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STARFISH SAPONINS, PART 43.[1] STRUCTURES OF TWO NEW SULFATED STEROIDAL FUCOFURANOSIDES (IMBRICATOSIDES A AND B) AND SIX NEW POLYHYDROXYSTEROIDS FROM THE STARFISH *DERMASTERIAS IMBRICATA*

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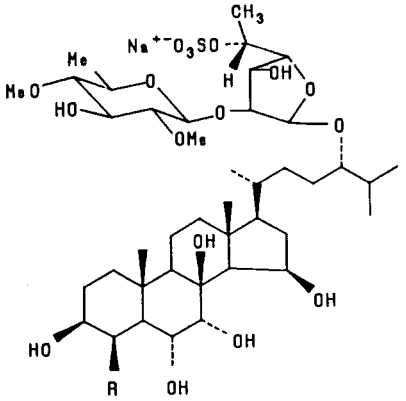
ABSTRACT.—Two new sulfated steroidal glycosides, imbricatoside A [**1**] and imbricatoside B [**2**], which inhibited cell division of fertilized sea urchin eggs, have been isolated from the H_2O extracts of the whole bodies of the starfish *Dermasterias imbricata*. They contain the same disaccharide chain, i.e., 2,4-di-*O*-methyl-β-D-quinovopyranosyl-(1→2)-5-*O*-sulfate-β-D-fucopyranosyl, which is linked to C-24 of 5α-cholestane-3β,4β,6α,7α,8,15β,24-heptaol and 5α-cholestane-3β,6α,7α,8,15β,24-hexaol aglycones.

The extracts also contained six new polyhydroxysteroids, to which the structures **3–8** were assigned.

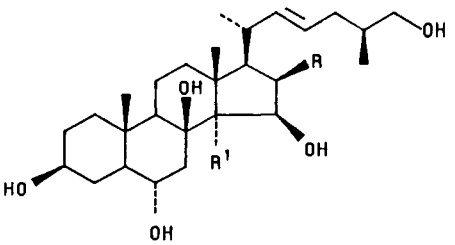
Compound **8**, (22*E*,24*R*)-5α-cholest-22-ene-3β,6α,8,15β,24-pentaol, showed strong antifungal activity and also inhibited cell division of fertilized sea urchin eggs.

Pathirana and Andersen (1) isolated from the starfish *Dermasterias imbricata* Grube a benzyltetrahydroisoquinoline alkaloid imbricatine, which can elicit the swimming response in its prey, the sea anemone *Stomphia coccinea*. Continuing with our work on biologically active compounds from echinoderms (2) we also analyzed the extractives from the whole bodies of *D. imbricata* (family Asteropseidae, order Phanerozonia) and have now isolated two new sulfated steroid glycosides, designated imbricatosides A [**1**] and B [**2**], and eight polyhydrosteroids **3–10**, of which six are new [**3–8**]. Imbricatosides A [**1**] and B [**2**], which contain an unusual 5-*O*-sulfate-β-D-fucofuranosyl unit in their disaccharide moieties, caused inhibition of cell division of fertilized sea urchin eggs. Of the new isolated steroids, compound **8** inhibited growth of the pathogenic fungus *Cladosporium cucumerinum* at a level of less than 1 μg and also inhibited cell division of fertilized sea urchin eggs.

In this paper, we describe isolation and structure elucidation of the new compounds.



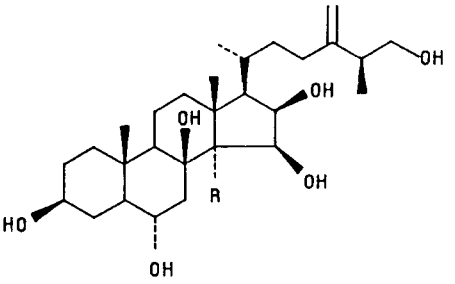
1 R=OH
2 R=H



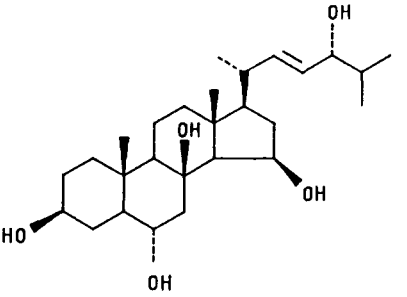
3 R=H, R′=H
4 R=OH, R′=H

5 R=OH, R′=OH, 22,23-dihydro
9 R=OH, R′=H, 22,23-dihydro

[1]For Part 42, see M.V. D'Auria, A. Fontana, L. Minale, and R. Riccio, *Gazz. Chim. Ital.*, in press.



