistent with three sulfate functions (Table III).

Comparison of the data in Tables I and III makes it abundantly clear that 5 and 6 are steroids related by the introduction of a 5(6) double bond in 6. Exploitation of this relationship aided the interpretation of the NMR results, which led eventually to the structures indicated in 5 and 6.

The ^{13}C NMR spectrum (Table I) of 5 and DEPT measurements were consistent with a trioxygenated 5α-cholestane structure including two >CHO– and one –CH_2O–carbon. The ^{1}H NMR spectrum (Table III) showed the presence of two tertiary methyl groups [δ 0.71 (s, 3 H), 1.02 (s, 3 H)], two secondary methyl groups, which appeared as three overlapping doublets [δ 0.96 (J = 6.5 Hz), 0.98 (J = 6.5 Hz), and 0.99 (J = 7.0 Hz)] integrating together for 6 H, one oxygen-bearing methylene group [δ 3.80 (1 H, dd, J = 9.5, 6.3 Hz) to 3.90 (1 H, dd, J = 9.5, 5.5 Hz)], and two oxygen-bearing methine groups [narrow 1 H multiplets at δ 4.72 and 4.74]. Each signal of the double doublet at δ 3.90 appeared as a doublet with separations between the peaks of δ 0.01. We assign these duplicate signals and the duplicate signal for one secondary methyl group to the presence of two stereoisomers. Analysis of ^{13}C NMR data and comparison with those of 21-sulfoxy steroids (Table I) and 26-hydroxy steroids[14] clearly established the location of the primary sulfoxy group at C-26. Thus the satisfactory explanation of the slight differences in the ^{1}H NMR spectral data is a mixture of 25R and 25S isomers. The major support for this proposal is found in the ^{13}C NMR spectrum, where each signal of the side-chain carbons appeared as doublet with separation between the peaks ranging from 0.04 to 0.2 ppm. One epimer is present in relatively major amount, even if we cannot establish which epimer it is (25R or 25S). Also compound 6 is a mixture of 25R and 25S isomers (see Table I).

The position and configuration of the two sulfoxy groups located on the tetracyclic nucleus were mainly deduced from a 500-MHz ^{1}H NMR study of 6 in CD_3OD, including double resonance experiments. There were two >CHO– signals as narrow multiplets at δ 4.75 (3β-H) and 4.81 (2α-H) and one olefinic proton signal as broad doublet (J = 5.5 Hz) at δ 5.39 (6-H). Irradiation of the 6-H at δ 5.39 collapsed the C-4 proton at δ 2.84 (dt, J = 15, 2 Hz) to a doublet of doublets (J = 15, 2 Hz). Irradiation of the 3β-H at δ 4.75 collapsed the C-4 geminal protons at δ 2.84 (dt, J = 15, 2 Hz) and δ 2.33 (br d, J = 15 Hz) to a doublet of doublets (J = 15, 2 Hz) and a sharp doublet (J = 15 Hz). Irradiation of the 2α-H at δ 4.81 sharpened the C-1 geminal protons at δ 2.22 (br d, J = 13 Hz, 1-H_{ax}, downfield shifted by the 3-sulfoxy group axially oriented) and 1.63 (dd, J = 13, 2 Hz). Thus the 2β,3α-disulfoxy feature was established. This structure was further confirmed by the ^{13}C NMR data (Table I). In the spectrum of 5 the ^{13}C resonances of nuclear carbons C-5 through C-18 match those of 5α-cholestan-3α-ol[6] while the shifts at carbons C-1, C-2, C-3, and C-19 were very similar with those published for sulfated halistanol, 24ξ,25-dimethylcholestane-3β,3α,6α-triyl sodium sulfate, a bioactive steroid isolated from the sponge *Halichondria panicea*.[15] The signals for the side-chain carbons were assigned on the basis of published data for 26-hydroxy steroids[14] and the expected sulfation shifts. Thus compound 5 is suggested to be a 25R and 25S mixture of 5α-cholestane-2β,3α,26-triyl sulfate, and compound 6 a 25R and 25S mixture of the Δ^5 analogue.

