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STARFISH SAPONINS, PART 41.[1] STRUCTURE OF TWO NEW STEROIDAL GLYCOSIDE SULFATES (MINIATOSIDES A AND B) AND TWO NEW POLYHYDROXYSTEROIDS FROM THE STARFISH *PATIRIA MINIATA*

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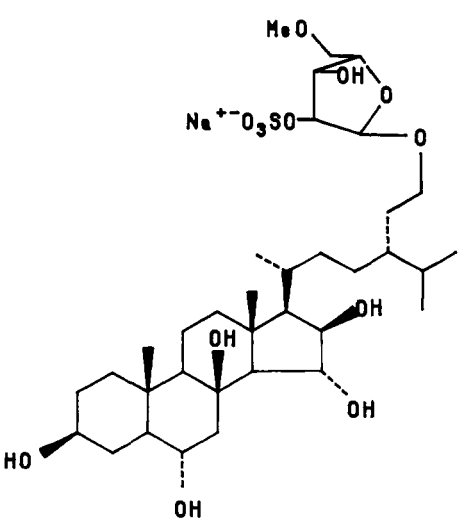
ABSTRACT.—Two new sulfated steroidal glycosides, miniatoside A [**1**] and miniatoside B [**2**], have been isolated from the H_2O extracts of the whole bodies of the starfish *Patiria miniata*. They co-occur with two known sulfated steroidal monoglycosides **6** and **7** and seven polyhydroxysteroids, among which two are new (**3** and **5**). The structures of the new compounds were determined as (24*R*)-29-*O*-(5′-*O*-methyl-2′-*O*-sulfate-α-L-arabinofuranosyl)-24-ethyl-5α-cholestane-3β,6α,8,15α,16β,29-hexaol (miniatoside A [**1**]), (24*S*)-24-*O*-[β-D-xylopyranosyl-(1→2)-3′-*O*-sulfate-α-L-arabinofuranosyl]-5α-cholestane-3β,6α,8,15α,24-pentaol (miniatoside B [**2**]), (22*E*,24*R*,25*R*)-24-methyl-5α-cholest-22-ene-3β,4β,6α,7α,8,15β,16β,25,26-nonaol 6-sulfate [**3**], and (25*S*)-24-methyl-5α-cholest-24(28)-en-3β,4β,6α,7α,8,15β,16β,26-octaol [**5**].

In the preceding paper (1) we reported the occurrence of two steroidal hexaglycoside sulfates, patiriosides A and B, along with two known asterosaponins, pectinioside G (2) and acanthaglycoside C (3), from the starfish *Patiria miniata* Brandt (Asterinidae). We now describe the isolation and structure determination of two new steroid oligoglycoside sulfates, miniatosides A [**1**] and B [**2**], which co-occur with two known steroid monoglycoside sulfates **6** and **7**, previously isolated from *Patiria pectinifera* (4) and *Oreaster reticulatus* (5), and seven polyhydroxysteroids, among which two (**3** and **5**) are new compounds.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—For instruments used, see Riccio *et al.* (6).

EXTRACTION AND ISOLATION.—The animals, *Pa. miniata*, were collected off the Gulf of California,



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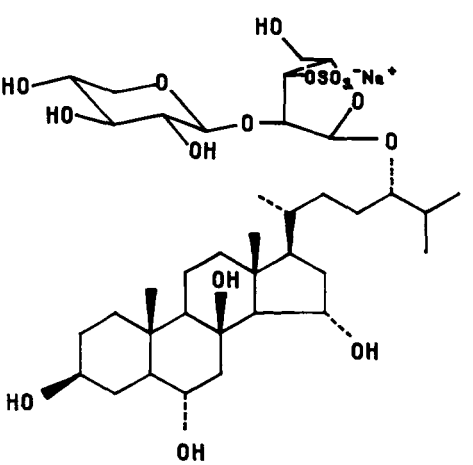