6 R=H
7 R=OH



8
10 22,23-dihydro

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Bruker WM-250 (^{1}H at 250 MHz, ^{13}C at 62.9 MHz), δ (ppm), *J* in Hz spectra referred to *CH*D_2OD signal at 3.30 ppm and to central carbon CD_3OD signal at 49.0 ppm, mass spectra on a Kratos MS 50 mass spectrometer equipped with FAB source [in glycerol or glycerol-thioglycerol (3:1) matrix, Xe atoms of 2–6 kV]; optical rotations on a Perkin-Elmer model 241 polarimeter, glc on a Carlo Erba fractovap 2900 for capillary column (SE-30, 25 m, 140°, helium carrier flow 5 ml/min), reversed-phase hplc on a C_{18} μ-Bondapak column (30 cm × 8 mm i.d.; flow rate 5 ml/min), Waters Model 6000 A pump equipped with a U6K injector and a differential refractometer, model 401, and on a 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, *D. imbricata* were collected off the Gulf of California in November 1985, and frozen (1 kg); identification was done by the zoologists of the Scripps Institution of Oceanography, La Jolla, California; 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 (3 × 1 liter). 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 then eluted with MeOH (2 liters). The MeOH eluate was taken to dryness to give a glassy material (7 g), which was then chromatographed in two runs on a column of Sephadex LH-60 (4 × 100 cm) with MeOH-H_2O (2:1) as eluent. Fractions (8 ml) were collected and analyzed by tlc on SiO_2 with *n*-BuOH–HOAc–H_2O (12:3:5).

Fractions 40–80 (1.5 g in total) contained the sulfated steroid glycosides **1** and **2** together with large amounts of nucleosides and other polar compounds. Fractions 80–105 mainly contained the polyhydroxysteroids, and the residue (0.3 g in total) from these latter fractions was subjected to dccc using $CHCl_3$-MeOH-H_2O (7:13:8) in the ascending mode (the lower phase was the stationary phase, flow rate 10 ml/h; 5-ml fractions were collected and monitored by tlc) (Table 1).

Each fraction was then submitted to hplc with MeOH-H_2O (7:3 or 7.5:2.5) to give pure compounds.

The first eluting fractions (40–80) from the column of Sephadex LH-60 combined with the more polar fractions (6–41) eluted from the previous dccc fractionation (Table 1) were subjected to dccc using *n*-BuOH–Me_2CO–H_2O (3:1:5) in the ascending mode (the lower phase was the stationary phase, flow rate 15 ml/h; 5-ml fractions were collected and monitored by tlc). Fractions 11–23 (35 mg) were evaporated, and the residue was subjected to reversed-phase hplc, MeOH-H_2O (6:4), to collect imbricatoside B [**2**] (5 mg)

TABLE 1. Dccc Fraction of the Polyhydroxysteroids Fraction (fractions 80–105 from Sephadex LH-60).

Fractions	Amounts (mg)	Compounds[a]
6–41	83	**1** + **2** + other polar compounds
68–86	15	**5** (10 mg)
87–124	19	**7** (6 mg)
125–134	7	**9** (5.5mg)
135–157	18	**3** (8 mg), **4** (3 mg), **9** (7 mg)
158–200	23	**6** (4 mg), **8** + **10** (12 mg)

[a]Figures in parentheses are the amounts of each compound after hplc separation.

TABLE 2. Selected 250-MHz ^{1}H-nmr (CD_3OD) Signals (δH) for the Steroids **1–7** and **8 + 10**; *J* (Hz) in parentheses.

