12, 76866-96-1; **13a**, 116437-34-4; **13b**, 116437-35-5; **14a**, 69352-30-3; **14b**, 116437-35-5; **15**, 116437-36-6; **16a**, 116437-37-7; **16b**, 116437-38-8; **16c**, 116437-39-9; **16d**, 116437-40-2; **17a**, 116437-41-3; **17b**, 116437-42-4; **18**, 1671-75-6; **19**, 5454-21-7; **20**, 120-51-4; **21**, 10361-39-4; **22**, 41084-78-0; **26b**, 116437-43-5; **26c**, 116437-44-6; **26d**, 116437-45-7; **27**, 116437-46-8; **30a**, 116437-48-0; **30b**, 5832-25-7; **30c**, 116437-49-1; **31a**, 700-91-4; **31b**, 57050-07-4; **32**, 1604-01-9; **33**, 95018-40-9; **36a**, 532-12-7; **36b**, 3471-05-4; **37**, 4593-27-5; *t*-BuCOCl, 3282-30-2; PhCOCl, 98-88-4; $PhCH_2OCOCl$, 501-53-1; $(t\text{-BuOCO})_2O$, 24424-99-5; $n\text{-}C_6H_{13}Br$, 111-25-1; PhBr, 108-86-1; *n*-BuLi, 109-72-8; $H_3C(CH_2)_5CO(CH_2)_5NH_2$, 116466-11-6; $n\text{-}C_{11}H_{23}Br$, 693-67-4; 2-bromopyridine, 109-04-6; 3-bromopyridine, 626-55-1; 1-aminotridecan-7-one, 116437-47-9; 1-bromopropane, 106-94-5; benzyl alcohol, 100-51-6.

Isolation and Structure Elucidation of Seven New Polyhydroxylated Sulfated Sterols from the Ophiuroid *Ophiolepis superba*†

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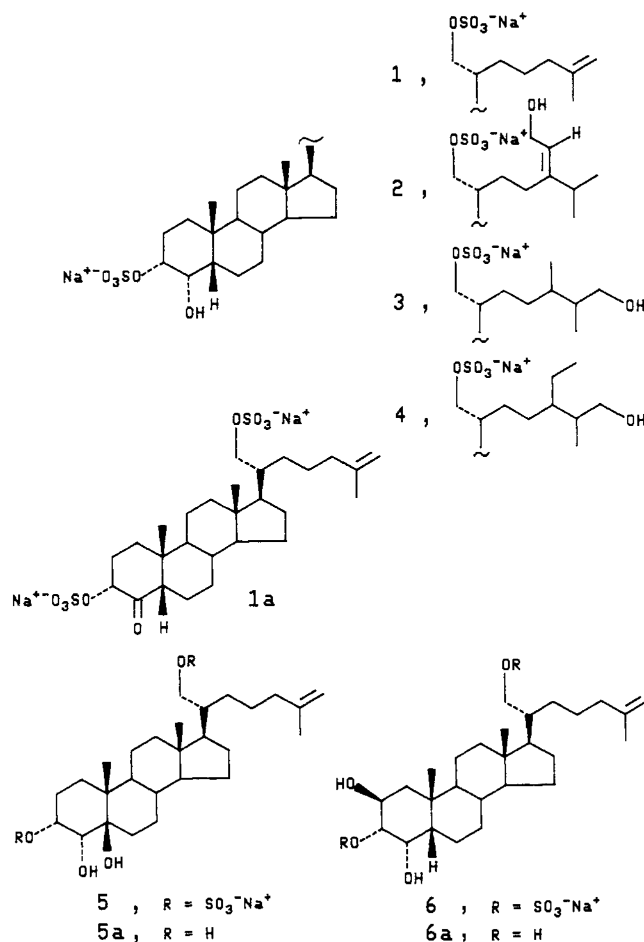
Seven new polyhydroxylated sulfated sterols, all possessing 3α,21-disulfoxy-4α-hydroxy substituents and the A/B cis ring junction, have been isolated from the ophiuroid *Ophiolepis superba*, collected at Okinawa, Japan. Four sterols possessed identical nuclei (i.e., 3α-sulfoxy-4α-hydroxy) but differed in the side chain. Two possessed one more hydroxyl group in the nucleus located at C-2β, and one had the extra hydroxyl group at C-5β. Their general structure was deduced from spectral data (^1H and ^{13}C NMR and FABMS), and the stereochemistry of some of them was determined by correlating their respective spectral data with those of synthetic sterols.

Sterols with hydroxy and sulfoxy functionalities have been recently reported from marine ophiuroids (commonly known as brittle stars). Apart from a group of 5α-H steroids possessing 2β,3α,26-trisulfoxy substituents, isolated from *Ophiorachna incrassata*,[1] all the isolated polyhydroxylated sulfated sterols possessed a 21-sulfoxy substituent. 5β-Cholestane-3α,4α,11β,21-tetrol 3,21-disulfate (**8**) is the major polar steroid component of the Pacific *Ophiocoma dentata*, *O. incrassata*, and *Ophiarthrum elegans*, the latter of which also contained the 11-keto derivative.[1] The Mediterranean *Ophioderma longicaudum* contained a group of cytotoxic disulfated 3α,21-dihydroxy-5α-H steroids along with the moderately cytotoxic 5β-cholestane-3α,4α,11β,12β,21-pentol 3,21-disulfate.[2]

Our investigation of the Pacific ophiuroid *Ophiolepis superba* collected near Zampa, Okinawa, led to the isolation of seven new polyhydroxylated sulfated sterols (**1–7**), all with 3α,21-disulfoxy-4α-hydroxy substituents and the A/B cis ring junction. The polar sterol mixture also contained the known **8**[1] and **9**.[2]

Since spectral data indicated that the steroids **1–4** possessed virtually identical nuclei and the same 21-sulfoxy substituent, but differed in the side chain, it was only necessary to settle the nuclear substitution pattern of the steroid **1**.