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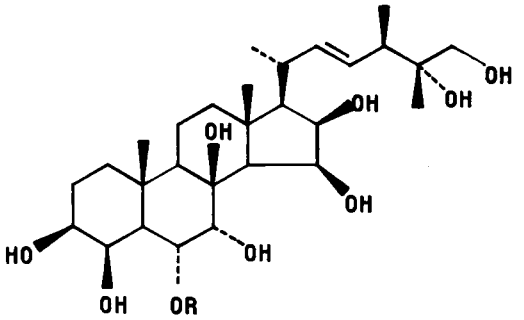
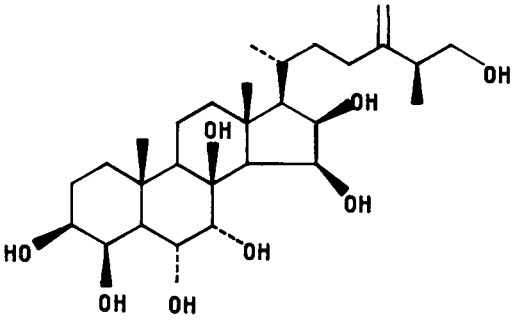
[1]For Part 40, see D'Auria *et al.* (1).

[2]On leave from the Departamento de Quimica Organica, Santiago de Compostela, Spain.



3 $R=SO_3^-Na^+$
4 R=H



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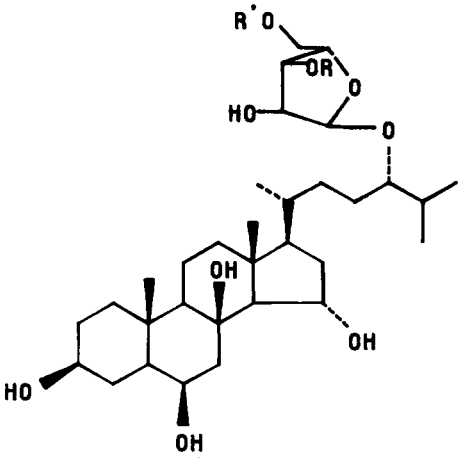

6 $R=Me$, $R'=SO_3^-Na^+$
7 $R=SO_3^-Na^+$, R'=H

in November 1985, and frozen (3.5 kg); identification was done by the zoologist of the Scripps Institution of Oceanography, La Jolla, California, and a voucher specimen is preserved at the Dipartimento di Chimica delle Sostanze Naturali, University of Naples. The animals were chopped and soaked in H_2O (2 × 2 liters). The aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (1 kg). This column was washed with distilled H_2O (1 liter) and eluted with MeOH (2 liters). The MeOH eluate was taken to dryness to give a glassy material (3 g) which was then chromatographed on a column of Sephadex LH-60 (4 × 80 cm) with MeOH-H_2O (2:1) as eluent. Fractions (8 ml) wre collected and analyzed by tlc on SiO_2 with *n*-BuOH–HOAc–H_2O (12:3:5).

Fractions 56–79 (0.9 g) mainly contained the asterosaponins (1), while fractions 85–96 (0.7 g) and 97–123 (0.4 g) mainly contained steroid mono- and diglycoside sulfates and polyhydroxysteroids, respec-

tively. Fractions 85–96 were submitted to dccc using *n*-BuOH–Me_2CO–H_2O (3:1:5) (ascending mode: the lower phase was the stationary phase, flow rate 15 ml/h; 5-ml fractions were collected and monitored by tlc). The steroid glycoside sulfates **1**, **2**, **6**, and **7** and the sulfated polyhydroxysteroid **3** were spread out among fractions 67–145, which were combined into five major fractions after tlc analysis and submitted to hplc on a C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.) with MeOH-H_2O (1:1) to give pure compounds. Similarly the polyhydroxysteroid-containing fractions (97–123) as eluted from the Sephadex LH-60 column were submitted to dccc using $CHCl_3$-MeOH-H_2O (7:13:8) (ascending mode; the lower phase was used as the stationary phase, flow rate 10 ml/h; 5-ml fractions were collected and monitored by tlc). Polyhydroxysteroids were spread out among fractions 40–95, which were combined into three major fractions after tlc analysis and submitted to hplc on a C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.) with MeOH-H_2O (7:3) to give pure compounds. The amounts of each isolated compound are in Table 1. The known compounds were identified based on direct comparison (fabms, ^{1}H nmr, and hplc) with authentic samples. The ^{1}H- and ^{13}C-nmr data for the new compounds are in Tables 2 and 3; rotations and fabms data are reported in the text.