Proton	Compound							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8 + 10**
H-3	3.55 m	3.50 m	3.50 m	3.50 m	3.50 m	3.50 m	3.50 m	3.52 m
H-4	4.22 brs	—	—	—	—	—	—	—
H-5	1.53 dd (2.5,11)	—	—	—	—	—	—	—
H-6	4.27 dd[a]	3.85 dd[d]	3.72 ddd (10.5,10.5,4)	3.75 ddd (10.5,10.5,4)	3.77 ddd (10.5,10.5,4)	3.75 ddd (10.5,10.5,4)	3.77 ddd (10.5,10.5,4)	3.71 ddd (10.5,10.5,4)
H-7	3.96 d[b] (3)	3.88 d[d]	2.40 dd (4,12)	2.42 dd (3.75,12.5)	2.14 dd (4,12.5)	2.42 dd (4,12.5)	2.14 dd (4,12.5)	2.42 dd (4,12)
H-14	1.43 d[c]	1.43 d (6)	1.05 d (6)	1.05 d (6)	—	1.06 d[f] (5)	—	—
H-15	4.58 dd (6,7)	4.58 brt (6)	4.40 bt (6)	4.38 dd (6,7)	4.0 d (6.25)	4.40 dd (6,7.5)	3.99 d (6.7)	4.43 m
H-16	2.44 m	2.43 m	2.2 m	4.15 t (6.7)	4.37 t (6.7)	4.27 t (6.7)	4.39 t (6.7)	—
H-18	1.30 s	1.30 s	1.31 s	1.30 s	1.35 s	1.28 s	1.36 s	1.30 s–1.33 s
H-19	1.19 s	1.02 s	1.02 s	1.02 s	1.04 s	1.02 s	1.04 s	1.02 s
H-21	0.97 d (6.5)	0.98 d (6.5)	1.04 d (6.5)	1.06 d (6.5)	0.95 d (6)	1.00 d (6)	0.95 d (5.5)	1.04 d (6.5)
H-22	—	—	5.31 m	5.60 dd (6.5,16)	—	—	—	5.46 dd (16,7.5)
H-23	—	—	5.31 m	5.51 dt (16,6.5)	—	—	—	5.39 dd (16,6.5)
H-24	3.26 m	3.26 m (7)	—	—	—	—	—	3.24 m–3.73 t (7)
H-26	0.93 d (7)	0.94 d (7)	3.46 dd (6.25,10.5)[e]	3.46 dd (6.25,10.5)	3.46 dd (1H) (6,10.5)[e]	3.63 dd (11,6) 3.41 dd (11,7.5)	3.61 dd (11,6) 3.41 dd (11,7.5)	(1H)
H-27	0.935 d (7)	0.94 d (7)	1.00 d (7)	0.92 d (7)	0.95 d (7)	1.10 d (7.5)	1.10 d (7.5)	
H-28	—	—	—	—	—	4.78–4.85 bs	4.79–4.87 bs	

[a]Partially overlapping with the signal for H-3 of the fucofuranose unit. [b]Partially overlapping with the signal for H-4 of fucofuranose unit.
[c]Partially overlapping with the signal for 5-Me of the fucofuranose unit. [d]Overlapping signals.
[e]The remaining H-26 partially obscured by the solvent signal. [f]Overlapping with the signal for 21-Me.

after 16 min; fractions 24–36 (21 mg) and 37–74 (90 mg) were evaporated and similarly subjected to reversed-phase hplc with MeOH-H_2O (6:4) to collect imbricatoside A [**1**] (35 mg in total) after 14.5 min. The ^{1}H- and ^{13}C-nmr data for the new compounds are in Tables 2–4. Rotations and fabms data are reported in the text.

SOLVOLYSIS OF IMBRICATOSIDE A [**1**].—A solution of **1** (5 mg) in pyridine (0.2 ml) and dioxane (0.2 ml) was heated at 140° for 4 h in a stoppered reaction vial. After solution was cooled, H_2O (2 ml) was added and the solution was extracted with *n*-BuOH (2 × 2 ml). The combined extracts were evaporated to dryness under reduced pressure to give desulfated material. The residue was analyzed by fabms (negative ion), *m/z* 803, and by ^{1}H nmr assisted by decoupling, δ (CD_3OD) of the fucofuranosyl unit, 5.14 (bs, H-1′), 4.10 (d, J = 3.5 Hz, H-2′), 4.06 (dd, J = 3.5, 5.5 Hz, H-3′), 3.78 (dd, J = 3.5, 7.5 Hz, H-4′), 3.85 (m, H-5′) and 1.32 (3H, s, 5′-Me). The remaining signals were virtually identical with those of **1**. A solution of the desulfated imbricatoside A (1 mg) was then dissolved in 2 M HCl/MeOH (0.5 ml) and heated at 80° in a stoppered reaction vial for 8 h. After having been 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 Co.) for 15 min at room temperature. Glc analysis gave peaks which co-eluted with those of methyl fucoside standard (Rt 4.4 and 4.8 min); peaks with shorter retention time (Rt 1.4 and 1.5 min) were assigned to 2,4-di-*O*-methyl-α- and β-quinovopyranosides.