Hydroxylation at C-26 is a common feature among the polyhydroxy steroids isolated from starfishes,[4] also encountered in the shark-repelling pavonins, steroidal glycosides from the defense secretion of the sole *Pardachirus pavonius*.[16] Interestingly the stereochemistry at C-25 of pavonins was established to be 25R,[16] while in polyhydroxy steroids from starfishes the 25S configuration was determined.[17] The 2β,3α-dihydroxy function is rather unusual and biosynthetically intriguing, encountered among marine steroids only in sulfated halistanol from a sponge.[15]

This study adds evidence that polyoxygenated steroids, which are ubiquitous in starfishes[4] and also found less

(13) Elliot, W. H. In *Biochemical Applications of Mass Spectrometry*; Waller, G., Ed.; Witey: New York, 1972; p 291.
(14) Riccio, R.; Minale, L.; Pagonis S.; Pizza, C.; Zollo, F.; Pusset, J. *Tetrahedron* **1982**, *38*, 3622.
(15) Fusetani, N.; Matsunaga, S.; Konosu, S. *Tetrahedron Lett.* **1981**, *22*, 1985.
(16) Tachibana, K.; Sakaitani, M.; Nakanishi, K. *Tetrahedron* **1985**, *41*, 1027.
(17) (a) D'Auria, M. V.; Minale, L.; Pizza, C.; Riccio, R.; Zollo, F. *Gazz. Chim. Ital.* **1984**, *114*, 469. (b) Riccio, R.; Iorizzi, M.; Minale, L. *Bull. Soc. Chim. Belg.* **1986**, *95*, 869.

Table IV. Sephadex LH-20 Fractions

	fractions no.	amount (mg)	compounds (mixture of)
Ophiocoma dentata[a] (3 kg wet)	155–200	220	1, 2, 3
Ophiarthrum elegans (1 kg wet)	78–85	80	1, 3, 4
	86–118	85	1
Ophiarachna incrassata (1 kg wet)	66–71	39	1
	72–132	44	1, 5, 6

[a] The 1-butanol extract was chromatographed in two runs.

frequently in sponges,[15] soft corals,[18] gorgonians,[18a] nudibranchs,[19] and hydroids,[20] are also characteristic of the class Ophiuroidea (phylum Echinodermata), where they occur as sulfate esters. The biological role of these sulfated polyhydroxy steroids in ophiuroids is not yet known; some of them[2] showed moderate cytotoxicity;[3] The major steroid sulfate 1 now isolated showed some activity in an antitumoral test using mouse T-cell lymphoma cells.[3]

Experimental Section

General Methods. ^1H and ^{13}C NMR spectra were recorded on a Brüker WM-250 or WM-500 spectrometer. The DEPT experiments[21] were made by using polarization transfer pulse of 90° and 135°, respectively, obtaining in the first case only the CH group and in other case positive signals for CH and CH_3 and negative ones for CH_2 groups. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. FAB mass spectra were recorded on a Kratos MS 50 mass spectrometer equipped with Kratos FAB source. The spectra were obtained by dissolving the samples in a glycerol matrix and placing them on a copper probe tip prior to bombardment with Xe atoms of energy 2.6 kV. EI mass spectra were recorded on an AEI MS 30 spectrometer. The IR spectrum of compound 5 was run on a Nicolet 50X FT IR instrument. The CD spectrum of the ketone 4 was run on a Jasco J 500 A spectropolarimeter. Rotations were measured on a Perkin-Elmer Model 141 polarimeter. Reverse-phase HPLC was performed by using Waters equipment (M 6000 A pump, U6K injector, R401 refractometer), a Waters μ-Bondapak C_{18} column (7.8 mm i.d., 30 cm), and a Whatman Partisil M9 10/25 ODS column (10 mm i.d., 50 cm).

Collection and Extraction of Ophiuroids. *Ophiocoma dentata* (3 kg wet) was collected in July 1984 on the reef of Touaourou, New Caledonia. *Ophiarthrum elegans* (1 kg wet) and *Ophiarachna incrassata* (1 kg wet) were collected on the reef of Ricaudy at Nouméa, New Caledonia.

Each sample was stored frozen and then chopped in small pieces and extracted 2× with methanol (1 L for 1 kg of animals). Removal of solvent under reduced pressure left the residues, which were partitioned between water and *n*-hexane. The aqueous residues were then extracted 2× with 1-butanol. Removal of solvent under reduced pressure left viscous residues (*O. dentata*, 3.2 g; *O. elegans*, 1.57 g; *O. incrassata*, 0.56 g), which by TLC analysis (silica gel; 1-BuOH/AcOH/H_2O 20:15:25) were found to contain polar metabolites (the sulfated polyhydroxy steroids: R_f 0.3–0.5).

