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POLYOXYGENATED MARINE STEROIDS FROM THE DEEP WATER STARFISH *STYRACASTER CAROLI*

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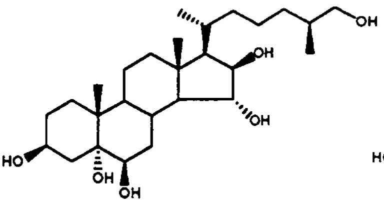
ABSTRACT.—Ten marine polyhydroxysteroids, **1–10**, of which two, **1** and **5**, are known compounds previously isolated from starfish, have been isolated from the deep water starfish *Styracaster caroli*, collected at a depth of 2000 m off New Caledonia. The 3β,5,6β-trihydroxy functionality is the common element in these steroids, and additional hydroxyl groups were found at positions 8,15α (or β) and 16β. Greater differences are observed in the structure of the side-chains, which showed multiple functionalities and different alkylation patterns. Characterization was accomplished by fabms and ^{1}H- and ^{13}C-nmr spectroscopy, with the assignments of the configurations to the stereogenic centers of the side-chains being made by ^{1}H-nmr comparison with appropriate models and on analysis of their derivatives with a chiral reagent.

Steroidal oligoglycosides, which are the predominant metabolites of starfish, are often accompanied by various polyhydroxysteroids (1). Polyhydroxysteroids are not uncommon in marine species, and have been isolated from every marine invertebrate phylum and also from algae and fish (2,3) but, starfish (phylum Echinodermata) appear to be their richest source (1–3). They have been found in almost all the species examined, usually as complex mixtures, and more than eighty polyhydroxysteroids have already been reported. The 3β,6α (or β),8,15α (or β),16β-pentahydroxycholestane structure is a common feature with additional hydroxyl groups found at positions 4β,5α,7α (or β) and occasionally at 14α, all disposed on one side of a steroidal nucleus, thus giving an amphiphilic character to the molecules with hydrophilic and hydrophobic regions (4). The major group possesses a 26-hydroxyl function, whereas in a less common group the side-chain is hydroxylated at C-24. In part, the polyhydroxysteroids of starfish occur in sulfated form.

In a preceding note we have described the occurrence in the starfish *Styracaster caroli* (Ludwig, family Porcellanasteridae) of a novel group of polyhydroxysteroid constituents, carolisterols A–C (**11–13**), characterized by a polyhydroxycholanic acid moiety, in which the 24-carboxylic acid function is found as an amide derivative of D-cysteinolic acid (5). In this report we describe the isolation and structural elucidation of ten more polyhydroxysteroids (of which eight are new compounds) from *Styracaster caroli* collected at a depth of 2000 m between Thio and Lifou (New Caledonia).

RESULTS AND DISCUSSION

Separation of the crude mixture of polar steroids from the aqueous and Me_2CO extracts of *S. caroli*, was achieved by chromatography on a column of Sephadex LH-60, using $MeOH-H_2O$ (2:1) as eluent. This separated the polyhydroxysteroids **1–4** from the sulfated polyhydroxysteroids **5–10**, and from the more polar compounds **11–13**. The final purification was accomplished by droplet counter current chromatography (dccc) followed by hplc on a C_{18}-bonded phase to obtain the steroids **1–10**. The results of this separation are shown in Table 1, and the polyhydroxysteroids **1–4** and the sulfated polyhydroxysteroids **5–10** are discussed in turn.



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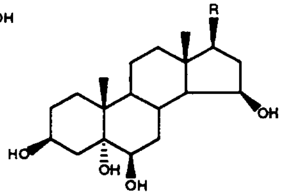
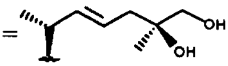
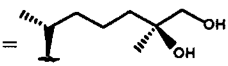
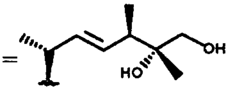
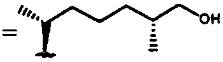


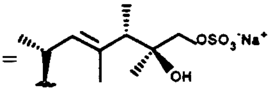
2 R= 

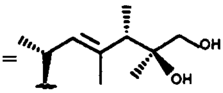
2a R= 

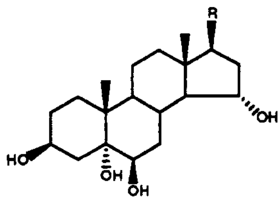
3 R= 

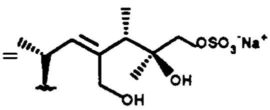
(the stereochemistry at C-24 and C-25 in **3** is relative)

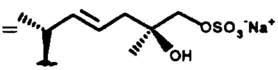
4 R= 

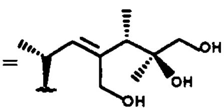
7 R= 

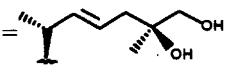
7a R= 

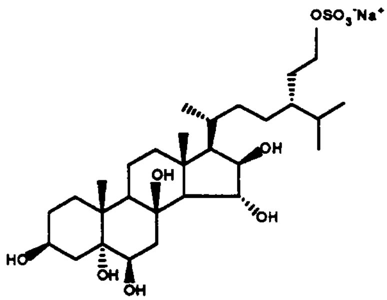


9 R= 

10 R= 

9a R= 

10a R= 



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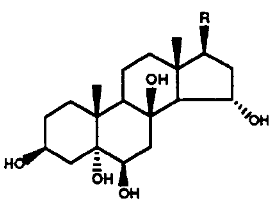
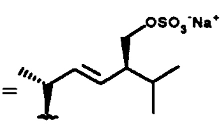
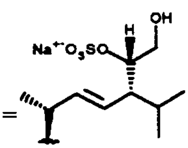
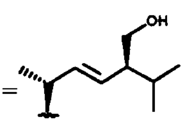
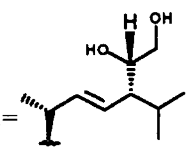
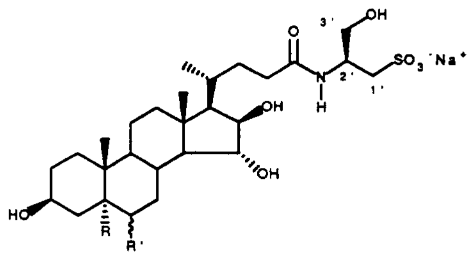


6 R= 

8 R= 

6a R= 

8a R= 



11 Carolisterol A R=OH; R′=◀OH
12 Carolisterol B R=OH; R′==O
13 Carolisterol C R=H; R′=ıııOH

The major polyhydroxysteroid has been identified as (25*S*)-5α-cholestane-3β,5,6β,15α,16β,26-hexaol [**1**], previously found in the starfish *Luidia maculata* (6) and *Myxoderma platyacanthum* (7). The 25*S* configuration was assigned by direct ^{1}H-nmr comparison with both stereoisomers: the 25*S*-isomer [**1**], isolated from *Myxoderma platyacanthum* (7) and the 25*R*-isomer, later isolated from *Tremaster novaecaledoniae* (8). We observed very small differences in the spectra of the two isomers, the major one concerning the signals, at 500 MHz, of the 27-methyl protons seen at δ 0.934 ppm in the spectrum of the (25*S*)-isomer, shifted to 0.925 ppm in that of (25*R*)-isomer. Although the difference is small (0.009 ppm) it is enough to distinguish them when a direct comparison of both stereoisomers is made. Assignment of the *S* configuration at C-25 in **1** was supported by the ^{1}H-nmr pattern of the 26-methylene proton signals in the 3β,26-di-(+)-MTPA[1] ester (2H, doublet at δ_H 4.21, J=5.5 Hz) and in the 3β,26-di-(−)-MTPA[1] ester (two 1H double doublets at δ_H 4.28 and 4.12, J=10.0, 5.5 Hz) (1,9).