Structure Elucidation of 5β-Cholest-25-ene-3α,4α,21-triol 3,21-Disulfate (1). The negative-ion fast atom bombardment (FAB) mass spectrum exhibited molecular ion species at *m/z* 577, 599 (major), and 615, corresponding to $[M(SO_3H)SO_3^-]$, $[M(SO_3Na)SO_3^-]$, and [M-



† This paper is dedicated to Professor Edgar Lederer (Gif-Sur-Yvette, France) on the occasion of his 80th birthday.

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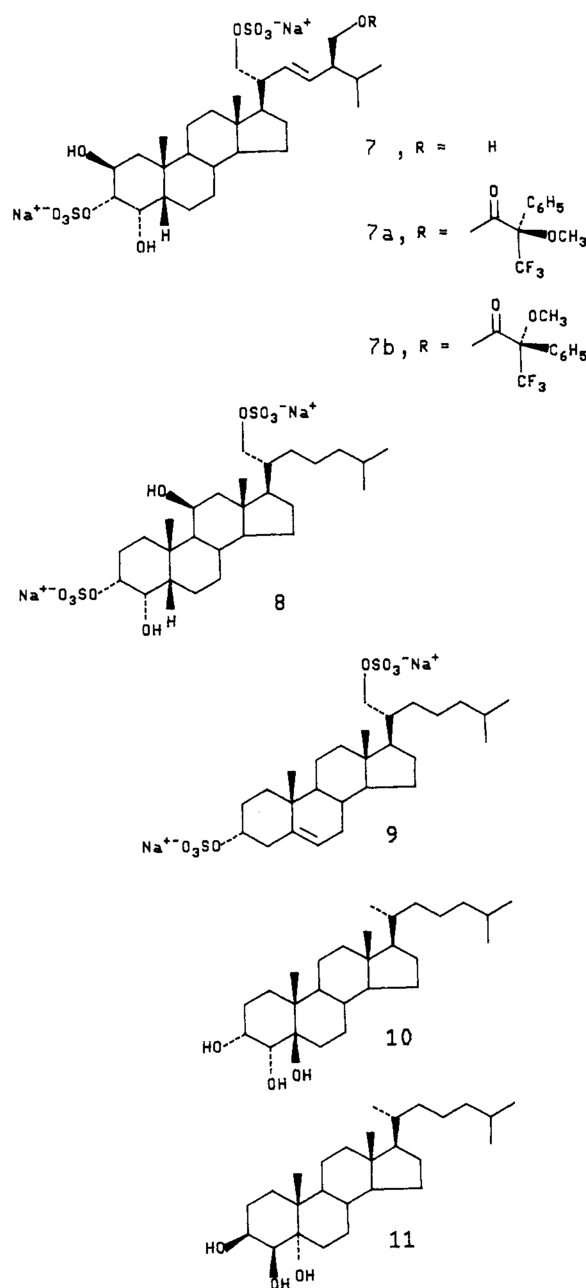

$(SO_3K)SO_3^-]$, respectively. Intense fragmentation peaks at m/z 497 and 479 were interpreted as losses of $NaSO_3$ (+H) and $NaHSO_4$ from m/z 599, respectively. The ^{13}C NMR spectrum measured in CD_3OD at 62.9 MHz and DEPT measurements (Table I) indicated the presence of 27 carbon atoms and revealed the presence of three methyl groups, 11 methylenes, six methines, two quaternary carbons, two —OCH<, one —OCH_2—, and a >C=CH_2. Taken together, these data indicated a disulfated trihydroxycholestane structure with one of the five methyl groups typical of a sterol oxidized to hydroxymethylene and one oxidized to exo methylene.

The 250-MHz ^1H NMR spectrum (Table II) of 1 supported the existence of a terminal methylene (2 H, at 4.69 ppm) and showed the presence of an olefinic methyl singlet at δ 1.74, thus suggesting the presence of a terminal isopropylidene group in the side chain. Also present were singlets at 0.75 ppm (18-CH_3) and at 0.96 ppm (19-CH_3). Two one-proton signals at 4.06 ppm (dd, J = 10, 6.5 Hz) and at 4.22 ppm (dd, J = 10, 3.7 Hz), coupled to each other by 10 Hz, were suggestive of the presence of a 21-sulfoxy group. These signals were already observed in the spectra of the many 21-sulfoxy steroids isolated from ophiuroids[1,2] and starfishes.[3] Two overlapping signals (2 H) at 4.19 ppm were reminiscent of those observed in the spectrum of 5β-cholestane-3α,4α,11β,21-tetrol 3,21-disulfate (**8**)[1] and assigned to the protons at C-3 and C-4. The 5β-cholestane-3α,4α-diol 3-sulfate structure was supported by ^{13}C NMR signals at 82.4 and 75.2 ppm virtually identical with those observed in the spectrum of **8** and assigned to C-3 and C-4, respectively, and by the low-field chemical shifts of the C-19 methyl carbon (δ_C 24.0 ppm),[1,4] strongly indicative of the cis-A/B ring fusion.[4] An independent confirmation of the ring A was provided by a simple chemical transformation of steroid **1**. Oxidation with chromium trioxide–pyridine reagent[5] afforded the ketone **1a** showing a very weak negative CD Cotton curve [$\Delta\epsilon_{290}$ = –0.11] consistent with the cis-A/B ring junction[6] (for the alternative, 3β-hydroxy-5α-cholestan-4-one structure, a strong negative Cotton curve is expected).[6] Its ^1H NMR spectrum was in excellent agreement with the assigned structure; the resonance frequency of the proton at C-3 moved downfield to 4.80 ppm as a dd with J = 12, 7 Hz (axial proton), and most significantly, the 19-CH_3 signal was observed downfield shifted to 1.16 ppm [calcd[7] 1.15; for the alternative 3β-hydroxy-5α-cholestan-4-one structure, calcd by using Arnold's substituent increment parameters,[8] 19-CH_3, 0.79 ppm; to the best of our knowledge, the presence of sulfate at 3-C(OH) only slightly (ca. 0.05 ppm) affects the shift of the 19-CH_3 in ^1H NMR].