Chromatography. Each 1-butanol extract was chromatographed on a column of Sephadex LH-20 (2 × 60 cm; MeOH, 6-mL fractions were collected) to give the major polar compounds dispersed in the fractions listed below (Table IV).

Separation of the Sulfated Polyhydroxy Steroid Mixtures. The polar fractions obtained from chromatography on the Sephadex LH-20 column were dissolved in methanol and subjected to preparative reverse-phase HPLC on a C_{18} μ-Bondapak column with MeOH/H_2O (45:55 or 40:60 as mobile phase). The composition of sulfated steroids and HPLC mobilities and rotations are in Table V.

Table V. Composition of Sulfated Polyhydroxy Steroids

no.	mobility[a] HPLC (min)	$[\alpha]_D$[b] (deg)	amount (mg) *O. dentata* (3 kg wet)	*O. elegans* (1 kg wet)	*O. incrassata* (1 kg wet)
1	12.4	+41.3	96	89	45
2	11.2	+15.8	7.2		
3	10.0	+2.4	5.3	4	
4	13.2	+25		7	
5	8.2				10
6	7.6				4

[a] MeOH/H_2O (45:55) as mobile phase for compounds 1–4; MeOH/H_2O (40:60) as mobile phase for compounds 5 and 6. [b] Taken from solutions in MeOH (*c* ranging from 1 to 0.4).

The ^{13}C NMR spectra are reported in Table I, the ^1H NMR spectra in Tables II and III. The FAB mass spectrometry (negative ion) data are in the text. IR of compound 5: 3468, 2934, 1630, 1240, 1220 cm^{-1}.

5β-Cholestane-3α,4α,11β,21-tetrol (1a). A solution of 1 (12 mg) in dioxane (0.2 mL) and pyridine (0.2 mL) was heated at 120 °C for 4 h in a stoppered reaction vial. After the solution had cooled, H_2O (2 mL) was added, and the solution was extracted 3× with 1-butanol. Removal of solvent left the glassy material of tetrol 1a. Purification by reverse-phase HPLC on the ODS column using 22% aqueous methanol afforded 7.5 mg of the tetrol 1a: $[\alpha]_D$ +46.4° (c 0.7); EIMS, *m/z* (relative intensity) 436 (M^+, 2), 418 (100), 403 (30), 410 (10), 385 (8), 382 (5), 367 (15); 250-MHz ^1H NMR; see Table II; ^{13}C NMR, see Table I.

24-Methylene-5β-cholestane-3α,4α,11β,21-tetrol (2a). Compound 2 was solvolyzed as above to give the tetrol 2a, which was purified by reverse-phase HPLC on the ODS column using 22% aqueous methanol: EIMS, *m/z* (relative intensity) 430 ($M^+ - H_2O$, 15), 418 (7), 387 (100), 369 (20), 351 (30), 287 (15), 269 (10); 250-MHz ^1H NMR, see Table II.

Conversion of 1 to 4. 1 (7 mg) was treated with chromium trioxide–pyridine complex in dry pyridine (1 mL) for 2 h at room temperature. The reaction mixture was passed through a Waters SEP-PAK C-18 cartridge. The cartridge was washed with water (3 mL) and then the adsorbed material was eluted with MeOH (2 mL) to give 6 mg of the ketone 4, which was purified by reverse-phase HPLC (Waters μ-Bondapak C_{18} column, MeOH/H_2O, 45:55, as eluent). It was identical with the native 4 in HPLC and ^1H NMR.

Conversion of 4 to 1. 4 (2 mg) in 0.5 mL of methanol was treated with excess sodium borohydride and stirred at room temperature overnight. The usual workup (1-butanol for extraction) afforded the glassy material of sulfated tetrahydroxy steroid 1, identical with native 1 in HPLC and ^1H NMR.

5α-Cholestane-2β,3α,26-triol (5a). A solution of 5 (2 mg) in 10% aqueous HCl (0.5 mL) was heated at 80 °C for 2 h in a stoppered reaction vial. After the solution had cooled, H_2O (0.5 mL) was added and the solution was extracted 2× with $CHCl_3$. Evaporation of solvent to dryness left a residue of 5a, which was characterized without further purification: EIMS, *m/z* (relative intensity) 420 (M^+, 26), 402 (20), 387 (28), 384 (11), 369 (11), 249 (100), 231 (66); 250-MHz ^1H NMR, see Table III.