SOLVOLYSIS OF COMPOUNDS **1**, **2**, AND **3**.—A solution of **1** (1 mg) in pyridine (0.1 ml) and dioxane (0.1 ml) was heated at 140° for 24 h in a stoppered reaction vial. After the solution was cooled, H_2O (1 ml) was added, and the solution was extracted with *n*-BuOH (2 × 1 ml). The combined extracts were evaporated to dryness under reduced pressure. The residue was purified by hplc [C_{18} μ-Bondapak (30 cm × 3.8 mm i.d.), MeOH-H_2O (7:3)] to give a desulfated material, fabms (negative ion) *m/z* 641 $[M-H]^-$. Solutions of **2** (2 mg) and **3** (2 mg) were similarly solvolyzed affording the corresponding desulfated derivatives with fabms (negative ion) *m/z* 715 $[M-H]^-$ (100%), 583 $[M-H-132]^-$ (22%), and *m/z* 527 $[M-H]^-$.

METHANOLYSIS OF MINIATOSIDE B [**2**].—*Sugar analysis*: A solution of miniatoside B [**2**] (0.5 mg) in anydrous 2 M HCl/MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 8 h. After having cooled, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2. The residue was trimethylsilylated with trisil Z (Pierce Chemical) for 15 min at room temperature. Glc analysis (25 m, SE-30 capillary column, 132°; helium carrier flow 5 ml/min^{-1}) gave peaks which co-eluted with those of the methyl xyloside and methyl arabinoside standards.

3,26-DI-(*R*)-(+)-MTPA ESTER AND 3,26-DI-(*S*)-(−)-MTPA ESTER OF **5**.—The steroid **5** (1.5 mg) was treated with freshly distilled (+)-methoxytrifluoromethylphenyl acetyl chloride (2 μl) in dry pyridine (0.2 ml) for 1 h at room temperature. After removal of solvent, the product was analyzed by ^{1}H nmr: δ

TABLE 1. Asterosaponins, Monoglycosides, Diglycosides, and Polyhydroxysteroids from the Starfish *Patiria miniata* (3.5 kg fresh wt).

Compound	Amount (mg)	Reference
Asterosaponins		
Patirioside A	10	D'Auria *et al.* (1)
Patirioside B	7	D'Auria *et al.* (1)
Pectinioside G	18	Iorizzi *et al.* (2)
Acanthaglycoside C	39	Itakura and Komori (3)
Monoglycosides and Diglycosides		
Asterosaponin P-1 [**6**]	10	Kicha *et al.* (4), Segura de Correa *et al.* (5)
Compound **7**	2	Segura de Correa *et al.* (5)
Miniatoside A [**1**] (new)	2	
Miniatoside B [**2**] (new)	6	
Polyhydroxysteroids		
Compound **3** (new)	9	
Compound **5** (new)	5	
(25*S*)-5α-Cholestane-3β,6α,8,15α,16β,26-hexaol	4	Riccio *et al.* (7)
(25*S*)-5α-Cholestane-3β,6α,7α,8,15α,16β,26-heptaol	30	Riccio *et al.* (7)
(25*S*)-5α-Cholestane-3β,4β,6α,8,15α,16β,26-heptaol	2.5	Minale *et al.* (8)
(25*S*)-5α-Cholestane-3β,4β,6α,7α,8,15α,16β,26-octaol	200	Riccio *et al.* (7)
(25*S*)-5α-Cholestane-3β,4β,6α,7α,8,15β,16β,26-octaol	18	Iorizzi *et al.* (2), Higuchi *et al.* (9)

TABLE 2. Selected ^{1}H-nmr Signals[a] (δH in CD_3OD) for Compounds **1–5**. *J* (Hz) Values are Shown in Parentheses.