The configuration at C-20 is suggested to be R (natural) on the basis of the comparison of ^1H and ^{13}C NMR data with those of **8**.[1]

^1H NMR data of sterols **1–4** (Table II) show conclusively that they all have the 3α,4α,21-triol, 3,21-disulfate structure in a 5β-H steroid, but different side chains. In the ^1H NMR spectra of compounds **2**, **3**, and **4**, signals due to the terminal isopropylidene group at 4.69 (2 H) and 1.74 (3 H) ppm disappeared, while a doublet due to the isopropyl methyls was observed at δ 1.06 (6 H, d, J = 7 Hz) in **2**. In addition to the above doublet, a 2 H doublet (J = 7 Hz) and one-proton triplet (J = 7 Hz), coupled to each other, appeared at 4.18 and 5.34 ppm, respectively, indicating a 24-hydroxyethyl 24(28)-ene structure. The stereochemistry of the side-chain double bond was suggested to be E on the basis of the chemical shift value of the C-25 methine proton at δ 2.32 (septet; in the Z isomer it would be expected to give a signal downfield shifted to δ 3.0)[9,10] and confirmed by NOEDS experiments [enhancement of 28-H (7%) was observed by irradiation of 25-H]. We note that a steroidal structure with a 24-hydroxyethyl 24(28)(E)-ene functionality has been found in dehydrooogoniol-1, a very active hormone of the aquatic fungus *Achlya*,[10] and in the 29-hydroxy-7-oxofucosterol, which was later synthesized[11] as a model compound. The above-

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

C	1	2	3	4	5	6[b]	6a	7	10	11
1	36.2	36.2	36.2	36.2	31.0	44.6	44.6	44.6	31.0	32.0
2	22.4	22.5	22.5	22.5	22.3	65.6 (−1.8)	67.3	65.5	23.1	26.8
3	82.4	82.4	82.4	82.4	79.5	87.0 (+7.9)	79.1	87.0	70.5	69.2
4	75.2	75.4	75.5	75.5	79.5	76.5 (−1.9)	78.4	76.5	81.2	79.2
5	46.7	46.7	46.7	46.7	76.0	46.5	46.7	46.4	75.9	76.5
6	27.6	27.6	27.6	27.6	37.5	26.9	26.8	26.9	37.4	32.4
7	28.7	28.7	28.7	28.7	28.5	28.6	28.4	29.3	29.6	26.8
8	36.3	36.2	36.2	36.2	35.2	36.3	36.3	36.3	35.2	39.4
9	42.9	43.1	43.1	43.1	41.8	44.0	44.1	44.0	41.7	47.7
10	36.2	36.2	36.2	36.2	40.8	38.2	38.5	38.2	40.8	37.0
11	22.5	22.5	22.9	22.9	22.9	22.6	22.6	22.6	22.4	21.6
12	40.7	40.7	40.8	40.8	40.5	40.7	40.9	40.7	40.7	40.6
13	43.6	43.6	43.6	43.6	43.4	43.6	43.7	43.6	43.6	43.9
14	57.8	57.8	57.8	57.8	57.8	57.8	57.8	57.7	57.8	57.7
15	25.0	25.1	25.1	25.1	25.1	25.1	25.1	25.1	25.1	25.1
16	29.3	29.3	29.3	29.3	29.4	29.3	29.5	29.4	29.3	29.3
17	52.2	52.2	52.4	52.4	52.2	52.3	52.3	52.3	58.0	57.7
18	12.7	12.6	12.7	12.7	12.6	12.7	12.8	13.0	12.6	12.6
19	24.0	24.0	24.0	24.0	17.7	24.0	24.1	24.1	17.8	16.2
20	41.3	41.8	41.9	41.9	41.3	41.2	43.6	46.9	37.4	37.4
21	69.7	69.8	70.0	70.0	69.9	69.9	63.6	71.2	19.3	19.2
22	30.5	32.8	28.8	29.4	30.5	30.6	30.6	136.0	37.0	36.1
23	24.8	35.6	30.2	27.6	25.0	25.0	25.5	131.8	25.0	24.9
24	39.3	150.2	36.7	43.4	39.3	39.3	39.4	53.7	41.5	41.5
25	147.1	35.6	41.6	38.6	147.2	147.2	147.2	29.7	29.0	29.0
26	110.3	22.4	66.2	66.8	110.2	110.2	110.2	19.5	22.9	22.9
27	22.4	22.5	14.3	13.4	22.4	22.4	22.4	21.4	23.0	23.1
28		122.7	17.4	25.1				65.0		
29		59.8		12.6						

[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 sulfatation shifts.

Table II. Selected 250-MHz Chemical Shifts of 5β-Steroids 1–7[a]