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(18) (a) Schmitz, F. J. In *Marine Natural Products, Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic Press: New York, San Francisco, 1978; Vol. I, pp 241–297 and references cited therein. (b) Yamada, Y.; Suzuki, S.; Iguchi, H.; Tsukitani, Y.; Horiai, H.; Nakanishi, H. *Chem. Pharm. Bull.* **1980**, *28*, 473. (c) Sjöstrand, U.; Bohlin, L.; Fisher, L.; Calin, M.; Djerassi, C. *Steroids* **1981**, *38*, 347. (d) Kobayashi, M.; Lee, N. K.; Son, B. W.; Yanagi, K.; Kyogoku, Y.; Kitagawa, I. *Tetrahedron Lett.* **1984**, *25*, 5925. (e) Jagodzinska, B. M.; Trimmer, J. S.; Fenical, W.; Djerassi, C. *J. Org. Chem.* **1985**, *50*, 1435; (f) **1985**, *50*, 2988.

(19) Cimino, G.; De Rosa, S.; De Stefano, S.; Sodano, G. *Tetrahedron Lett.* **1980**, *21*, 3303.

(20) (a) Cimino, G.; De Rosa, S.; De Stefano, G.; Sodano, G. *Tetrahedron* **1980**, *21*, 303. (b) Fattorusso, E.; Lanzotti, V.; Magno, S.; Novellino, E. *J. Org. Chem.* **1985**, *50*, 2868; (c) *J. Nat. Prod.* **1985**, *48*, 784.

(21) Bendall, M. R.; Pegg, D. T.; Doddrell, D. M.; Williams, D. H. J. *J. Org. Chem.* **1982**, *47*, 3021. Bendall, M. R.; Doddrell, D. M.; Pegg, D. T.; Hull, W. E. *DEPT Bruker Information Bullettin*; Bruker Analyticshe Masstechnik: Karlsruhe; 1982. Bendall, M. R.; Pegg, D. T.; Doddrell, D. M.; Johns, S. R.; Willing, R. I. *J. Chem. Soc., Chem. Commun.* **1982**, 1138.

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Registry No. 1 acid, 109152-31-0; 1a, 109152-37-6; 2 acid, 109152-32-1; 2a, 109152-38-7; 3 acid, 109152-33-2; 4 acid, 109152-34-3; 5 acid, 109152-35-4; 5a, 109152-39-8; 6 acid, 109152-36-5.

Modification of Photochemical Reactivity by Cyclodextrin. Difference in Photobehavior between Short Chain and Long Chain Benzoin Alkyl Ethers: Conformational Effect

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The photochemical behavior of long chain benzoin alkyl ethers when complexed to β-cyclodextrin has been investigated with the view to understand their chemical behavior when included in the above host matrix. Photolysis results in benzil and pinacol ethers as the main products in solid β-cyclodextrin complex and benzaldehyde, benzil, pinacol ethers, and deoxybenzoin in aqueous β-cyclodextrin complex. The results on long chain benzoin alkyl ethers 4–6 are distinctly different from those of short chain benzoin alkyl ethers 1–3.

Introduction

A remarkable effect was recently reported on the photoreactivity of benzoin alkyl ethers[1] and alkyl deoxybenzoins[2] upon cyclodextrin complexation. Benzoin alkyl ethers are known to undergo Norrish type I reaction as the only photoprocess in organic solvents.[3] The competing type II hydrogen abstraction process, though feasible in these substrates, is not observed at all in organic solvents. Quite interestingly, the solid β-cyclodextrin complexes of substrates 1–3 (Scheme I) upon irradiation were found to yield only the type II products in quantitative yields.[1] The photolysis of aqueous solutions of the above complexes afforded a mixture of the type I and the type II products. The substantial difference in photoreactivity of benzoin alkyl ethers in β-cyclodextrin was attributed to a combination of the "cage effect" and "conformational control", afforded by the cyclodextrin cavity. The "cage effect" due to cyclodextrin has recently been established with several examples.[4] On the contrary, "conformational control" has not received due attention.[5] Therefore, in order to investigate the validity of proposing such a conformational control in cyclodextrin, the photobehavior of benzoin hexyl ether (4), benzoin octyl ether (5), and benzoin decyl ether (6) was investigated. We envisioned that by introducing longer alkyl chains in the alkoxy moiety, it should be possible to reverse the photobehavior as conformer B (Scheme II) would be populated to increasing extents as the longer alkyl chain on the alkoxy moiety would prefer to reside in the interior of the cyclodextrin cavity. In such a case, even in the presence of cage control there is no possibility of observing the type II reaction. results presented here confirm our prediction and provide support to our earlier postulate on the conformational control using cyclodextrin.[1] These results on benzoin alkyl ethers 4–6 when viewed in conjunction with the photobehavior of 1–3 clearly indicate that with carefully tailored molecules even crude binding sites such as those of the cyclodextrins are capable of profoundly influencing the conformations of included guests.[6]