Proton	Compound				
	1	Ref[b]	**2**	**3**[c]	**5**
H-3	3.50		3.53 m	3.53 m	3.48 m
H-4	—		—	4.26 b (W½ 9 Hz)	4.22 br (W½ 9 Hz)
H-5	—		—	1.76 dd (11.5,2.5)	1.53 dd (11.5,2.5)
H-6	3.65 dt (3.5,9)		3.65 dd (3.5,9)	5.09 dd (11.5,2.5)	4.32 dd (11.5,2.5)
H-7	2.44 dd (12.5,3.5)[d]		2.42 dd (12.5,3.5)[d]	4.30 d (3)	3.98 d (2.5)
H-14	—		—	1.45 d (5.5)	1.44 d (5.5)
H-15	4.08 dd (11,2.5)		4.23 dt (3.5,10)	4.50 dd (7.0,5.5)	4.52 dd (7.0,5.5)
H-16	4.01 dd (8,2.5)		—	4.14 t (7.0)	4.26 t (7.0)
H-18	1.14 s		1.00 s	1.30 s	1.27 s
H-19	1.05 s		1.05 s	1.27 s	1.19 s
H-21	0.96 d (7)		0.93 d (7)	1.07 d (7)	1.00 d (7)
H-26	0.88 d (7)		0.93 d (7)	3.42–3.50 (each d, 11)	1.09 d (7)
H-27	0.90 d (7)		0.93 d (7)	1.10 s	1.09 d (7)
H-28	—		—	1.01 d (7)	4.87 bs–4.79 bs
H-29	3.76 m		—	—	—
H-1′	5.17 bs	4.95 d (1.5)	5.18 bs		
H-2′	4.59 bd (3)	4.04 m	4.39 bd (3)		
H-3′	4.04 dd (3,6)	3.87 dd (6.5,3.5)	4.65 dd (5.8,3)		
H-4′	4.06 m	4.04 m	4.19 m		
H-5′	3.63 dd (12,3.5)	3.75 dd (12.5,3.5)	3.88 dd (12.5,3.5)		
	3.58 dd (12,5.8)	3.68 dd (12.5,5)	3.78 dd (12.5,5)		
OMe	3.42 s				
H-1″			4.53 d (7.5)		
H-2″			3.18 dd (10,7.5)		
H-3″			3.35 t (10)		
H-4″			3.51 m		
H-5″			3.85 dd (11,5)		
			3.23 dd (11,9)		

[a]Spectra of **1** and **2** were taken at 500 MHz, those of **3** and **5** at 250 MHz.
[b]A steroidal α-L-arabinofuranoside has been used as reference compound (5).
[c]Other signal centered at δ 5.54 (olefinic's).
[d]This signal correspond to 7β-H; the 7α-H signals is confused in the high field region of the spectrum.

(CD_3OD) 0.99 (3H, d, *J* = 7 Hz, H_3-21), 1.12 (3H, d, *J* = 7 Hz, H_3-27), 1.20 (3H, s, H_3-19), 1.29 (3H, s, H_3-18), 2.62 (1H, m, H-25), 4.00 (1H, d, *J* = 2.5 Hz, H-7), 4.26 and 4.36 (each dd, *J* = 11, 7 Hz, H_2-26), 4.23 (m, partially overlapped with H-26, H-16), 4.40 (m, H-4), 4.52 (dd, *J* = 7.0, 5.5, H-15), 4.82–4.90 (each 1H, s, $=CH_2$), 4.92 (m, H-3).

(−)-MTPA ester of **5** was prepared by using (−)-methoxytrifluoromethylphenyl acetyl chloride; ^{1}H-nmr results were very similar to those of the (+)-MTPA ester except the signals for 26-CH_2 which appeared as two dd at δ 4.20 and 4.41 ppm.

RESULTS AND DISCUSSION

Separation and isolation of the individual compounds from the aqueous extracts of the animals followed the steps described previously (1,6). The results of our analysis are summarized in Table 1. The identification of the known compounds was based on direct comparison (^{1}H nmr, fabms, and hplc) with authentic samples.

MINIATOSIDE A [**1**].—*Compound* **1**: [α]D + 19.0° (*c* = 0.5, MeOH). The fabms (negative ion mode) showed a molecular anion peak at *m/z* 721. On solvolysis in a dioxane-pyridine mixture (10) it afforded a desulfated derivative: fabms (negative ion mode) *m/z* 641 $[M-H]^-$. Examination of the spectral data (Tables 2 and 3) and comparison with those of the many polyhydroxysteroids and glycosides of polyhydroxysteroids isolated in our laboratory indicated that **1** contains a 24-(β-hydroxyethyl)-3β, 6α,8,15α,16β-pentahydroxycholestane aglycone. The 3β,6α,8,15α,16β-hydroxylation pattern was encountered before in the (25*S*)-5α-cholestane-3β,6α,8,15α,16β,26-hexaol, first isolated from the starfish *Protoreaster nodosus* (7), while a (24*R*)-24-ethyl-5α-cholestane-3β-6α,8,15α,16β,29-hexaol has been isolated as 29-sulfate derivative from