sterol	2-H	3β-H	4β-H	$18-H_3$	$19-H_3$	$21-H_2$	other signals
1		4.19 m	4.19 m	0.75 s	0.96 s	396 dd (10.5, 6.5) 4.22 dd (10.5, 3.7)	$26-H_2$ 4.69 br s; $27-H_3$ 1.74 s
2		4.19 m	4.19 m	0.75 s	0.96 s	3.97 dd (10.5, 6.5) 4.23 dd (10.5, 3.7)	25H 2.32 m; $26,27-H_3$ 1.06 d (7) 28-H 5.34 t (7); $29-H_2$ 4.18 d (7)
3		4.19 m	4.19 m	0.75 s	0.96 s	3.94 dd (10.5, 6.5) 4.22 dd (10.5, 3.7)	$26-H_2$ 3.62 dd (11, 6), 3.34 dd (11, 6) $27,28-H_3$ 0.92 (d (7)
4		4.19 m	4.19 m	0.74 s	0.96 s	3.94 dd (10.5, 6.5) 4.23 dd (10.5, 3.7)	$26-H_2$ 3.58 dd (11, 6), 3.35 dd (11, 7) $27-H_3$ 0.92 d (7); $29-H_3$ 0.86 t (7)
5		4.69 m	3.99 m	0.74 s	0.92 s	3.66 dd (10.5, 6.5) 4.22 dd (10.5, 3.7)	$26-H_2$ 4.69 br s; $27-H_3$ 7.74 s
5a		3.89 dt (11.5, 3.7)	3.63 d (3.7)	0.72 s	0.92 s	3.53 dd (11, 6.5) 3.72 dd (11, 3.5)	$26-H_2$ 4.70 br s; $27-H_3$ 1.74 s
6	4.03 m	4.03 m	4.33 t (3.4)	0.75 s	1.02 s	3.97 dd (10.5, 6.5) 4.23 dd (10.5, 3.7)	$26-H_2$ 4.69 br s; $27-H_3$ 1.74 s
6a	3.81 m ($W_{1/2}$ 18)	3.23 dd (9.5, 3.5)	3.94 t (3.5)	0.73 s	1.01 s	3.53 dd (11, 6.5) 3.73 dd (11, 6.5)	$26-H_2$ 4.70 br s; $27-H_3$ 1.74 s
7	4.03 m	4.03 m	4.33 t (3.4)	0.78 s	1.02 s	3.80 dd (10, 8.7) 4.24 dd (10, 3.7)	22-H 5.29 dd (14,9); 23-H 5.34 dd (15, 7) 26,27-H 0.95 d (7), 0.89 d (7) $28-H_2$ 3.57 dd (10.5, 6), 3.49 dd (10.5, 7.5)

[a] The spectra were measured in CD_3OD. The chemical shift values are given in parts per million and were reffered to CD_3OD (3.34 ppm). The coupling constants are given in hertz and are enclosed in parentheses.

discussed spectral data of compound **2** are in good agreement with those reported.[10,11] Negative-ion FABMS, m/z 621 and 643 [$M(SO_3H)SO_3$; $M(SO_3Na)SO_3^-$] and the ^{13}C NMR spectrum (Table I) supported the structure of 24-ethyl-5β-cholest-24(28)(*E*)-ene-3α,4α,21,29-tetrol 3,21-disulfate for compound **2**.

The ^1H NMR spectrum of **3**, negative-ion FABMS, m/z 609 [$M(SO_3H)SO_3^-$], 631 [$M(SO_3Na)SO_3^-$], showed one methyl doublet integrating for 6 H at δ 0.92 (J = 7 Hz) and two double doublets at δ 3.62 (1 H, J = 11, 6 Hz) and 3.34 (1 H, J = 11, 7 Hz, partially overlapping with CHD_2OD signal), characteristic for a $HCCH_2OH$ group. Irradiation at δ 1.61 (25-H) transformed one of the methyl doublets at δ 0.92 into a singlet, leaving part of the signal as a doublet, and the two dd at δ 3.62 and 3.34 into two doublets with J = 11 Hz. Thus the structure of **3** was elucidated as 24(ξ)-methyl-5β-cholestane-3α,4α,21,26-tetrol 3,21-disulfate. The ^{13}C NMR spectrum in CD_3OD confirmed the 24-methyl-26-hydroxy structure. The C-26 signal was observed at 66.2 ppm, 2.3–2.4 ppm upfield relative to 26-hydroxy steroids (C-26: 68.5–68.6 ppm),[12] as expected upon introduction of a methyl group at C-24 (γ-effect). Very recently (24*R*)- and (24*S*)-24-(hydroxymethyl)cholesterols have been synthesized,[13] and the ^{13}C NMR signals for the hydroxymethyl carbon were observed at 63.8 and 64.1 ppm, respectively, which are substantially different from our value.

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Negative-ion FABMS of the steroid **4** exhibited molecular ion species at *m/z* 623 [$M(SO_3H)SO_3^-$], 645 [$M(SO_3Na)SO_3^-$], and 661 [$M(SO_3K)SO_3^-$], 14 mass units higher than **3** (*m/z* 609, 631). In addition to the signals ascribable to a hydromethylene group at δ 3.58 (dd, J = 11, 6 Hz) and 3.34 (dd, J = 11, 7 Hz) already observed in **3** and assigned to $-C(26)H_2OH$, the ^1H NMR spectrum showed one methyl doublet at δ 0.92 (3 H, J = 7 Hz) and one methyl triplet at δ 0.86 (3 H, J = 7 Hz), this latter indicating the presence of an ethyl group. Thus the 24-(ξ)-ethyl-5β-cholestane-3α,4α,21,26-tetrol 3,21-disulfate structure was suggested for the steroid **4**. This conclusion was supported by ^{13}C NMR data (Table I) and especially by the chemical shift of the hydroxymethyl carbon at 66.8 ppm, very close to the shift observed in **3**. The stereochemistry at C-24 and C-25 of **3** and **4** remains to be determined.