TABLE 1. Polyhydroxysteroids from the Starfish *Styracaster caroli* (2.0 kg fresh wt).

Compound	Amount (mg)	Specific rotation[a] ([α]D)	Hplc mobility[b] (MeOH/H_2O)	Fabms (negative-ion)
1	80.0	+12.1°	7:3	467 [M−H]$^-$
2	15.5	−14.6°	7:3	465 [M−H]$^-$
3	1.4	−10.6°	7:3	479 [M−H]$^-$
4	0.4	+21.4°	7:3	451 [M−H]$^-$
5	90.0	+25.0°	1:1	577 [MSO_3^-]
6	10.0	+2.5°	1:1	559 [MSO_3^-]
7	2.9	+2.7°	1:1	573 [MSO_3^-]
8	19.4	−0.7°	45:55	589 [MSO_3^-]
9	0.5	−3.3°	35:65	589 [MSO_3^-]
10	0.4	+5.2°	35:65	545 [MSO_3^-]
11	6.0	+5.3°	4:6	576 [M]$^-$
12	3.3	−3.6°	4:6	574 [M]$^-$
13	2.7	—	4:6	560 [M]$^-$

[a]From solutions in MeOH (*c* ranging from 0.2 to 0.6).
[b]On a Waters C_{18} μ-Bondapak column (30×7.8 mm i.d., flow rate 5 ml/min).

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[1]MTPA=α-methoxy-α-(trifluoromethyl)-phenylacetic acid; Mosher's reagent (10); the term (+)- or (−)-MTPA ester refers to an ester prepared using the acid chloride derived from *R*-(+)- or *S*-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid, respectively.

Steroid **2**, (22*E*,25*S*)-5α-cholest-22-en-3β,5,6β,15β,25,26-hexaol, showed in its fabms (negative-ion mode) a quasi-molecular ion at *m/z* 465 [M−H]$^-$. Examination of its ^{1}H-nmr spectrum (Table 2) indicated the presence of the common 3β,5α,6β-trihydroxy functionality. One more hydroxymethine signal was seen at δ 4.17 with the splitting pattern consistent with a 15β-hydroxy group (11) [$J_{(14\text{-H}/(15\alpha\text{-H})}$=5.5 Hz]; the downfield shift of H_3-18 to δ_H 1.02 supported the 15β stereochemistry. Further support came from the comparison of the ^{1}H-nmr spectrum of **2** (Table 2) with that of its 15α-hydroxy isomer **10a** (Table 4) and from the ^{13}C-nmr spectrum of **2** (Table 3) and its comparison with those of steroids with the 15α-hydroxyl group (e.g., **7**, Table 3). The major differences in their ^{13}C-nmr spectral data are observed for the resonances of the γ carbons at C-8 (27.4 vs. 31.1 ppm, **2** vs. **7**), and C-17 (57.3 vs. 55.5 ppm, **2** vs. **7**) confirming the 15β-OH stereochemistry in **2** and the 15α-stereochemistry in **7**. The two well-separated olefinic protons in the ^{1}H-nmr spectrum of **2** at δ 5.34 (1H, dd, *J*=15.7 and 8.2 Hz, H-23) and 5.45 (1H, m, H-22) indicated the presence of a $\Delta^{22}E$ double bond in the side-chain. The spectrum also contained a 3H signal at δ_H 1.13 and a 2H singlet at δ_H 3.38, thereby also indicating a 25,26-dihydroxy functionality in the side-chain. ^{13}C-Nmr signals at δ 73.7 (quaternary carbon, DEPT) and 69.8 (CH_2, DEPT) ppm supported this conclusion.

Assignment of the *S* configuration at C-25 was based on the ^{1}H-nmr pattern of the 26-methylene proton signals in the 3β,26-di-(+)-MTPA (two 1H doublets at δ_H 4.27 and 4.11, *J*=11.2 Hz) and in the 3β,26-di-(−)-MTPA (2H singlet at δ_H 4.19) esters of the dihydro derivative of **2** [**2a**] and their comparison with the ^{1}H-nmr data of the 26-(+)-MTPA esters of the stereoisomeric models (25*S*)-25,26-dihydroxycholesterol (δ H_2-26: 4.26 and 4.09, each 1H, d, *J*=12.0 Hz) and (25*R*)-25,26-dihydroxycholesterol (δ H_2-26: 4.17 s, 2H), synthesized by Ikekawa and coworkers (12).

Steroid **3**, (22*E*)-24-methyl-5α-cholest-22-en-3β,5,6β,15β,25,26-hexaol, the 24-methyl analogue of **2**, in the fabms (negative-ion mode) showed a quasi-molecular ion at *m/z* 479 [M−H]$^-$, 14 mass units different from **2** (*m/z* 465). The nmr spectra of **3** (Tables 2 and 3) contained a signal at δ_H 1.02 (d, *J*=7.0 Hz) and at δ_C 15.5, for the methyl group located at C-24. The remaining signals were close to those assigned to **2** except for the resonances due to the side-chain protons, which are in agreement with those expected for a $\Delta^{22}E$-24-methyl-25,26-dihydroxysteroidal side-chain. The same $\Delta^{22}E$-24-methyl-25,26-dihydroxysteroidal side-chain was found in a polyhydroxysteroid isolated from the starfish *Archaster typicus* (13). The assignments of the configurations at C-24 and C-25 were made by the synthesis of side-chain models, the enantiomeric pairs (2*R*,3*R*)/(2*S*,3*S*)- and (2*S*,3*R*)/(2*R*,3*S*)-2,3-dimethylpentane-1,2-diol (13). These compounds exhibit easily recognizable ^{1}H-nmr spectra which, when compared with that of the 22,23-dihydro derivative of natural **3** allow recognition of its relative stereochemistry on the basis of the good agreement between its C-26, C-27, and C-28 proton signals (δ_H 3.44 d–3.50 d, 1.06 s, 0.90 d) and the corresponding signals (δ_H 3.42 d–3.49 d, 1.04 s, 0.89 d) of the (2*R*,3*R*)/(2*S*,3*S*)-2,3-dimethylpentane-1,2-diol enantiomeric pair. The corresponding signals of the enantiomeric pair (2*S*,3*R*)/(2*R*,3*S*)-2,3-dimethylpentane-1,2-diol (δ_H 3.45 ABq, 1.07 s and 0.96 d) are very different from those of the 22-dihydro derivative of natural **3**. The small amount available of the 22-dihydro derivative of **3** did not allow us to prepare the (+)- and (−)-MTPA esters and compare their ^{1}H-nmr spectra with those of the corresponding esters of the enantioselectively prepared (2*R*,3*R*) and (2*S*,3*S*)-2,3-dimethylpentane-1,2-diols (13).

(25*R*)-5α-Cholestan-3β,5,6β,15α,26-pentaol (steroid **4**) is a minor component of *Styracaster caroli*. In the negative-ion fabms spectrum of **4** a quasi-molecular ion was observed at *m/z* 451 [M−H]$^-$, corresponding to a pentahydroxycholestane structure.