MTPA ESTERS OF COMPOUNDS **4** AND **5**.—The steroid **4** (1.5 mg) was treated with freshly distilled (+)-methoxytrifluoromethylphenyl acetyl chloride (5 ml) in dry pyridine (0.15 ml) for 1 h at room temperature. After removal of solvent the product was analyzed by ^{1}H nmr, δ (CD_3OD) 0.97 (3H, d, J = 7 Hz, H_3-27), 1.06 (3H, d, J = 6 Hz, H_3-21), 1.08 (3H, s, H_3-19), 1.30 (3H, s, H_3-18), 4.10 (1H, t, J = 7 Hz, H-16), 4.21 (2H, d, J = 6 Hz, H_2-26), 4.38 (1H, dd, J = 7, 5.5 Hz, H-15), 4.93 (1H, m, H-3), 5.33 (1H, dt, J = 4, 11 Hz, H-6), 5.50 (2H, m, H-22, -23), establishing that esterification had occurred at C-3, C-6, and C-26 and that the stereochemistry at C-25 is 25*S*.

Similarly the steroid **5** was treated with (+)-methoxytrifluoromethylphenyl acetyl chloride and the corresponding ester analyzed by ^{1}H nmr, δ (CD_3OD) 3.97 (1H, d, J = 7 Hz, H-15), 4.21 (2H, d, J = 6 Hz, H_2-26), 4.35 (1H, t, J = 7 Hz, H-16), 4.92 (1H, m, H-3), 5.33 (1H, dt, J = 4, 11 Hz, H-6).

HYDROGENATION OF THE MIXTURE OF **8** + **10** TO GIVE **10**.—The mixture of the steroids **8** + **10** (1.0 mg) dissolved in EtOH (1 ml) was hydrogenated over PtO_2 (Adams catalyst) (1 mg) in the presence of trace $NaNO_2$ (3) for 12 h to give pure **10** (4): fabms *m/z* $[M-H]^-$ 451, ^{1}H nmr δ (CD_3OD) 0.92 and 0.94 (each d, J = 6.8 Hz, H_3-26, -27), 1.00 (3H, d, J = 7 Hz, H_3-21), 1.19 (3H, s, H_3-19), 1.30 (3H, s, H_3-18), 3.24 (1H, m, H-24), 3.50 (1H, m, H-3α), 3.73 (1H, dt, J = 4, 10.5 Hz, H-6β), 4.45 (t, J = 7.5 Hz, H-15α), identical with that of an authentic sample.

BIOASSAYS.—Compounds were tested for antifungal activity by using the direct bioautography on tlc method (5). *C. cucumerinum* was used as test organism. For the sea urchin eggs assays see Ruggieri and Nigrelli (6).

RESULTS AND DISCUSSION

Separation and isolation of the individual compounds from the aqueous extracts of the animals followed the steps described previously (7).

TABLE 3. Assignments of nmr (CD_3OD) Signals of the Disaccharide Moiety of Glycoside **1**. [J (Hz) values are shown in parentheses].

Position	2,4-di-*O*-Methyl-β-quinovopyranosyl		Lit.[a]	5-*O*-Sulfate-β-fucofuranosyl	
	^{1}H	^{13}C		^{1}H	^{13}C
1	4.47 d (7.5)	104.0	104.3	5.12 brs	107.7
2	2.89 dd (7.5,9)	85.4	74.5	4.10 br d (3)	92.1
3	3.44 t (9)	74.1	76.7	4.30 dd (3,6)	77.2
4	2.76 t (9)	86.8	76.2	3.96 dd (2,6)	86.8
5	3.30 m	72.3	73.0	4.65 m	77.4
Me	1.30 d (7)	17.3	17.8	1.46 d (7)	18.4
OMe	3.58 s	60.6	—		
	3.62 s	61.2	—		

[a]Values for methyl-β-D-quinovopyranoside taken from Bock and Pederson (8).