Structure Elucidation of 5β-Cholest-25-ene-3α,4α,5,21-tetrol 3,21-Disulfate (5). The negative-ion fast atom bombardment (FAB) mass spectrum exhibited molecular ion species at *m/z* 593 [$M(SO_3H)SO_3^-$], 615 [$M(SO_3Na)SO_3^-$; major], and 631 [$M(SO_3K)SO_3^-$], 16 mass units higher than **1** (*m/z* 577, 599, 615). Analysis of the ^1H NMR spectrum (Table II) revealed that **5** possessed the same side chain as **1**. Also present were two methyl singlets at δ 0.74 and 0.92 due to the C-18 and C-19 methyl groups, respectively, and two methine proton signals at δ 3.99 (1 H, d, J = 3 Hz, partially overlapped with 21-H) and 4.69 (br m, overlapped with the 26-H_2 br s) each coupled to the other. In the ^1H NMR spectrum (CD_3OD) of the tetrol **5a**, derived from **5** upon solvolysis, the multiplet at δ 4.69 was observed as an isolated double triplet (J = 11.5, 3.7 Hz) upfield shifted to δ 3.89, and the doublet at δ 3.99 also appeared as an isolated signal at δ 3.63 (d, J = 3.7 Hz). These data indicated the presence of two cis-vicinal groups, one adjacent to a carbon bearing two protons and the other to one without protons. The large coupling constants associated with the signal at δ 4.69 in **5** (3.89 in **5a**) are consistent with an axial proton, and the small coupling constant of the doublet at δ 3.99 in **5** (δ 3.68 in **5a**) is consistent for an equatorial proton. These data can be best rationalized by placement of a sulfoxy group at C-3α and the remaining hydroxyls at C-4α and C-5 in a 5β-steroid skeleton or alternatively by placement of a sulfoxy group at C-3β and the remaining hydroxyls at C-4β and C-5 in a 5α-steroid skeleton. The ^{13}C NMR spectrum in CD_3OD (Table I) and DEPT measurements supported the presence of a *tert*-hydroxyl group (δ_C 76.0 ppm) and were in good agreement with the structure **5**, all signals well corresponding to those expected upon introduction of a hydroxyl group at C-5 in **1**. Even stronger evidence for the cis-A/B ring fusion was the chemical shift of the C-19 methyl protons (0.92 ppm), which appeared at too high a field for a 3β,4β,5α-trihydroxy structure (calcd by using Arnold's substituent increment parameters,[8] 19-CH_3, 1.29 ppm).

As a confirmation of the structure assignment, we synthesized an analogous compound, 5β-cholestane-3α,4α,5-triol (**10**), and the alternative 5α-cholestane-3β,4β,5-triol (**11**) in order to compare their spectral data with those of the tetrol **5a**, derived from the natural material **5**. Compound **10** could be synthesized in five steps from cholest-4-en-3-one by known procedures, which include alkaline epoxidation[14] to the β-epoxy ketone and reduction with sodium borohydride[15] to the 4β,5β-epoxycholestan-3α-ol, followed by acetylation and ring opening of the epoxide with perchloric acid to give a mixture of 3α-acetoxy-5β-cholestane-4α,5-diol and 4α-acetoxy-5β-cholestane-3α,5-diol (neighboring α-acetoxy effect),[16] which were then hydrolyzed to **10**, mp 168–70 °C. Compound **11** was similarly synthesized by starting from 4α,5α-epoxycholestan-3β-ol,[17] which was acetylated and then treated with perchloric acid to give a mixture of 3β-acetoxy-5α-cholestane-4β,5-diol and 4β-acetoxy-5α-cholestane-3β,5-diol, which were finally hydrolyzed to **11**, mp 187–9 °C. The ^1H NMR spectrum of the 5β-cholestane-3α,4α,5-triol (**10**) showed the same shifts and coupling constants as the product **5a** (Table II) for 3-H (δ 3.90, dt, J = 11, 3.5 Hz), 4-H (δ 3.65, d, J = 3.5 Hz), and 19-CH_3 (δ 0.92, s) while in the spectrum of 5α-cholestane-3β,4β,5-triol (**11**) the shifts for 3-H, 4-H, and 19-CH_3 were found at δ 4.09 (dt, J = 11, 3 Hz), 3.47 (d, J = 3 Hz), and 1.18 (s), substantially different from our values. Thus, the 5β-cholest-25-ene-3α,4α,5,21-tetrol 3,21-disulfate structure was established for the steroid **5**.

Since ^1H (Table II) and ^{13}C NMR (Table I) spectra indicated that the steroids **6** and **7** possessed identical nuclei and the common 21-sulfoxy substituent, but differed in the side chain, the structure analysis to settle the nuclear substitution was pursued on the major **6**.

Structure Elucidation of 5β-Cholest-25-ene-2β,3α,4α,21-tetrol 3,21-Disulfate (6). The FABMS (negative-ion mode) showed molecular ion species at *m/z* 593, 615, and 631 and suggested **6** to be isomeric with **5**. An examination of ^1H and ^{13}C NMR spectra immediately suggested that **6** possessed the same side chain as **1** and **5**. Further analysis of ^{13}C NMR data (Table I) also established that the remaining sulfoxy and hydroxy functionalities in **6** are located on ring A, as the signals for the carbons 6–27 were virtually identical with those of the corresponding signals of **1**, except for some deviations for C-6 (–0.7 ppm in **6**), C-9 (+1.1 ppm in **6**), and C-10 (+2.0 ppm in **6**), confirming the presence of a perturbation on ring A. Continuing now with the examination of ^1H NMR data of compound **6**, signals for three protons at δ 4.33 (1 H, apparent t with J = 3.4 Hz) and δ 4.03 (2 H, complex) indicated the presence of three methine protons on oxygen-bearing carbons. When we measured the spectrum of the tetrol **6a**, derived from **6** upon solvolysis, the three methine protons appeared as isolated signals at δ 3.23 (dd, J = 9.5, 3.5 Hz), 3.81 (m, $W_{1/2}$ = 18 Hz), and 3.94 (t, J = 3.5 Hz) and double-resonance experiments proved that they were located next to each other. The double doublet at δ 3.23 (3-H) was coupled with the signal at δ 3.81 (2-H) by 9.5 Hz and with the other one at δ 3.94 (4-H) by 3.5 Hz. The signal at δ 3.81 (2-H) was also found to be coupled by 3.5 Hz with a double doublet at δ 2.13 (1 H, dd, J = 13.5, 3.5 Hz), assigned to 1-H_{eq}. These data can be best rationalized by locating the secondary hydroxyls in **6a** at positions 2β (δ 3.81), 3α (δ 3.23), and 4α (δ 3.94) in a 5β-H steroidal skeleton. The sulfate group in **6** is located at C-3 by consideration of chemical shift in comparison with ^1H NMR data for the desulfated derivative **6a** [(e.g., 3-H 4.03 vs 3.23; **6** vs **6a**), while 2-H and 4-H were shifted upfield by only 0.2–0.4 ppm in **6a** (δ 2-H, 3.81; δ 4-H, 3.94) relative to **6** (δ 2-H, 4.03; δ 4-H, 4.33)] and comparison of ^{13}C NMR spectra (Table I). Assignments of carbon signals in **6** were aided by heteronuclear selective decoupling, which allow us to correlate the isolated proton signal at δ 4.33 (4-H) with the carbon resonance at 76.5 ppm (C-4). Of the two