TABLE 3. ^{13}C-nmr Shifts (62.9 MHz, CD_3OD) of Compounds **1–3** (in ppm).[a]

Carbon	Compound			
	1	**2**	**3**	methylglycosides[b]
C-1	39.6	39.7	39.5	
C-2	31.5	31.5	26.7	
C-3	72.2	72.2	73.2	
C-4	32.4	32.4	69.3	
C-5	53.7	53.7	46.8	
C-6	67.7	67.7	76.6	
C-7	49.0	50.0	75.4	
C-8	76.0	76.1	79.2	
C-9	57.4	57.4	51.3	
C-10	37.9	37.9	38.4	
C-11	19.4	19.6	18.6	
C-12	43.2	43.6	43.0	
C-13	45.3	45.6	44.4	
C-14	64.5	67.2	55.2	
C-15	80.8	69.9	71.3	
C-16	83.0	41.8	73.0	
C-17	60.6	56.1	63.5	
C-18	17.0	15.4	17.9	
C-19	14.2	14.2	15.9	
C-20	31.2	36.2	35.2	
C-21	18.5	19.0	20.6	
C-22	35.1	33.0	138.9	
C-23	28.9	28.8	131.3	
C-24	42.6	84.5	44.9	
C-25	31.0	31.6	75.7	
C-26	19.3	18.4	69.2	
C-27	19.9	18.1	21.2	
C-28	31.9	—	16.8	
C-29	67.9	—	—	
C-1′	107.8	107.9		109.2
C-2′	89.1	90.3		81.8
C-3′	78.4	84.1		77.5 (F)ara(α-L)
C-4′	83.5	83.4		84.9
C-5′	74.0	62.8		62.4
OMe	59.4			
C-1″		104.9		105.1
C-2″		75.2		74.0
C-3″		77.8		76.9 (P)xyl(β-D)
C-4″		71.2		70.4
C-5″		67.0		66.3

[a]Assignments were aided by DEPT measurements; the shifts of the reference methylglycosides are from spectra recorded for solutions in D_2O.

[b]Bock and Pedersen (16).

the starfish *Poraster superbus* (11). Furthermore 24-(β-hydroxyethyl)-cholestane aglycones were already found in (24*R*)-29-*O*-glycosides isolated from the starfishes *Hacelia attenuata* (12), *Halityle regularis* (13), *Gomophia watsoni* (14), and *Pa. pectinifera* (15). Examination of the ^{13}C-nmr spectrum of **1** also clarified that the sugar moiety is attached at C-29 of the aglycone. In addition to the aglycone moiety, the ^{13}C nmr assisted by DEPT measurements showed one methyl carbon signal at 59.4 ppm (-OMe), one methylene carbon at 74.0 ppm, and four methine carbons at 107.8, 89.1, 83.5, and 78.4 ppm, indicative of a methylated sulfated pentose moiety. Comparison of the above

data with those reported for methyl pentafuranosides (16) suggested a 5′-*O*-methyl-2′-*O*-sulfated-arabinofuranoside as the most probable structure for the sugar moiety of **1**, an hypothesis which received sound support from analysis of the ^{1}H-nmr spectrum at 500 MHz with double resonance experiments. The spectrum showed four methine protons at δ 5.17 (broad s, H-1′), 4.59 (d, J = 3 Hz, H-2′), 4.04 (m, H-3′), and 4.06 (m, H-4′); the last methine proton is further coupled to methylene protons at δ 3.64 (dd, J = 12, 3.5 Hz) and 3.58 (dd, J = 12 and 5.8 Hz). The coupling constants of the methine signals indicated a furanosyl structure; the value of the chemical shift for H-4′ (δ 4.06) was diagnostic for an arabinose structure [in a xylofuranose structure H-4 is expected to resonate at lower field, owing to the 1,3-syn interaction with the $OSO_3^-Na^+$ group at C-2; for example in a β-xylofuranoside the signal for H-4 was found at δ 4.27 ppm (17)]; the downfield shift of H-2′ indicated that C-2′ bears the sulfate. Thus we propose for miniatoside A the (24*R*)-29-*O*-(5′-*O*-methyl-2′-*O*-sulfate-α-L-arabinofuranosyl)-24-ethyl-5α-cholestane-3β,6α,8,15α,16β,29-hexaol structure [**1**]. The L configuration of arabinose is preferred because L-arabinose occurs in many steroid oligoglycosides isolated from starfishes (12,18,19).