TABLE 4. ^{13}C-nmr Shifts (δ ppm) of Steroids **1** and **3**–**7** in CD_3OD at 62.9 MHz.

Carbon	Compound					
	1	**3**	**4**	**5**	**6**	**7**
C-1	39.7	39.6	39.3	39.8	39.5	38.8
C-2	26.4	31.6	31.4	31.6	31.5	31.6
C-3	73.6	72.3	72.2	72.3	72.1	72.3
C-4	69.7	32.5	32.3	32.5	32.2	32.4
C-5	51.3	54.1	53.8	53.7	53.8	53.7
C-6	66.7	67.7	67.6	68.1	68.1	68.1
C-7	76.7	50.1	50.3	45.7	[a]	45.6
C-8	79.4	77.6	77.6	80.9	77.2	81.0
C-9	56.7	57.7	57.5	48.6	57.4	48.9
C-10	37.7	38.1	38.1	38.0	38.0	38.0
C-11	19.0	19.7	19.4	18.4	19.6	18.9
C-12	43.1	43.4	43.4	38.0	43.1	36.0
C-13	44.3	44.4	44.4	47.6	44.4	[a]
C-14	60.7	63.0	61.1	82.4	61.2	82.4
C-15	71.3	71.3	71.3	76.3	71.1	76.3
C-16	42.8	43.0	73.1	72.4	72.8	72.4
C-17	58.1	57.9	63.5	53.3	63.1	56.3
C-18	16.5	16.7	17.9	17.2	17.8	17.2
C-19	16.8	14.4	14.0	14.2	14.1	14.2
C-20	36.4	40.4	43.6	30.7	30.7	30.7
C-21	19.1	20.9	20.2	18.9	18.3	18.3
C-22	33.1	137.8	139.2	37.6	35.6	35.8
C-23	28.9	131.8	127.5	24.9	33.0	33.1
C-24	85.0	40.5	38.0	35.0	154.0	154.0
C-25	31.9	37.3	37.3	37.0	43.6	43.5
C-26	18.3	68.6	68.2	68.6	67.6	67.6
C-27	18.3	17.2	17.0	17.9	17.9	17.9
C-28					109.2	109.2

[a]Signal under CD_3OD signal.

IMBRICATOSIDE A [**1**].—[α]D −27.5° (*c* = 0.5, MeOH). The fabms (negative ion mode) showed a molecular anion peak at *m/z* 883 and a major fragment at *m/z* 709 $[M-174]^-$ corresponding to the loss of a dimethoxylated deoxyhexose unit. On solvolysis in a dioxane/pyridine mixture it afforded a desulfated derivative, fabms (negative ion mode), *m/z* 803 $[M-H]^-$, which, on acid methanolysis, liberated methyl fucosides identified by glc co-chromatography with standards and a second methylglycoside with shorter retention time (methyl 2,4-di-*O*-methyl quinovosides; see below). The ^{1}H-nmr spectrum (Table 3) of **1** and double resonance experiments showed five methine protons, at δ 4.47 (d, 7.5 Hz), 2.89 (dd, *J* = 7.5, 9 Hz), 3.44 (t, *J* = 9 Hz), 2.76 (t, *J* = 9 Hz), and 3.30 (m), which couple to their neighbors in this order. The last methine proton is further coupled to methyl protons at δ 1.30 (d, *J* = 7 Hz). The coupling constants of the methine signals indicated that the molecule bears a moiety equivalent to a β-quinovopyranoside. The presence in the spectrum of two methoxyl signals, at δ 3.58 s and 3.62 s, along with the high field shifts observed for H-2″ and H-4″ was indicative for a 2,4-di-*O*-methyl quinovosyl unit. This was confirmed by a ^{13}C-nmr spectrum and comparison of the chemical shifts assigned to the quinovopyranosyl unit with those of the methyl β-D-quinopyranoside (**8**) (Table 3). In addition to the signals for the 2,4-di-*O*-methyl-β-quinovopyranosyl unit, the ^{1}H nmr showed signals (Table 3) for a moiety equivalent to a β-fucofuranoside determined by