TABLE 2. Selected 500 MHz ^{1}H-Nmr Data in CD_3OD of Polar Steroids of *Styracaster caroli*.[a]

Proton	Compound							
	2	**3**	**4**	**6**	**7**	**8**	**9**	**10**
H-3α	4.05 m	4.05 m	4.04 m	4.10 m	4.04 m	4.10 m	4.04 m	4.04 m
H-6α	3.52 t (2.5)	3.51 t (2.5)	3.50 t (2.5)	3.59 t (2.5)	3.50 t (2.5)	3.59 t (2.5)	3.50 t (2.5)	3.50 t (2.5)
H-7β	—	—	—	2.20 dd (15.0, 2.5)	—	2.20 dd (15.0, 2.5)	—	—
H-15	4.17 t (5.5)	4.17 t (5.5)	3.89 td (10.0, 3.0)	4.26 td (10.0, 3.0)	3.87 td (10.0, 3.0)	4.27 td (10.0, 3.0)	3.85 td (10.0, 3.0)	3.86 td (10.0, 3.0)
H-16α	—	—	—	—	—	—	—	—
H_3-18	1.02 s	1.02 s	0.77 s	1.00 s	0.80 s	1.00 s	0.82 s	0.78 s
H_3-19	1.24 s	1.24 s	1.20 s	1.33 s	1.21 s	1.32 s	1.21 s	1.21 s
H_3-21	1.07 d (7)	1.05 d (7)	0.97 d (7)	1.03 d (7.0)	1.00 d (7.0)	1.04 d (7.0)	1.06 d (7.0)	1.06 d (7.0)
H-22	5.45 m	5.47 dd (15.0, 8.0)	—	5.33 dd (15.0, 8.6)	5.08 d (9.0)	5.33 dd (15.0, 8.0)	5.23 d (9.0)	5.45 dd (15.0, 8.0)
H-23	5.34 dd (15.7, 8.2)	5.30 dd (15.0, 8.5)		5.23 dd (15.0, 9.0)		5.11 dd (15.0, 9.0)		5.36 dd (15.0, 9.0)
H-24					2.32 q (7.0)	2.18 m	2.52 q (7.0)	2.28–2.19 dd (12.7, 7.0)
H-26	3.38 s	3.42–3.48 d (11.2)	3.34[b]–3.44 dd (11.2, 5.6)	0.94 d (7.0)	3.80–3.90 d (9.0)	0.88 d (7.0)	3.96–3.82 d (9.0)	3.85 ABq (9.0)
H_3-27	1.13 s	1.13 s	0.94 d (7)	0.88 d (7.0)	1.18 s	0.89 d (7.0)	1.25 s	1.17 s
H-28	—	1.02 d (7)	—	3.98 d (5.6)	1.10 d (7.0)	4.40 m	1.15 d (7.0)	—
H_3-29	—	—	—	—	1.70 s	3.92 dd (13.0, 2.0) 3.60 dd (13.0, 5.6)	4.28–4.02 d (11.2)	—
Other signals	—	—	—	—	H-4β: 2.10 t (13.0) H-20: 2.42 m	H-20: 2.06 m	H-4β: 2.09 t (13.0) H-20: 2.57 m	H-4β: 2.08 t (13.0)

[a]The chemical shift values are given in ppm and are referenced to CHD_2OD (3.34 ppm). The coupling constants are given in Hz and are enclosed in parentheses.
[b]Under solvent signal.

TABLE 3. ^{13}C-Nmr Chemical Shift Data (CD_3OD) of the Polar Steroids from *Styracaster caroli*.[a]

Carbon	Compound						
	2	**3**	**6**	**7**	**8**	**9**	**10**
1	31.5	31.5	34.5	31.5	34.5	31.7	31.5
2	33.3	33.3	31.0	33.4	30.9	33.5	33.4
3	68.1	68.1	68.3	68.2	68.3	68.3	68.2
4	41.1	41.1	41.1	41.4	41.9	41.5	41.0
5	76.5	76.5	76.5	76.5	76.4	76.4	76.3
6	76.5	76.5	78.0	76.3	77.9	76.3	76.3
7	33.9	33.9	40.0	35.2	41.0	35.3	35.1
8	27.4	27.4	77.3	31.1	77.3	31.3	31.1
9	46.6	46.6	49.6	46.5	49.1	46.6	46.5
10	39.4	39.4	39.2	39.2	39.2	39.5	39.2
11	21.9	21.9	19.7	22.0	19.7	22.1	22.0
12	42.2	42.4	42.8	41.3	42.8	41.5	42.1
13	43.2	43.2	45.4	44.7	45.4	44.9	44.7
14	61.8	61.8	66.6	63.5	66.6	63.7	63.5
15	70.7	70.7	69.9	74.4	70.0	74.2	74.1
16	42.6	42.5	42.1	41.2	40.4	41.5	43.0
17	57.3	57.5	55.6	55.5	55.7	55.2	54.6
18	15.3	15.3	15.6	14.0	15.5	14.3	13.8
19	17.2	17.1	18.0	17.3	18.1	17.4	17.3
20	41.4	41.5	40.9	35.1	41.1	35.4	41.3
21	21.2	21.2	21.2	20.6	21.2	21.3	21.0
22	141.2	138.5	140.7	135.6	142.6	137.2	141.5
23	124.0	130.7	126.8	134.5	124.6	140.2	123.7
24	42.5	44.6	49.0	49.0	51.2	49.0	41.4
25	73.7	75.3	29.2	74.4	27.7	73.7	72.6
26	69.8	69.0	18.8	74.1	17.4	74.0	74.9
27	23.6	21.7	21.0	22.6	22.0	24.4	23.9
28	—	15.5	70.7	13.7	82.1	14.2	—
29	—	—	—	15.3	64.8	73.3	—

[a]At 125 MHz; values relative to CD_3OD=49.0 ppm (central peak); assignments aided by DEPT technique.

The ^{1}H-nmr spectrum (Table 2) revealed signals almost identical with those observed in the spectrum of (25*S*)-5α-cholestane-3β,5,6β,15α,26-pentaol, isolated from the starfish *Myxoderma plathyacanthum* (7); slight differences were observed in the chemical shifts of the side-chain protons, especially those of the 26-methylene and 27-methyl protons, thus supporting **4** as the 25*R*-isomer. In confirmation, **4** was converted into the diastereomeric (+)- and (−)-MTPA esters and, in their ^{1}H-nmr spectra, the 26-methylene proton signals were closer to the spectrum of the (−)-MTPA ester (δ_H 4.26 dd–4.16 dd, J=11.0 and 6.0 Hz) than to that of the (+)-MTPA ester (δ_H 4.27 dd–4.13 dd, J=11.0 and 6.0 Hz) as expected for a 25*R* isomer (1,7,9).

The major component of the sulfated polyhydroxysteroids **5**–**10** has been identified as (24*R*)-24-ethyl-5α-cholestane-3β,5,6β,8,15α,16β,29-pentaol 29-sulfate [**5**], previously found in the starfish *Tremaster novaecaledoniae* (8).

Steroid **6**, (22*E*,24*S*)-5α-ergosta-22-en-3β,5,6β,8,15α,28-hexaol 28-sulfate, contained a sulfate function which was indicated by its polarity and by fabms (negative-ion mode), which gave the molecular anion species at *m/z* 559 [MSO_3^-]. Upon solvolysis with dioxane-pyridine, **6** was desulfated to **6a** of lower polarity. The fabms of this product showed a pseudomolecular ion at *m/z* 479 [M−H]$^-$, representing the loss of 80 mass units (SO_3) from **6**. Its molecular formula, $C_{28}H_{48}O_6$, which corresponded to a

TABLE 4. Selected ^{1}H-Nmr Chemical Shifts (CD_3OD) of the Desulfated Products **6a–10a**.[a]