MINIATOSIDE B [**2**].—*Compound* **2**: [α]D −12.3° (c = 0.5, MeOH). Examination of its spectral data (^{1}H and ^{13}C nmr, Tables 2 and 3) immediately indicated that **2** contains a 5α-cholestane-3β,6α,8,15α,24-pentaol aglycone, already found as the aglycone of asterosaponin P-1 [**6**] and of several other glycosides [e.g., **7**, isolated from *Pa. pectinifera* (4) and *O. reticulatus* (5)] and more recently isolated as free sterol from *Asterina pectinifera* (9). Acid methanolysis liberated methyl xylosides and methyl arabinosides. The ^{13}C-nmr spectrum indicated the location of the sugar moiety at C-24. In addition to the aglycone proton signals, the 500 MHz ^{1}H-nmr spectrum of **2** showed signals for a β-xylopyranosyl unit (Table 2) and four methine signals at δ 5.18 (broad s), 4.39 (d, J = 3 Hz), 4.66 (dd, 5.8, 3 Hz), and 4.19 (m), which couple with their neighbors in this order. The last methine proton is further coupled to methylene protons at δ 3.88 (dd, J = 12.5, 3 Hz) and 3.78 (dd, J = 12.5, 5 Hz). The coupling constants of the methine signals indicated a furanosyl structure, and the large downfield shift observed for H-3′ [δ 4.66 vs. 3.87 in α-arabinofuranosides (5)], accompanied by smaller shifts observed for the vicinal protons at C-2′ and C-4′ (δ 4.39 and 4.19 vs. 4.04 and 4.04 in α-arabinofuranosides) suggested the presence of a sulfate group at C-3′. The fabms (negative ion mode) showed a molecular anion peak at *m/z* 795 and a major fragment at *m/z* 663 corresponding to the loss of the xylosyl residue, thus indicating the sequence T xylose-arabinose(3′-*O*-sulfated). On solvolysis in dioxane-pyridine mixture, **2** afforded a less polar derivative, which showed in the fabms (negative ion mode) a quasi molecular ion at *m/z* 715, corresponding to a desulfated derivative of **2**. Finally, an analysis of the ^{13}C-nmr data of **2** based upon comparison of appropriate methyl glycosides (16), the known glycosidation (20–22), and sulfation shifts (23,24) and assignments reported for similar glycosides (4,5,9) established the β-xylopyranosyl residue to be attached at C-2′ of the (3′-*O*-sulfated)-arabinose unit and confirmed the structure for miniatoside B as (24*S*)-24-*O*-[β-D-xylopyranosyl-(1→2)-3′-*O*-sulfate-α-L-arabinofuranosyl]-5α-cholestane-3β,6α,8,15α,24-pentaol [**2**]. We prefer the D-configuration of xylose by analogy with other steroid D-xylosides isolated from starfishes; the L configuration of arabinose is based on the same considerations as before.

(22*E*,24*R*,25*R*)-24-METHYL-5α-CHOLEST-22-ENE-3β,4β,6α,7α,8,15β,16β,25,26-NONAOL 6-SULFATE [**3**].—*Compound* **3**: [α]D +22.9° (c = 0.5, MeOH). The fabms (negative ion mode) gave a molecular anion peak at *m/z* 607; on solvolysis in dioxane/pyridine it gave a less polar compound **4**, fabms (negative ion mode) *m/z* 527, corresponding to the desulfated derivative. Examination of the ^{1}H-nmr spectrum of the de-