sequential decoupling. Upfield shift of H-5^1 from 4.65 in **1** to 3.85 ppm in the desulfated derivative clarified that C-5^1 bears the sulfate. Finally the glycosidation shift observed in **1** for C-2^1 of fucofuranose (92.1, Table 3) established the location of the terminal 2,4-di-*O*-methylquinovose at C-2^1 of the 5′-*O*-sulfate fucofuranose. Thus the disaccharide moiety of imbricatoside A [**1**] was established as 2,4-di-*O*-methyl-β-D-quinovopyranosyl-(1→2)-5-*O*-sulfate-β-D-fucofuranosyl. By subtracting those signals due to the carbons of the disaccharide moiety from the ^{13}C-nmr signals of **1**, there were remaining 27 signals (Table 4), of which seven (six CH and one C) were due to oxygen-carrying carbon atoms. Thus, the molecular formula $C_{27}H_{48}O_7$, which corresponds to a saturated cholestane with seven hydroxyl groups, was established for the aglycone moiety. In agreement with a steroid structure the ^{1}H nmr showed the presence of two tertiary and three secondary methyls (Table 2) and also the 3β,4β,6α,7α,8-hydroxylation pattern, which has been determined by sequential decoupling (chemical shifts and coupling constants are listed in Table 2). The same hydroxylation pattern has been already encountered in (25*S*)-5α-cholestane-3β,4β,6α,7α,8,15α,16β,26-octaol, first isolated from *Protoreaster nodosus* (9), and in the corresponding octaol having the 15β-OH instead of the 15α-OH, isolated from *Asterina pectinifera* (10,11). The two remaining hydroxyl groups were located at C-15β and C-24, which are common hydroxylation positions in polyhydroxysteroids and steroid glycosides isolated from starfishes (2,12). The double doublet ($J = 7$, 6 Hz) downfield shifted to δ 4.58, already observed in the spectrum of indicoside A, a steroidal glycoside from *Astropecten indicus* (13) having a 6α,7α,8,15β-hydroxylation pattern, was assigned to H-15α, while the signal at δ 3.26 has the chemical shift and the shape typical (broad triplet) for H-24 in 24-hydroxysteroids (14). These assignments were corroborated by ^{13}C-nmr spectrum (Table 4) and comparison with reference steroids (9–11, 13). The ^{13}C nmr also established that the disaccharide moiety is located at C-24. The signals for the side chain carbons are identical with those assigned to the previous (24*S*)-24-*O*-α-L-arabinofuranosyl steroids isolated from starfishes (14). Thus we presume that imbricatoside A [**1**] has the same *S* configuration at C-24.

The novel imbricatoside A [**1**] can be defined as (24*S*)-24-*O*-[2,4-di-*O*-methyl-β-D-quinovopyranosyl-(1→2)-5-*O*-sulfate-β-D-fucofuranosyl]-5α-cholestane-3β,4β,6α,7α,8,15β-hexaol [**1**]. We prefer the D configuration for the quinovose and fucose by analogy with other steroid glycosides isolated from starfishes (12). Fucose is a common monosaccharide among the asterosaponins, but never found before in the furanose form.

IMBRICATOSIDE B [**2**].—Compound **2**, $[\alpha]_D$ +2° ($c = 0.5$, MeOH), is closely related to imbricatoside A [**1**], but lacks the 4β-hydroxy group. The fabms (negative ion mode) gave the molecular anion peak at *m/z* 867, sixteen mass units shifted relative to **1**. Examination of ^{1}H-nmr spectrum (Table 3) of **2** immediately indicated the presence of the 2,4-di-*O*-methyl-β-D-quinovopyranosyl-(1→2)-5-*O*-sulfate-β-D-fucofuranosyl. Further analysis of the ^{1}H-nmr data (Table 2) revealed the lack of the broad signal at δ 4.22 assigned to H-4α in **1** and also the upfield shifts to δ 3.85 of the double doublet assigned to H-6β (δ 4.27 in **1**) and to δ 1.02 of the singlet assigned to H_3-19 (δ 1.19 in **1**). The remaining aglycone signals were almost identical in both spectra.