Proton	Compound				
	6a	**7a**	**8a**	**9a**	**10a**
H-3α . . .	4.10 m	4.04 m	4.10 m	4.04 m	4.04 m
H-6α . . .	3.61 t (2.5)	3.50 t (2.5)	3.61 t (2.5)	3.50 t (2.5)	3.50 t (2.5)
H-7β . . .	2.20 dd (15.0, 2.5)	—	2.20 dd (15.0, 2.5)	—	—
H-15 . . .	4.26 td (10.0, 3.0)	3.87 td (10.0, 3.0)	4.28 td (10.0, 3.0)	3.87 td (10.0, 3.0)	3.87 td (10.0, 3.0)
H-16α . .	—	—	—	—	—
H_3-18 . .	1.00 s	0.80 s	1.00 s	0.82 s	0.78 s
H_3-19 . .	1.33 s	1.21 s	1.32 s	1.21 s	1.21 s
H-20 . . .	—	2.42 m	—	2.57 m	—
H_3-21 . .	1.04 d (7)	0.99 d (7)	1.02 d (7)	1.06 d (7)	1.05 d (7)
H-22 . . .	5.33 dd (14.0, 8.0)	5.05 d (9.0)	5.26 dd (14.0, 8.0)	5.22 d (9.0)	5.43 m
H-23 . . .	5.21 dd (14.0, 8.6)		5.08 dd (14.0, 8.6)		5.34 dd (14.0, 8.6)
H-24 . . .	—	2.30 q (7.0)	—	2.51 q (7.0)	2.21–2.15 dd (14.0, 7.0)
$H_{2(3)}$-26 .	0.94 d (7.0)	3.41–3.35 d (9.0)	0.88 d (7.0)	3.48 d (10.0)–3.34[b]	3.38 s
H_3-27 . .	0.86 d (7.0)	1.12 s	0.85 d (7.0)	1.21 s	1.12 s
H-28 . . .	3.57 dd (9.5, 6.7) 3.51 dd (9.5, 6.5)	1.08 d (7.0)	3.58 m	1.12 d (7.0)	—
$H_{2(3)}$-29 .	—	1.70 s	3.68 dd (11.0, 2.5) 3.58 dd (11.0, 5.0)	4.26 d (11.0) 4.04 d (11.0)	—

[a]The chemical shift values are given in ppm and are referred to CHD_2OD (3.34 ppm). The coupling constants are given in Hz and are enclosed in parentheses.
[b]Under solvent signal.

monounsaturated methylcholestane hexaol, was determined primarily by DEPT and ^{13}C-nmr spectroscopy. Analysis of the ^{1}H- and ^{13}C-nmr spectra of **6** (Tables 2 and 3) provided evidence for the 3β,5,6β,8,15α-pentahydroxycholestane tetracyclic nucleus found in nodososide, the first representative of this group of polyhydroxysteriods to be found in starfish, initially isolated from *Protoreaster nodosus* (14). In addition to the steroidal tetracyclic moiety, the ^{1}H-nmr spectrum contained signals for three secondary methyl groups (δ_H 1.03 d, 0.94 d, and 0.88 d), two 1H olefinic signals at δ 5.23 dd (J=15.0 and 9.0 Hz)–5.33 (J=15.0 and 8.6 Hz) and a 2H doublet signal (J=5.6 Hz) at δ_H 3.98 for an oxygenated methylene group. These features can be arranged in a $\Delta^{22}E$-28-(oxygenated)-methylcholestane side-chain. In the ^{1}H-nmr spectrum of the desulfated **6a** (Table 4), the signal for the oxygenated methylene group was observed as two 1H double doublets shifted upfield to δ 3.57 dd (J=9.5 and 6.7 Hz) and 3.51 dd (J=9.5 and 6.5 Hz), thus allowing the sulfate group to be assigned to C-28 in **6**. We also note that the chemical shift values of the hydroxymethylene protons in **6a** eliminated the alternative 24-methyl-26-hydroxy side-chain, in which the 26-protons are known to resonate at δ 3.28 dd–3.60 dd (threo) and 3.34 dd–3.53 dd (erythro) (1,15). The same $\Delta^{22}E$-24-hydroxymethylcholestane side-chain was found in coscinasteroside C and pisasteroside A, polyhydroxylated steroid glycosides isolated from the starfish *Coscinasterias tenuispina* (16) and the genus *Pisaster* (17), respectively. The assignment of the stereochemistry at C-24 of these two steroids required the stereoselective synthesis of (24*S*)- and (24*R*)-24-hydroxymethylcholesta-5,22(*E*)-dien-3β-ols as model compounds (18). The ^{1}H-nmr spectra of the two epimeric models were virtually identical, but the spectra of their (+)- and (−)-MTPA esters showed diagnostic differences useful for assignment of configuration. Thus **6a** was converted to its 3β,28-di-(+)-MTPA and 3β,28-di-(−)-MTPA derivatives. In the ^{1}H-nmr spectrum of the 3β,28-di-(+)-MTPA derivative of **6a** the resonances of the C-28 protons were observed as two well-separated doublets at δ_H 4.21 (J=10.5 and 5.0 Hz) and 4.39 (J=10.5 and 7.0 Hz), while in the ^{1}H-nmr spectrum of the 3β,28-di-(−)-MTPA derivative of **6a** the same signals were observed as two overlapping double doublets at δ 4.31 (J=10.5 and 5.0 Hz) and 4.33 (J=10.5 and 7.0 Hz), in agreement with a 24*S* configuration (1,18).

Steroid **7**, (22*E*,24*S*,25*S*)-23-methyl-5α-ergost-22-en-3β,5,6β,15α,25,26-hexaol 26-sulfate, in the fabms spectrum (negative-ion mode) showed a molecular anion peak at *m/z* 573 [MSO_3^-]. On solvolysis in a dioxane-pyridine mixture it afforded a desulfated derivative **7a**, fabms (negative-ion mode) *m/z* 493 $[M-H]^-$, 80 mass units shifted relative to **7**, thus suggesting the presence of a sulfate group in natural **7**. Analysis of the ^{1}H-nmr spectrum of **7** (Table 2) established the 3β,5α,6β,15α-hydroxylation pattern, already encountered in **4**, and also indicated the presence of a 25,26-dihydroxydinosterol side-chain. This structure was confirmed by sequential decoupling (chemical shifts and coupling constants are listed in Table 2), and NOEDS experiments [irradiation of the olefinic proton at δ_H 5.08 (d, *J*=9.0 Hz), produced an enhancement of the 24-H signal at δ_H 2.32 (q, *J*=7.0 Hz) and vice versa] established the *E* stereochemistry of the Δ^{22}-double bond. An upfield shift of the 26-methylene proton signals from δ_H 3.80 d–3.90 d (*J*=9.0 Hz) in **7** to δ 3.35 d–3.41 d (*J*=9.0 Hz), in the desulfated derivative **7a** (Table 4) clarified that C-26 bears the sulfate. The 25*S* configuration is based on the pattern of the 26-methylene proton signals, which appeared as two close doublets at δ 4.22 d–4.11 d (*J*=9.7 Hz) in the spectrum of the 3β,26-di-(−)-MTPA of **7a** and as two well-separated doublets at δ 4.28– δ 4.06 (*J*=9.7 Hz) in the spectrum of the 3β,26-di-(+)-MTPA ester of **7a**, as expected for the 25*S*-isomer (12). The configuration at C-24 is suggested as *S* by analogy with dinosterol (19).