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Table III. Composition of Sulfated Polyhydroxy Steroids in *O. superba* (1.5 kg Wet) and Physical Data

steroid	amt, mg	HPLC mobility,[a] min	$[\alpha]_D$,[b] deg	FABMS (negative-ion mode) $M(SO_3H)SO_3^-$	$M(SO_3Na)SO_3^-$ (major)	$M(SO_3K)SO_3^-$
1	16	13.8	9.1	577	599	615
2	38	10.4	21.9	629	643	–
3	4	12.0	31.5	609	631	–
4	12	11.8	18.8	623	645	661
5	14	9.6	9.1	593	615	631
6	10	12.0	9.0	593	615	631
7	6.5	12.6	22.3	623	645	661
8	13	12.4	41.3	–	617	631
9	27	11.8	2.2	561	583	599

[a] $MeOH-H_2O$ (45:55) as the mobile phase and a C_{18} μBondapak column (7.8-mm i.d. × 30 cm). [b] Taken for solutions in MeOH (*c* ranging from 2 to 0.4).

remaining CH–O– signals at δ 87.0 and 65.5, the one at higher chemical shift (δ_C 87.0) was then assigned to the sulfoxymethine carbon. Comparison of the ^{13}C NMR data of **6** with those of the desulfated **6a** and of the compound **1** definitively established the 2β,4α-dihydroxy-3α-sulfoxy structure. Thus, the 5β-cholest-25-ene-2β,3α,4α,21-tetrol 3,21-disulfate structure is established for compound **6**, related to **1** by introducton of an "extra" hydroxyl group at C-2β.

Comparison of ^1H and ^{13}C NMR data of sterols **6** and **7** showed conclusively that both had 2β,3α,4α,21-tetrol 3,21-disulfate structure, but different side chains. The 250-MHz spectrum of **7** contains signals of olefinic protons: 1 H, dd at 5.34 ppm (J = 15, 7 Hz) and 1 H, dd at 5.29 ppm (J = 15, 9 Hz) indicative of a Δ^{22E}-double bond; two 3 H doublets associated with the terminal isopropyl group (0.95 ppm, J = 7 Hz and 0.89 ppm, J = 6.5 Hz); and two double doublets associated with a 24-hydroxymethyl group [δ 3.57 (1 H, J = 10.5, 6 Hz); 3.49 (1 H, J = 10.5, 7.5 Hz)]. The signals for 21-H_2 were observed shifted to δ 3.80 (dd, J = 10, 8.7 Hz) and 4.24 (dd, J = 10, 3.7 Hz). Sequential decoupling, which also allowed assignment of the signals for 20-H (m, 2.40 ppm), 24-H (m, 1.98 ppm), and 25-H (m, 1.78 ppm), established the structure of the side chain as in **7**. FABMS (negative-ion mode), m/z 623, 645, and 661, and ^{13}C NMR data (Table I) supported the structure **7** (δ_C 24–28, 53.7, 29.7, 19.5, 21.4, and 65.0 ppm are in good agreement with those measured for synthetic 24-(hydroxymethyl)-Δ^{22E}-cholesterols;[13] 24*R* isomer, δ_C 24–28, 52.9, 29.3, 19.7, 21.4, 65.2; 24*S* isomer, δ_C 24–28, 53.0, 29.5, 19.8, 21.6, 65.2 ppm).

The remaining feature needed to establish the structure fully is the stereochemistry at C-24. Recently we have synthesized stereoselectively at C-24. Recently we have synthesized stereoselectively 24(*R*)- and 24(*S*)-(hydroxymethyl)cholesta-5,22(*E*)-dien-3β-ol,[13] and they could be differentiated by the ^1H NMR spectra of their α-methoxy-α-(trifluoromethyl)phenylacetate (MTPA). The most noticeable feature was a large difference in the chemical shift of the C-21 methyl signal in both the (+)-(*R*)-MTPA and (–)-(*S*)-MTPA esters. In the spectra of the 24*S* isomer derivatives, the C-21 methyl signal is upfield shifted about 0.1 ppm, while in those of the 24*R* isomer derivatives it is virtually unshifted relative to the free alcohols. Thus we have prepared both the (+)-(*R*)-MTPA and (–)-(*S*)-MTPA esters of the natural **7** (**7a** and **7b**) and found that in their ^1H NMR spectra one of the two 21-methylene protons is upfield shifted to 4.16–4.17 ppm (4.24 in **7**). Reinforcing evidence for the 24*S* stereochemistry in **7** was the shifts of the isopropyl methyls of the (+)-(*R*)-MTPA (**7a**) and (–)-(*S*)-MTPA (**7b**) esters. Our values (in **7a** two doublets at δ 0.86 and 0.92, separated by 0.06 ppm, while in **7b** the two isopropyl methyls appeared as closer signals, triplet at δ 0.885 because of coincident overlap of the low-field arm of one doublet at 0.90 with the high-field arm of the other one at δ 0.87) compared better with those of esters of the 24*S* isomer [(+)-(*R*)-MTPA ester, δ 26,27-CH_3, 0.87–0.94 separated by 0.07 ppm; (–)-(*S*)-MTPA ester, δ 26,27-CH_3, 0.88–0.92, separated by 0.04 ppm]. In the (+)-(*R*)-MTPA ester of the 24*R* isomer, the shifts of the isopropyl methyls appeared as closer signals at δ 0.87–0.91, while they were observed separated by 0.06 ppm at δ 0.87–0.93 in the spectrum of the (–)-(*S*)-MTPA ester.