THE POLYHYDROXYSTEROIDS.—(22*E*,25*S*)-5α-Cholest-22(23)-ene-3β,6α,8,15β,26-pentaol [**3**], $[\alpha]_D$ +7.1° ($c = 0.5$, MeOH). The fabms (negative ion mode) exhibited a pseudomolecular ion peak at *m/z* 449. The ^{13}C-nmr spectrum (Table 4) and DEPT measurements indicated the presence of 27 carbon atoms and revealed the presence of four methyl groups, eight methylenes, six methines, two quaternary carbons, three -OCH<, one -OCH_2-, one -O-C-< and a CH=CH. Taken together, these data

indicated a pentahydroxycholestene with one of the five methyl groups typical of a sterol oxidized to hydroxymethylene. Further examination of ^{13}C- and ^{1}H-nmr data (Tables 4,2) and comparison with those of the many polyhydroxysteroids isolated in our laboratory indicated that **3** is a 3β,6α,8,15β,26-pentahydroxycholestane with a double bond in the side chain. The 3β,6α,8,15β-hydroxylation pattern was encountered before in (24*S*)-5α-cholestane-3β,6α,8,15β,24-pentaol, isolated from the starfish *Gomophia watsoni* (4). The double bond was placed at the common 22(23) biogenetic position from ^{1}H- and ^{13}C-nmr spectra, and the *E*-configuration is mainly derived from ^{13}C-nmr data (15). The 25*S* configuration is assumed by analogy with the steroids **4** and **5**, for which the 25*S* configuration has been determined (see below).

The steroid **3** showed moderate antifungal activity (inhibition of growth of the fungus *C. cucumerinum*, active at 5 μg).

(22*E*,25*S*)-5α-CHOLEST-22(23)-ENE-3β,6α,8,15β,16β,26-HEXAOL [**4**].—[α]D +12° (*c* = 0.5, MeOH), fabms (negative ion mode), *m/z* 465 $[M-H]^-$. This steroid is related to **3** by introduction of an "extra" hydroxyl group at position 16β, and also is related to the known **9**, previously isolated from the starfish *Halityle regularis* (16), by introduction of a double bond at position 22(23) (*E* configuration). Its structure was derived from ^{1}H and ^{13}C spectroscopy (Tables 2 and 4) and comparison with the above reference steroids.

In order to determine the stereochemistry at C-25, **4** was treated with (+)-methoxytrifluoromethylphenyl acetyl chloride [Mosher reagent (17)] in pyridine affording the corresponding 3,6,26-tri-(*R*)-(+)-methoxytrifluoromethylphenyl acetate (MTPA). The ^{1}H-nmr spectrum showed a signal at δ 4.21 (2H, d, *J* = 6 Hz) for H_2-26, thus indicating the configuration to be 25*S* like other 26-hydroxysteroids from starfishes (2). In the ^{1}H-nmr spectrum of (*R*)-(+)-MTPA ester of (25*R*)-26-hydroxysteroids, signals for H_2-26 were found at δ 4.24 (dd, *J* = 11, 6 Hz) and 4.08 (dd, *J* = 11, 7 Hz) (18).

(25*S*)-5α-CHOLESTANE-3β,6α,8,14α,15β,16β,26-HEPTAOL [**5**].—[α]D +41° (*c* = 0.5, MeOH). This sterol is related to the known **9** (16), by introduction of an "extra" hydroxyl group at position 14α. The fabms (negative ion mode) showed the pseudomolecular ion peak at *m/z* 483 $[M-H]^-$, sixteen mass units shifted relative to **9**. The ^{13}C-nmr spectrum contained seven signals in the region for oxygen-containing carbons, two of which were tertiary (->C-O-H). The ^{1}H-nmr spectrum (Table 2) showed the H-15α signal as a doublet (*J* = 6.25 Hz) coupled with a triplet at δ 4.37 (H-16α), thus indicating the 15β-OH to be adjacent to a carbon without protons. The location of the "extra" hydroxyl group at position 14α was confirmed by the analysis of the ^{13}C-nmr data and comparison with those of **4** (Table 4). Particularly significant for the location of the "extra" hydroxyl group at position 14α were the upfield shifts exhibited by C-7 (45.7 vs. 50.3 ppm; **5** vs. **4**), C-9 (48.6 vs. 57.5 ppm; **5** vs. **4**), C-12 (38.0 vs. 43.4 ppm; **5** vs. **4**) and C-17 (53.3 vs. 63.3 ppm; **5** vs. **4**).