Steroid **8**, (22*E*,24*R*,28*S*)-5α-stigmast-22-en-3β,5,6β,8,15α,28,29-heptaol 28-sulfate, in the fabms (negative-ion mode) showed a molecular anion peak at *m/z* 589 [MSO_3^-]. On solvolysis in a dioxane/pyridine mixture it afforded a desulfated derivative **8a**, fabms (negative-ion mode) *m/z* 509 $[M-H]^-$. A comparison of spectral data of **8** and **6** [Tables 2 and 3] makes it clear that steroid **8** possesses the same polyhydroxylation pattern in the tetracyclic nucleus as does **6**. A 2D-COSY nmr experiment allowed the determination of the C-20 to C-29 connectivities, thus indicating a 28,29-di-oxygenated side-chain, which is unique among the marine polyhydroxysteroids. An upfield shift of the 28-methine proton signal from δ_H 4.40, m, in **8** to δ_H 3.58 in the desulfated derivative **8a**, established that C-28 bears the sulfate. This consideration was further supported by the upfield shift of the C-28 signal from δ_C 82.1 in **8** to δ_C 73.6 in the desulfated **8a**. The absolute configuration of C-28 was assigned as *S* after the application of the Mosher method (10) to the 5α-stigmast-22*E*-ene-3β,5,6β,8,15α,28,29-heptaol 3,15,29-triacetate [**8f**], obtained from **8** by acetylation followed by solvolysis in dioxane/pyridine mixture to remove the sulfate group (Figure 1). The downfield shift to δ_H 2.23 of the H-24 signal and the upfield shift to δ_H 4.41 of one of the H_2-29 signals in the 28-(+)-MTPA ester, when compared with the shifts observed in the spectrum of the 28-(−)-MTPA spectrum (H-24: δ_H 1.99; H-29: δ_H 4.51), were suggestive of the 28*S* configuration by assuming the steroid side-chain as the higher [L_3] and the 29-acetoxy group as the lower [L_2] substituent. In order to determine the configuration at C-24 we took advantage of the recent stereoselective synthesis of (24*S*)- and (24*R*)-24-(hydroxymethyl)-3α,5-cyclo-6β-methoxy-5α-cholestanes, which can be differentiated directly by their ^{1}H-nmr spectra (18). Thus, the desulfated steroid **8a** was hydrogenated, followed by $NaIO_4$ oxidation of the 28,29-diol **8b** to give the 24-formyl derivative **8c**, which was eventually reduced with $NaBH_4$ to yield 5α-ergostane-3β,5,6β,8,15α,28-hexaol (**8d**, Figure 1). In the ^{1}H-nmr spectrum of **8d**, the C-28 methylene protons resonated as two well-separated double doublets at δ_H 3.46 and 3.55 and the 26- and 27-methyl protons appeared as two overlapping very close doublets at δ_H 0.94 as in the spectrum of the 24*S*- synthetic model [i.e., (24*S*)-6β-methoxy-24-methyl-3α,5-cyclo-5α-cholestane-28-ol] (18). In the ^{1}H-nmr spectrum of the 24*R*- synthetic model, the C-28 methylene protons resonated as a broad doublet at δ_H 3.52, whereas the 26- and 27-

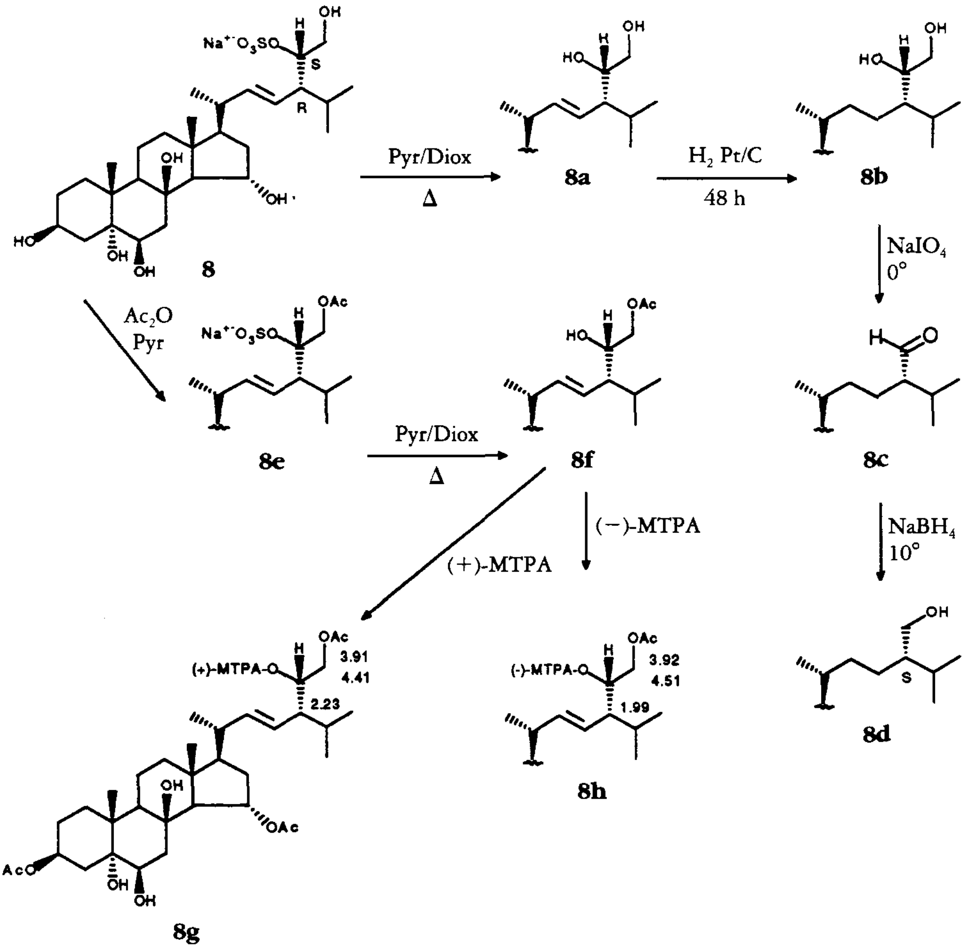

FIGURE 1. Determination of the Stereochemistry at C-24 and C-28 of the Steroid **8**.

methyl protons appeared as a triplet at δ_H 0.925 because of the coincidental overlap of the low-field arm of one doublet (δ_H 0.91) with the high-field arm of the other (δ_H 0.94) (17). Based on these data the 24*R*,28*S* configuration has been suggested for **8**. Note that the specification of the configuration at C-24 changes on going from the Δ^{22}-steroid **8** to the 22(23)-dihydro analog.

Steroid **9**, (22*Z*,24*S*,25*S*)-23-methyl-5α-ergost-22-en-3β,5,6β,15α,25,26,29-heptaol 26-sulfate, is related to the oxygenated dinosterol side-chain containing steroid **7** by the introduction of a further hydroxyl group at position 29. The fabms spectrum (negative-ion mode) showed a molecular anion peak at *m/z* 589 [MSO_3^-] sixteen mass units shifted relative to **7** (*m/z* 573) and in the ^{1}H-nmr spectrum (Table 2), the CH_3-29 singlet at δ_H 1.70 in **7** was replaced by two 1H doublets at δ 4.02 and 4.28 (J=11.2 Hz) coupled to each other, assigned to an allylic hydroxymethylene group. By analogy in the ^{13}C-nmr spectrum (Table 3) the methyl carbon signal at δ_C 15.3 in **7** was replaced by a methylene carbon signal at δ_C 73.3. Introduction of the hydroxyl group at C-29 also produced in the ^{1}H-nmr spectrum a downfield shift to δ_H 5.23 of the olefinic H-22 signal, which showed an nOe, in a NOEDS experiment, on irradiation of the H-24 proton signal (δ_H 2.52, q, J=7.0 Hz), and vice versa, thereby fixing the *Z* stereochemistry of the double bond. An upfield shift of the 26-methylene proton signals from δ_H 3.82 and 3.96 (each d,J=9.0 Hz) in **9** to δ_H 3.34 and 3.48 (each d,J=10.0 Hz) in the desulfated **9a** (Table 4), and a fabms (negative-ion mode) at m/z 509 $[M-H]^-$, confirmed the location of the sulfate at C-26. The stereochemistry at C-24 and C-25 is based on the same data and considerations used for **7**.