Thus the (22*E*,24*S*)-24-methyl-5β-cholest-22-ene-2β,3α,4α,21,28-pentol 3,21-disulfate structure is suggested for compound **7**.

Experimental Section

General Methods. For general methods, see ref 1.

Collection and Extraction of Ophiuroid. *O. superba* (1.5 kg wet) was collected in Aug. 1986 at Zampa, Okinawa, Japan, and extracted by soaking in methanol (4 L × 3). Removal of solvent under reduced pressure left the residue (37.9 g), which was partitioned between water and ethyl acetate (100 mL × 3). The aqueous residues were then extracted 3× with 1-butanol (400 mL). Removal of solvent under reduced pressure left a viscous residue (4.5 g), which was chromatographed on a column of Sephadex LH-20 (2 × 60 cm, MeOH, 8-mL fractions were collected) to give three major fractions, 105–114 (187 mg), 115–124 (264 mg), and 125–135 (97 mg), combined after TLC on silica gel in 1-butanol–acetic acid–H_2O (12:3:5), which contained the sulfate polyhydroxy steroids.

Separation of the Sulfated Polyhydroxy Steroid Mixtures. The fractions 105–114 were dissolved in methanol and subjected to preparative reverse-phase HPLC on a C_{18} μBondapak column (7.8-mm i.d. × 30 cm) with $MeOH-H_2O$ (45:55) as the mobile phase to give the (20*R*)-cholest-5-ene-3α,21-diol 3,21-disulfate (**9**):[2] ^1H NMR (CD_3OD) δ 0.78 (3 H, s, 18-H_3), 0.91 (6 H, d, J = 7 Hz, 26,27-H_3), 1.06 (3 H, s, 19-H_3), 3.98 (1 H, dd, J = 10, 6.5 Hz)–4.22 (1 H, dd, J = 10, 3.5 Hz, 21-H_2), 4.64 (1 H, m, $W_{1/2}$ = 7.5 Hz, 3β-H), 5.34 (1 H, m, $W_{1/2}$ = 10 Hz, 5-H).

The last-eluted fractions (125–135) from the column of Sephadex LH-20 were also dissolved in methanol and directly subjected to HPLC as before to give the compounds **1**, **5**, **6**, and **8**. The fractions 115–124 were further purified by DCCC (DCC-A apparatus manufactured by Büchi equipped with 250 tubes) using as solvent system 1-butanol–acetone–H_2O (3:1:5) in which the stationary phase consisted of the lower phase (ascending mode). The sulfated polyhydroxy steroids were eluted in fractions 260–380, which were grouped in 11 major fractions, each consisting of 10 4-mL fractions. Each major fraction was then subjected to HPLC as before to give the compounds **2**, **3**, **4**, and **7**. Amounts of each isolated steroid, HPLC mobilities, rotations, and FAB mass spectral data are in Table III. Tables I and II report the ^{13}C and ^1H NMR data.

Conversion of 1 to 1a. Compound **1** (3 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 (2 mL), and then the adsorbed material was eluted with MeOH (3 mL) to give 2 mg of the ketone **1a**, which

was purified by reverse-phase HPLC (Waters μBondapak C_{18} column, MeOH–H_2O, 1:1, as eluent): FABMS (negative-ion mode), *m/z* 575, 597, 613; ^{1}H NMR (CD_3OD) δ 0.74 (3 H, s, 18-H_3), 1.16 (3 H, s, 19-H_3), 1.74 (3 H, s, 27-H_3), 3.94 (1 H, dd, *J* = 10, 6.5 Hz)–4.20 (1 H, dd, *J* = 10, 3.5 Hz, 21-H_2), 4.69 (2 H, br s, 26-H_2), 4.80 (1 H, dd, *J* = 12, 7 Hz, 3-H); CD (MeOH), $\Delta\epsilon_{290}$ = −0.11.

5β-Cholest-25-ene-3α,4α,5,21-tetrol (5a). A solution of **5** (5 mg) in dioxane (0.1 mL) and pyridine (0.1 mL) was heated at 130 °C for 3 h in a stoppered reaction vial. After the solution had cooled, H_2O (1 mL) was added, and the solution was extracted 3× with 1-butanol. Removal of solvent left the glassy material of tetrol **5a**. Purification by reverse-phase HPLC on a C_{18} μBondapak column using 16% aqueous methanol afforded 2.7 mg of tetrol **5a**, FABMS (negative ion) 433 [M − H]$^-$; ^{1}H NMR in Table II.

5β-Cholest-25-ene-2β,3α,4α,21-tetrol (6a). Compound **6** (7 mg) was solvolyzed as above to give the tetrol **6a**, which was purified by reverse-phase HPLC on a C_{18} μBondapak column using 16% aqueous methanol, FABMS (negative ion) 433 [M − H]$^-$; ^{1}H and ^{13}C NMR data in Tables II and I.