sulfated material and comparison with those of the many polyhydroxysteroids isolated in this laboratory indicated that **4** contains a 3β,4β,6α,7α,8,15β,16β-heptahydroxy-tetracyclic steroid nucleus, already found in (25*S*)-5α-cholestane-3β,4β,6α,7α,8,15β,16β,26-hexaol recently isolated from *As. pectinifera* (2,9), and a Δ^{22E},24-methyl-25,26-dihydroxy side chain, which has been found in a highly hydroxylated steroid, i.e., (22*E*,24*R*,25*R*)-24-methyl-5α-cholest-22-ene-3β,4β,5,6α,8,14,15α,25,26-nonaol, isolated from the starfish *Archaster typicus* (25). The 22*E*,24*R*,25*R* configuration was assigned to the steroid from *Ar. typicus* by comparison of its spectral data with those of model compounds (25); in the steroid **3**, now isolated from *Pa. miniata*, the signals for C-26, C-27, and C-28 protons are identical with those assigned to the previous steroid. Thus we presume that the two polyhydroxysteroids have the same stereochemistry for the asymmetric carbons in the side chain. Upfield shifts of H-6 from δ 5.09 (1H, dd, J = 12 and 2.5 Hz) in **3** to δ 4.32 (1H, dd, J = 12 and 2.5 Hz) in **4** indicated that C-6 bears the sulfate in **3**. The ^{13}C-nmr spectrum (Table 3) and comparison with the reference steroids confirmed the proposed structure **3**.

(25*S*)-24-METHYL-5α-CHOLEST-24(28)-EN-3β,4β,6α,7α,8,15β,16β,26-OCTAOL [**5**].—*Compound* **5**: [α]D +8.50; fabms (positive ion mode) *m/z* 643 [M + Na + tioglycerol]$^+$ and 621 [M + H + tioglycerol]$^+$. Examination of its ^{1}H-nmr spectrum (Table 2) immediately indicated the presence of the same 3β,4β,6α,7α,8,15β,16β-hydroxylation pattern as in **4** and of a 24-methylene-26-hydroxy side chain [olefinic signals at δ 4.79 and 4.87 (broad s) and signals for H-25, H_2-26, and H_3-27 shifted to δ 2.33 (1H, sextet), 3.61 (1H, dd, J = 10, 6 Hz), 3.39 (1H, dd, J = 10, 7 Hz) and 1.09 (3H, d, J = 7 Hz), respectively]. 24-Methylene-26-hydroxysteroids have been recently isolated from *Po. superbus* (11), *Sphaerodiscus placenta* (26), and *Thromidia catalai* (27). We now suggest the 25*S* configuration for **5** based on the following observations.

During our current work on polyhydroxysteroids from echinoderms we have isolated a series of 26-hydroxysteroids and have applied an extension of the Mosher's method for determining the configuration at C-25, consisting of the use of a shift reagent with (+)-(*R*) and (−)-(*S*)-MTPA [MTPA = α-methoxy-α-(trifluoromethyl)-phenylacetic acid; Mosher's reagent (28)] esters in ^{1}H nmr (29). We noted that the 26-methylene protons appeared in the ^{1}H-nmr spectrum of the (25*S*)-26-hydroxysteroids esterified with (+)-(*R*)-MTPA as a doublet at δ 4.16 (30), while in the spectrum of the (25*R*) isomer they appeared as two well separated double doublets at δ 4.08 and 4.24 ppm [e.g., pavonins (31)]. The reverse happened with the (−)-(*S*)-MTPA esters. The same tendency has been observed with a series of 24-alkyl-26-hydroxysteroids; i.e., in (+)-(*R*)-MTPA ester of the (25*S*) isomers the 26-methylene proton signals are always observed closer than in (+)-(*R*)-MTPA ester of the 25*R* isomers.[3] Thus we prepared the (+)-(*R*)-MTPA and the (−)-(*S*)-MTPA esters of **5** and recorded their ^{1}H-nmr spectra. The methylene proton signals of the (+)-(*R*)-MTPA ester appeared as dd at δ 4.36 and 4.26 (0.07 ppm between the inner lines of the two dd systems), while in the spectrum of the (−)-(*S*)-MTPA ester they appeared much more separated (0.15 ppm between the inner lines of the two dd system) at δ 4.20 and 4.41 ppm. On this basis we propose the 25*S* configuration for the polyhydroxysteroid **5**.

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