Steroid **10**, (22*E*,25*S*)-5α-cholest-22-en-3β,5,6β,15α,25,26-hexaol 26-sulfate, was obtained as a very minor component of the polar steroid mixture of *Styracaster caroli*, which structurally combines the tetracyclic nucleus of **4** with the side-chain of **2** sulfated at C-26. The fabms (negative-ion mode) showed a molecular anion peak at m/z 545 $[MSO_3^-]$. Upon solvolysis in a dioxane/pyridine mixture it afforded a desulfated derivative **10a** (Table 4); fabms (negative-ion mode) m/z 465 $[M-H]^-$. The comparison of the ^{1}H-nmr spectral data of **10a** and **2** made it clear that the two compounds are isomeric, differing in the stereochemistry at C-15 which in **10** is 15α instead of 15β. The major difference in the ^{1}H-nmr spectrum of **10a**, when compared with that of the isomeric **2**, was the splitting pattern of the H-15 proton at δ 3.87 appearing as a triple doublet with J=10.0 and 3.0 Hz [$J_{(H\text{-}14)/(H\text{-}15\beta)}$=10 Hz] and the upfield shift of H_3-18 to δ_H 0.78 s, supporting the 15α-stereochemistry. Again, an upfield shift of the 26-methylene proton signals from δ_H 3.85 (ABq,J=9.0 Hz) in **10** to δ_H 3.38 s, in **10a**, confirmed the location of the sulfate at C-26. The 25*S* configuration is suggested by analogy with the 25,26-dihydroxysteroids isolated from the same organism.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were obtained on the following instruments: Bruker WM-250 (^{1}H at 250 MHz, ^{13}C at 62.9 MHz) and Bruker AMX-500 (^{1}H at 500 MHz, ^{13}C at 125 MHz). Spectra were referred to CHD_2OD (proton signal at 3.34 ppm) and to $^{13}CD_3OD$ (central carbon signal at 49.0 ppm) and $CHCl_3$ (proton signal at 7.27 ppm) and to $^{13}CDCl_3$ (central carbon signal at 77.0 ppm). Mass spectra were recorded on a VG ZAB mass spectrometer equipped with FAB source [in glycerol or glycerol-thioglycerol (3:1) matrix; Xe atoms of energy 2–6 kV]. Optical rotations: Perkin Elmer model 241 polarimeter. Reversed-phase hplc, C_{18} μ-Bondapak column (30 cm×7.8 mm i.d., flow rate 5 ml/min), Waters Model 6000 A or 510 pump equipped with a U6K injector and a differential refractometer, model 401. Droplet counter-current chromatography (dccc): DCC-A apparatus manufactured by Tokyo Rikakikai Co., equipped with 250 tubes and Buchi apparatus equipped with 300 tubes.

EXTRACTION AND ISOLATION.—The animals, *Styracaster caroli*, 2.0 kg (fresh wt), were collected between Thio and Lifou (New Caledonia) at a depth of 2000 m during the Biogeocal oceanographic campaign and then identified by Dr. Catherine Vadon, Museum Nationale d'Histoire Naturelle, Paris, where a voucher specimen (EA282) is preserved. The animals were chopped and soaked in H_2O (2.0 liters, 4 h), the H_2O was decanted and the residual solid mass was then treated with 3 liters of Me_2CO (residue after concentration: 7.5 g). The aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (1 kg). This column was washed with distilled H_2O and eluted with MeOH to give, after removal of solvent, a partially purified steroid mixture (1.0 g). The residue from the Me_2CO extraction was partitioned between MeOH and *n*-hexane. The MeOH extracts (2.48 g) were combined with the steroid mixture coming from Amberlite XAD-2 and chromatographed on a column of Sephadex LH-60 (5×100 cm) with MeOH-H_2O (2:1) as eluent. Fractions (8 ml) were collected and monitored by tlc on SiO_2 with *n*-BuOH-AcOH-H_2O (12:3:5) and $CHCl_3$-MeOH-H_2O (80:18:2).

Fractions 50–59 (0.5 g) mainly contained very polar polyhydroxysteroids (**11–13**), fractions 60–65 (0.7 g) contained a crude mixture of sulfated steroids **5–10**, and fractions 70–118 (1.0 g) mainly contained the polyhydroxylated steroids **1–4**.

The last-eluting fractions (1.0 g) were submitted to droplet counter-current chromatography (dccc) using the solvent system $CHCl_3$-MeOH-H_2O (7:13:8) in the ascending mode (the lower phase was used as stationary phase, flow rate 20 ml/h). Fractions (5 ml) were collected, monitored by tlc on SiO_2 with $CHCl_3$-MeOH-H_2O (80:18:2), and accordingly combined. Each of the above fractions was then submitted to hplc with MeOH-H_2O (7:3) on a C_{18} μ-Bondapak column (30 cm×7.8 mm i.d.), to give pure compounds **1–4**.

Fractions 60–65 from the LH-60 column, containing sulfated compounds, were submitted to dccc using *n*-BuOH-Me_2CO-H_2O (3:1:5) in the ascending mode (the lower phase was used as stationary phase; flow rate 10 ml/h); 5 ml fractions were collected and monitored by tlc. The first-eluted fractions (87–150, 400 mg) contained a mixture of **5** and **6**, while fractions 161–165 (32.4 mg) contained **7**. Each fraction was purified by hplc with MeOH-H_2O (1:1) on a C_{18} μ-Bondapak column (30 cm×7.8 mm i.d.). Fractions 166–188 (38.4 mg) contained **8** along with minor compounds and were purified by hplc with MeOH-H_2O (45:55); the last-eluting fractions (189–220, 19.1 mg) contained a mixture of **9** and **10** and were purified by hplc with MeOH-H_2O (35:65) on a C_{18} μ-Bondapak column (30 cm×3.9 mm i.d., flow rate 2 ml/min), to give pure compounds.

The amounts and the physical (hplc mobility, [α]) and spectrometric (fabms) data for each compound are reported in Table 1.

The ^{1}H- and ^{13}C-nmr spectral data of the new compounds are given in Tables 2 and 3. Steroids **1** and **5** were identified by direct comparison (^{1}H- and ^{13}C-nmr data) with authentic samples.

MTPA ESTERS OF STEROID **1**.—Steroid **1** (2.0 mg) was treated with freshly distilled α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (4 μl), obtained from the *R*-(+)-acid, in dry pyridine (100 μl) for 1 h at room temperature to give, after removal of the solvent, the 3β,26-di-(+)-MTPA ester. ^{1}H nmr (CD_3OD) δ 0.93 (3H, s, H_3-18), 0.96 (6H, d, *J*=7 Hz, H_3-21 and H_3-27), 1.20 (3H, s, H_3-19), 3.76 (1H, dd, *J*=9.0 and 2.5 Hz, H-15β), 4.00 (1H, dd, *J*=7.5 and 2.5 Hz, H-16α), 4.21 (2H, d, *J*=5.5 Hz, H_2-26), 5.50 (1H, m, H-3α).

The 3β,26-di-(−)-MTPA ester of **1** was prepared using α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride obtained from the *S*-(−)-acid. The ^{1}H-nmr (CD_3OD) spectrum was identical with values reported for the (+)-MTPA ester except for the signals of H_2-26 at δ 4.12 (1H, dd, *J*=10.0 and 5.5 Hz) and 4.28 (1H, dd, *J*=10.0 and 5.5 Hz).