Synthesis of 5β-Cholestane-3α,4α,5-triol (10). Cholest-4-en-3-one (1.0 g) in methanol was oxidized with hydrogen peroxide and sodium hydroxide according to Henbest and Jackson.[14] Chromatography of the neutral material through a column of silica gel in petroleum ether–ethyl ether, 98:2, gave 0.7 g of 4β,5β-epoxycholestan-3-one: mp 115–116 °C (lit.[14] mp 116–116.5 °C); δ 4-H, 2.97, s; δ 18-CH_3, 0.69, s; δ 19-CH_3, 1.14, s. The epoxy ketone (0.7 g) in dioxane–H_2O, 3:1 (40 mL), was reduced[15] with an excess of sodium borohydride (2 h, 0 °C) to give the 4β,5β-epoxycholestan-3β-ol (0.58 g), which, without further purification, was acetylated with an excess of acetic anhydride in 4 mL of pyridine at room temperature for 10 h. After removal of the excess reagent in vacuo, the residue was purified by chromatography on silica gel in petroleum ether–ethyl ether, 94:6, to give 0.35 g of 4β,5β-epoxycholestan-3α-yl acetate: mp 69–70 °C (lit.[18] mp 68–70 °C); δ 4-H, 2.79, br s; δ 3-H, 4.90, dd (*J* = 9, 7 Hz); δ 18-CH_3, 0.67, s; δ 19-CH_3, 0.99, s. The epoxy acetate (150 mg) in THF (5 mL) was treated[16] with 60% perchloric acid (0.01 mL) at room temperature for 3 h to give a crude product, which was hydrolyzed with 10% methanolic KOH (3 h, reflux). Usual workup gave 5β-cholestane-3α,4α,5-triol, which was crystallized from MeOH: mp 168–70 °C; FABMS (negative ion) 419 [M − H]$^-$; ^{1}H NMR (CD_3OD) δ 0.71 (3 H, s, 18-H_3), 0.91 (6 H, d, *J* = 7 Hz, 26,27-H_3), 0.92 (3 H, s, 19-H_3), 0.95 (3 H, d, *J* = 6.2, 21-H_3), 3.65 (1 H, d, *J* = 3.5 Hz, 4-H), 3.90 (1 H, dt, *J* = 11, 3.5 Hz, 3-H); ^{13}C NMR in Table I.

Synthesis of 5α-Cholestane-3β,4β,5-triol (11). 4α,5α-Epoxycholestan-3β-yl acetate[17] (100 mg) (δ 4-H, 2.88, s; δ 3-H, 4.95, t (*J* = 10), δ 18-CH_3, 0.67, δ 19-CH_3, 1.12) in THF (3 mL) was treated[16] with 60% perchloric acid (0.01 mL at room temperature for 4 h) to give a crude product, which was hydrolyzed with 10% methanolic KOH (3 h, reflux). Usual workup gave 5α-cholestane-3β,4β,5-triol (11), which was crystallized from MeOH: mp 187–90 °C; FABMS (negative ion) 419 [M − H]$^-$; ^{1}H NMR (CD_3OD) δ 0.72 (3 H, s, 18-H_3), 0.91 (6 H, d, *J* = 7 Hz, 26,27-H_3), 0.96 (3 H, d, *J* = 7 Hz, 21-H_3), 1.18 (3 H, s, 19-H_3), 3.47 (d, *J* = 3.5 Hz, 4-H), 4.09 (1 H, dt, *J* = 11, 3.5 Hz, 3-H); ^{13}C NMR in Table I.

(+)-(*R*)-MTPA and (−)-(*S*)-MTPA Esters of 7. Compounds 7a and 7b. Compound **7** (2 mg) was treated with freshly distilled (+)-(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (5 μL) in 0.2 mL of dry pyridine at room temperature for 3 h. After solvent removal, the product was purified by reverse-phase HPLC on a C_{18} μBondapak column (3.8-mm i.d. × 30 cm) in MeOH–H_2O, 55:45, to give after 13.6-min elution **7a**: FABMS (negative-ion mode), *m/z* 839 [M(SO_3H)SO_3^-], 861 [M-(SO_3Na)SO_3^-], 877 [M(SO_3K)SO_3^-]; ^{1}H NMR (CD_3OD) δ 0.75 (3 H, s, 18-H_3), 0.86 (3 H, d, *J* = 7 Hz, 26-H_3 or 27-H_3), 0.92 (3 H, d, *J* = 7 Hz, 27-H_3 or 26-H_3), 1.02 (3 H, s, 19-H_3), 3.56 (3 H, s, OCH_3), 3.84 (1 H, apparent t, *J* = 9 Hz, 21-H), 4.03 (2 H, m, 2-H, 3-H), 4.16 (1 H, dd, *J* = 10, 3.5 Hz, 21-H), 4.28–4.37 (each 1 H, dd, *J* = 11, 6.0 and *J* = 11, 6.5 Hz, 28-H_2), 4.32 (1 H, partially overlapped with 28-H_2, 4-H), 5.26–5.38 (1 H each, dd, *J* = 16, 7.5, *J* = 16, 7 Hz, 22,23-H), 7.48–7.53 (m, Ph H's).

Compound **7b** was similarly prepared from **7** (2 mg) and freshly distilled (−)-(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride and purified as above (elution time: 12.5 min): FABMS (negative ion), *m/z* 839, 861, 877; ^{1}H NMR (CD_3OD) δ 0.75 (3 H, s, 18-H_3), 0.88 (6 H, t, *J* = 7 Hz, 26,27-H_3), 1.02 (3 H, s, 19-H_3), 3.84 (1 H, apparent t, *J* = 9 Hz, 21-H), 4.03 (2 H, m, 2-H, 3-H), 4.17 (1 H, dd, *J* = 10, 3.5 Hz, 21-H)8 4.28–4.40 (each 1 H, dd, *J* = 11, 6.5 Hz, *J* = 11, 6.0 Hz, 28-H_2), 4.32 (1 H, m, 4-H), 5.33–5.38 (2 H, 2 dd partially overlapping, *J* = 16, 7.5 Hz, 22,23-H), 7.48–7.53 (m, Ph H's).

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