Results

Photolysis of benzoin alkyl ethers 4–6 in N_2-saturated benzene solution and as a neat liquid resulted in the formation of benzaldehyde, benzil, pinacol ethers, and deoxybenzoin. While the first three products result from the Norrish type I α-cleavage process, deoxybenzoin arises from the intramolecular γ-hydrogen abstraction. Solution results on 4–6 are similar to the analogous systems reported in the literature.[3,7] The Norrish type I rearrangement product—*p*-benzoylbenzyl alkyl ether—was not obtained both in isotropic solvent and in cyclodextrin media. It is to be noted, however, that in micellar media such a product is obtained in major amount.[6] Results obtained upon photolysis of 4–6 in cyclodextrins (aqueous solution and solid state) along with those in benzene are summarized

(1) Dasaratha Reddy, G.; Usha, G.; Ramanathan, K. V.; Ramamurthy, V. *J. Org. Chem.* **1986**, *51*, 3085.

(2) Dasaratha Reddy, G.; Ramamurthy, V. *J. Org. Chem.*, in press.

(3) Lewis, F. D.; Lauterbach, R. L.; Heine, H. G.; Hartmann, W.; Rudolph, H. *J. Am. Chem. Soc.* **1975**, *97*, 1519. Adams, S.; Gusten, H.; Schulte-Frohlinde, D. Tetrahedron **1974**, *30*, 4249. Sander, M. R.; Osborn, C. L. *Tetrahedron Lett.* **1974**, 415. Heine, H. G. *Tetrahedron Lett.* **1972**, 4755. Pappas, S. P.; Chattopadhyay, A. *J. Am. Chem. Soc.* **1973**, *95*, 6484.

(4) Nageswer Rao, B.; Turro, N. J.; Ramamurthy, V. *J. Org. Chem.* **1986**, *51*, 460. Sharat, S.; Usha, G.; Tung, C. H.; Turro, N. J.; Ramamurthy, V. *J. Org. Chem.* **1986**, *51*, 941. Syamala, M. S.; Dasaratha Reddy, G.; Nageswer Rao, B.; Ramamurthy, V. *Curr. Sci.* **1986**, *55*, 875. Nageswer Rao, B.; Syamala, M. S.; Turro, N. J.; Ramamurthy, V. *J. Org. Chem.*, in press.

(5) For a few examples in which conformational effect has been invoked, see: Emert, J.; Kodal, D.; Catena, R. *J. Chem. Soc., Chem. Commun.* **1981**, 758. Turro, N. J.; Okubo, T.; Weed, G. C. *Photochem. Photobiol.* **1982**, *35*, 325. Arad-Yellin, R.; Eaton, D. F. *J. Phys. Chem.* **1983**, *87*, 5051. Vander Jagt, D. L.; Killiam, F. L.; Bender, M. L. *J. Am. Chem. Soc.* **1970**, *92*, 1016. Straub, T. S.; Bender, M. L. *J. Am. Chem. Soc.* **1972**, *94*, 8881. Griffiths, D. W.; Bender, M. L. *J. Am. Chem. Soc.* **1973**, *95*, 1679.

(6) Modification of the photobehavior of benzoin alkyl ethers through "conformational control" using micelles as reaction medium has recently been achieved in our laboratory. Ramamurthy, V. *Tetrahedron* **1986**, *42*, 5753. Devanathan, S.; Dasaratha Reddy, G.; Ramamurthy, V. In *Surfactants in Solution-Modern Aspects*; Mittal, K. L., Ed.; Plenum: New York, in press. Devanathan, S.; Ramamurthy, V., submitted for publication in *J. Org. Chem.*

(7) De Mayo, P.; Ramnath N. *Can. J. Chem.* **1986**, *64*, 1293. De Mayo, P.; Nakamura, A.; Tsang, P. W. K.; Wong, S. K. *J. Am. Chem. Soc.* **1982**, *104*, 6824.

0022-3263/87/1952-3952$01.50/0