HYDROGENATION OF STEROID **2** TO PRODUCE **2a**.—Steroid **2** (10.0 mg) was hydrogenated at atmospheric pressure over 10% Pt/C in 3.5 ml of MeOH for 24 h. Removal of the catalyst by filtration and evaporation of the solvent gave the saturated compound **2a**. Compound **2a** was purified by hplc with MeOH-H_2O (7:3) on a C_{18} μ-Bondapak column (30 cm×3.9 mm i.d., flow rate 2 ml/min). Fabms (negative-ion mode) *m/z* 467 $[M-H]^-$. ^{1}H nmr (CD_3OD) δ 0.99 (3H, d, *J*=7 Hz, H_3-21), 1.00 (3H, s, H_3-18), 1.16 (3H, s, H_3-27), 1.23 (3H, s, H_3-19), 2.41 (1H, m, H-16β), 3.38 (2H, s, H_2-26), 4.04 (1H, m, H-3α), 4.21 (1H, t, *J*=5.5 Hz, H-15α).

MTPA ESTERS OF STEROID **2a**.—Steroid **2a** (2.0 mg) was treated with freshly distilled (+)-MTPA chloride (4 μl). The reaction was carried out as reported for steroid **1** to give the 3β,26-di-(+)-MTPA ester. ^{1}H nmr ($CDCl_3$) δ 0.92 (3H, d, *J*=7.0 Hz, H_3-21), 1.00 (3H, s, H_3-18), 1.20 (3H, s, H_3-27), 1.23 (3H, s, H_3-19), 4.27 and 4.11 (1H each, d, *J*=11.2 Hz, H_2-26), 4.22 (1H, t, *J*=5.5 Hz, H-15α), 5.45 (1H, m, H-3α). The 3β,26-di-(−)-MTPA ester of **2a** was prepared using (−)-MTPA chloride, and the ^{1}H-nmr ($CDCl_3$) spectrum was identical with that recorded for the (+)-MTPA ester except that the signals of H_2-26 resonated as a 2H singlet at δ 4.19.

HYDROGENATION OF STEROID **3**.—Steroid **3** (1.2 mg) was hydrogenated and purified as previously described for compound **2**. Fabms (negative-ion mode) *m/z* 481 $[M-H]^-$. ^{1}H nmr (CD_3OD) δ 0.90 (3H, d, *J*=7 Hz, H_3-28), 1.00 (3H, d, *J*=7.0 Hz, H_3-21), 1.01 (3H, s, H_3-18), 1.06 (3H, s, H_3-27), 1.24 (3H, s, H_3-19), 2.42 (1H, m, H-16β), 3.44 and 3.50 (1H each, d, *J*=12.2 Hz, H_2-26), 4.05 (1H, m, H-3α), 4.21 (1H, t, *J*=5.5 Hz, H-15α).

MTPA ESTERS OF STEROID **4**.—Steroid **4** (0.2 mg) was treated with freshly distilled (+)-MTPA chloride (4 μl). The reaction was carried out as reported for steroid **1** to give the 3β,26-di-(+)-MTPA ester. ^{1}H nmr (CD_3OD) δ 0.97 (3H, d, *J*=7.0 Hz, H_3-21), 4.27 and 4.13 (1H each, dd, *J*=11.0 and 6.0 Hz, H_2-26). The 3β,26-di-(−)-MTPA ester of **4** was prepared using (−)-MTPA chloride. The ^{1}H-nmr (CD_3OD) spectrum was identical with that recorded for the (+)-MTPA ester except for signals of H_2-26 resonating at δ 4.26 and 4.16 (each 1H, dd, *J*=11.0 and 6.0 Hz).

SOLVOLYSIS OF STEROIDS **6–10**.—A solution of **6** (5.5 mg) in 200 μl of pyridine-dioxane (1:1) was heated at 140° for 4 h in a stoppered reaction vial. The residue was purified by hplc with MeOH-H_2O (7:3) on a C_{18} μ-Bondapak column (30 cm×3.9 mm i.d., flow rate 2 ml/min), to give desulfated **6a** (5.0 mg). Fabms (negative-ion mode) *m/z* 479 $[M-H]^-$; ^{1}H nmr (CD_3OD), see Table 4.

Compounds **7** (2.5 mg), **8** (10.5 mg), **9** (0.4 mg), and **10** (0.3 mg) were solvolyzed in the same way to give, respectively, **7a** (2.0 mg), fabms *m/z* 493 $[M-H]^-$, **8a** (8.0 mg), fabms *m/z* 509 $[M-H]^-$, **9a** (0.3 mg), fabms *m/z* 509 $[M-H]^-$, **10a** (0.2 mg), fabms *m/z* 465 $[M-H]^-$. ^{1}H-nmr (CD_3OD) spectral data of all the desulfated compounds are reported in Table 4; ^{13}C nmr of **8a** (CD_3OD) δ 34.5 (C-1), 30.9 (C-2), 68.3 (C-3), 42.2 (C-4), 76.4 (C-5), 77.9 (C-6), 41.1 (C-7), 77.3 (C-8), 49.1 (C-9), 39.2 (C-10), 19.7 (C-11), 42.8 (C-12), 45.4 (C-13), 66.4 (C-14), 70.1 (C-15), 40.4 (C-16), 55.7 (C-17), 15.5 (C-18), 18.0 (C-19), 41.1 (C-20), 21.3 (C-21), 141.8 (C-22), 125.3 (C-23), 53.0 (C-24), 27.7 (C-25), 16.8 (C-26), 22.0 (C-27), 73.6 (C-28), 67.0 (C-29).

MTPA ESTERS OF THE STEROID **6a**.—Steroid **6a** (2.0 mg) was treated with freshly distilled (+)-MTPA chloride (4 μl). The reaction was carried out as reported for steroid **1** to give the 3β,28-di-(+)-MTPA ester. ^{1}H nmr (CD_3OD) δ 0.85 (3H, d, *J*=7.0 Hz, H_3-27), 0.94 (3H, d, *J*=7.0 Hz, H_3-26), 0.97 (3H, d, *J*=7.0 Hz, H_3-21), 4.21 (1H, dd, *J*=10.5 and 5.0 Hz, H-28), 4.39 (1H, dd, *J*=10.5 and 7.0 Hz, H'-28), 5.28 and 5.38 (1H each, dd, *J*=15.0 and 9.0 Hz, H-22 and H-23). The 3β,28-di-(−)-MTPA ester of **6a** was prepared using (−)-MTPA chloride. The ^{1}H-nmr (CD_3OD) spectrum was identical with that recorded for the (+)-MTPA ester except that the signals of H_2-26 resonated as two 1H double doublets at δ 4.31 (1H, dd, *J*=10.5 and 5.0 Hz, H-28), 4.33 (1H, dd, *J*=10.5 and 7.0 Hz, H'-28), and the signals H-22 and H-23 resonated as two 1H double doublets at δ 5.18 and 5.26 (each 1H, dd, *J*=15.0 and 9.0 Hz).

MTPA ESTERS OF STEROID **7a**.—Steroid **7a** (1.0 mg) was treated with freshly distilled (+)-MTPA chloride (2 μl). The reaction was carried out as reported for steroid **1** to give the 3β,26-di-(+)-MTPA ester. ^{1}H nmr (CD_3OD) δ 0.97 (3H, d, *J*=7.0 Hz, H_3-21), 1.02 (3H, d, *J*=7.0 Hz, H_3-28), 1.10 (3H, s, H_3-27), 1.66 (3H, s, H_3-29), 4.28 and 4.06 (1H each, d, *J*=9.7 Hz, H_2-26), 4.90 (1H, d, *J*=9.0 Hz, H-22). The 3β,26-di-(−)-MTPA ester of **7a** was prepared using (−)-MTPA chloride. ^{1}H nmr (CD_3OD) δ 0.91 (3H, d, *J*=7.0 Hz, H_3-21), 1.09 (3H, d, *J*=7 Hz, H_3-28), 1.10 (3H, s, H_3-27), 1.63 (3H, s, H_3-29), 4.22 and 4.11 (1H each, d, *J*=9.7 Hz, H_2-26), 4.80 (1H, d, *J*=9.0 Hz, H-22).

ACETYLATION AND SOLVOLYSIS OF STEROID **8** TO PRODUCE **8f**.—To a solution of **8** (5.0 mg) in 200 μl of dry pyridine was added 20 μl of Ac_2O. After 10 h at room temperature the solvent was removed to give 4.0 mg of the 3β,15α,29-acetylated product, **8e**, fabms (negative-ion mode) *m/z* 715 $[M]^-$. ^{1}H nmr (CD_3OD) δ 0.89 (3H, d, *J*=7 Hz, H_3-26), 0.90 (3H, d, *J*=7 Hz, H_3-27), 1.04 (3H, s, H_3-18), 1.05 (3H, d, *J*=7 Hz, H_3-21), 1.34 (3H, s, H_3-19), 2.03 (6H, s, $CH_3C{=}O$), 2.06 (3H, s, $CH_3C{=}O$), 3.60 (1H, t, *J*=2.5 Hz, H-6α), 3.99 (1H, dd, *J*=11.2 and 3.0 Hz, H-29), 4.51 (1H, m, H-28), 4.53 (1H, dd, *J*=11.2 and 2.0 Hz, H'-29), 5.14–5.29 (1H each, dd, *J*=14.0 and 8.0 Hz, H-22 and H-23), 5.20 (1H, td, *J*=10.0 and 3.0 Hz, H-15β), 5.25 (1H, m, H-3α). A solution of the acetylated product (4.0 mg) in 200 μl of pyridine-dioxane (1:1) was heated at 140° for 4 h in a stoppered reaction vial. The residue was purified by hplc with MeOH-H_2O (9:1) on a C_{18} μ-Bondapak column (30 cm×3.9 mm i.d., flow rate 2 ml/min), to give the desulfated **8f** (2.5 mg); fabms *m/z* 635 $[M-H]^-$. ^{1}H nmr (CD_3OD) δ 0.86 (3H, d, *J*=7 Hz, H_3-26), 0.88 (3H, d, *J*=7 Hz, H_3-27), 1.00 (3H, s, H_3-18), 1.01 (3H, d, *J*=7 Hz, H_3-21), 1.34 (3H, s, H_3-19), 2.01 (3H, s, $CH_3C{=}O$), 2.02 (3H, s, $CH_3C{=}O$), 2.07 (3H, s, $CH_3C{=}O$), 3.60 (1H, t, *J*=2.5 Hz, H-6α), 3.73 (1H, m, H-28), 3.84 (1H, dd, *J*=11.2 and 3.0 Hz, H-29), 4.15 (1H, dd, *J*=11.2 and 2.0 Hz, H'-29), 5.12–5.25 (1H each, dd, *J*=14.0 and 8.0 Hz, H-22 and H-23), 5.13 (1H, td, *J*=10.0 and 3.0 Hz, H-15β), 5.27 (1H, m, H-3α).

MTPA ESTERS OF STEROID **8f**.—Steroid **8f** (1.0 mg) was treated with freshly distilled (+)-MTPA chloride (2μl) to give **8g**. The reaction was carried out as reported for steroid **1** to give the 28-(+)-MTPA ester. ^{1}H nmr (CD_3OD) δ 0.85 (3H, d, *J*=7 Hz, H_3-26), 0.90 (3H, d, *J*=7 Hz, H_3-27), 0.95 (3H, d, *J*=7 Hz, H_3-21), 1.01 (3H, s, H_3-18), 1.33 (3H, s, H_3-19), 1.97 (3H, s, $CH_3C{=}O$), 2.02 (6H, s, $CH_3C{=}O$), 2.23 (1H, m, H-24), 3.91 (1H, dd, *J*=11.2 and 3.0 Hz, H-29), 4.41 (1H, dd, *J*=11.2 and 2.0 Hz, H'-29), 5.12–5.25 (1H each, dd, *J*=14.0 and 8.0 Hz, H-22 and H-23), 5.13 (1H, td, *J*=10.0 and 3.0 Hz, H-15β), 5.27 (1H, m, H-3α), 5.40 (1H, m, H-28). The 28-(−)-MTPA ester of **8f**, **8h** was prepared using (−)-MTPA chloride. The ^{1}H-nmr (CD_3OD) spectrum was identical with that recorded for the (+)-MTPA ester except for the signals: δ 0.78, 0.87, and 0.93 (3H each, d, *J*=7 Hz, H_3-26, -27, and -21), 1.00 (3H, s, H_3-18), 1.32 (3H, s, H_3-19) and H_2-29 resonating as two 1H double doublets at δ 3.92 (1H, dd, *J*=11.2 and 3.0 Hz, H-29), 4.51 (1H, dd, *J*=11.2 and 2.0 Hz, H'-29), and the signal for H-24 resonating as multiplet at δ 1.99 (Figure 1).

HYDROGENATION OF STEROID **8a**.—Steroid **8a** (7.0 mg) was hydrogenated and purified as previously described for compound **2** to give **8b**, fabms *m/z* 511 $[M-H]^-$. The ^{1}H-nmr (CD_3OD) spectrum was identical with values reported for **8a** (Table 2) except for the signals: δ 0.92 (3H, d, *J*=7 Hz, H_3-27), 0.96 (6H, d, *J*=7.0 Hz, H_3-21 and -26), 0.98 (3H, s, H_3-18), 3.59 (1H, m, H-28), 3.46 (1H, dd, *J*=11.0 and 5.5 Hz, H-29), 3.66 (1H, dd, *J*=11.0 and 2.5 Hz, H'-29), 4.30 (1H, td, *J*=10.0 and 3.0 Hz, H-15β).

PERIODATE OXIDATION OF STEROID **8b**.—To a solution of **8b** (1.0 mg) [solvent MeOH-H_2O (1:1), 300 μl], 0.5 mg of $NaIO_4$ were added. The reaction was cooled at 0° for 1 h, then, after removal of the excess of MeOH, 200 μl of H_2O were added and the precipitation of **8c** (0.6 mg) was observed. Fabms *m/z* 479 $[M-H]^-$. ^{1}H nmr (CD_3OD) δ 0.93 (3H, d, *J*=7 Hz, H_3-26), 0.97 (3H, s, H_3-18), 0.98 (6H, d, *J*=7 Hz, H_3-21 and H_3-27), 1.32 (3H, s, H_3-19), 3.62 (1H, t, *J*=2.5 Hz, H-6α), 4.11 (1H, m, H-3α), 4.29 (1H, td, *J*=10.0 and 3.0 Hz, H-15β), 9.60 (1H, d, *J*=3.0 Hz, H-28).

REDUCTION OF STEROID **8c** TO GIVE **8d**.—To a solution of **8c** (0.5 mg, solvent MeOH, 100 μl) 0.1 mg of $NaBH_4$ was added. The reaction was cooled at 0° for 1 h, then 20 μl of 2 N HCl were added. When the reaction was concentrated to eliminate excess of MeOH, the product **8d** (0.2 mg) precipitated. Fabms (negative-ion mode) *m/z* 481 [M−H]$^-$. ^{1}H nmr (CD_3OD) δ 0.94 (6H, d, *J*=7 Hz, H_3-26 and H_3-27), 0.97 (3H, d, *J*=7 Hz, H_3-21), 0.98 (3H, s, H_3-18), 1.32 (3H, s, H_3-19), 3.46–3.55 (1H each, dd, *J*=10.0 and 6.5 Hz, H_2-28), 3.62 (1H, t, *J*=2.5 Hz, H-6α), 4.11 (1H, m, H-3α), 4.30 (1H, td, *J*=10.0 and 3.0 Hz, H-15β).

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