The 25*S* configuration is assigned by the method used for steroid **4**.

24-METHYL-5α-CHOLEST-24(28)-ENE-3β,6α,8,15β,16β,26-HEXAOL [**6**].—Compound **6**, [α]D +35° (*c* = 0.5, MeOH), fabms (negative ion mode) *m/z* 479 $[M-H]^-$, is the 24-methylene derivative of the known **9** (15). Examination of its spectral data (Tables 2 and 4) immediately indicated the presence of the same 3β,6α,8,15β,16β-hydroxylation pattern as in **4** and **9** and a 24-methylene-26-hydroxy side chain. 24-Methylene-26-hydroxysteroids have been already encountered among polyhydroxysteroids isolated from starfishes [i.e., *Poraster superbus* (19), *Sphaerodiscus placenta* (20), and *Thromidia catalai* (21)] and very recently isolated from *Patiria miniata* (22).

24-METHYL-5α-CHOLEST-24(28)-ENE-3β,6α,8,14α,15β,16β,26-HEPTAOL [**7**].—

Compound **7**, $[\alpha]_D$ +30.5° (c = 0.5, MeOH), fabms (negative ion mode) m/z 495 $[M-H]^-$, is related to the previous **6** by introduction of an "extra" hydroxyl group at position 14α, as indicated by ^{1}H- and ^{13}C-nmr data and comparison with those of **6**.

(22*E*,24*R*)-5α-CHOLEST-22(23)-ENE-3β,6α,8,15β,24-PENTAOL [**8**].—This compound was isolated as a mixture with the known **10**, previously isolated from *G. watsoni* (4), resistant to attempts at separation by reversed-phase hplc. We pursued the structure determination on the mixture. The mass spectrum (negative ion mode) gave two pseudomolecular ions at m/z 449 $[M-H]^-$ and 451 $[M-H]^-$ in a ratio ca. 1:1.

The ^{1}H-nmr spectrum contained signals for a 3β,6α,8,15β-tetrahydroxy tetracyclic steroid nucleus [Table 2, compare Riccio *et al.* (4)]. In addition, the spectrum contained an eight-line pattern centered at δ 5.39 (dd, J = 16, 6.5 Hz) and 5.46 (dd, J = 16 and 7.5 Hz) which could be assigned to the Δ^{22}-*trans* protons. In the hydroxymethine proton region a triplet at δ 3.73 (J = 7 Hz) emerging from the dt at 3.70 ppm assigned to H-6β was found to be coupled with the olefinic signal at δ 5.39 (H-23), thus suggesting a Δ^{22E},24-hydroxycholesterol side chain for the compound **8** with the pseudomolecular ion at m/z 449. The corresponding 22,23-saturated structure **10** was then assigned for the compound (signal at δ 3.24, H-24) with the pseudomolecular ion at m/z 451. In the methyl region of the spectrum we observed one singlet (3H) for 19-Me protons at δ 1.02 and two isolated singlets (3H together) for the 18-Me at δ 1.33 (Δ^{22}-compound, **8**) and at δ 1.30 (saturated analogue **10**); an isolated doublet (J = 6.5 Hz) integrating for less than 3H at δ 1.04 was assigned to the 21-Me protons of the Δ^{22}-compound. The remaining methyl signals overlap between 0.97 and 0.90 ppm. In confirmation the mixture of **8** + **10** was hydrogenated on PtO_2 in the presence of a trace of $NaNO_2$ (3) to afford a single compound identical in all respects (tlc, hplc, and ^{1}H nmr) with an authentic sample of **10** (4). The 24*R* configuration proposed for the Δ^{22}-analogue **8** is also based on the chemical shift of the H-24 at δ 3.73. Indeed, in the course of our continuing work on marine steroids we had at our disposal two small samples of (22*E*,24*R*)- and (22*E*,24*S*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-24-ol (7), which showed in the ^{1}H-nmr spectrum signals for H-24 at δ 3.71 and 3.68 ppm, respectively. In the spectrum of our mixture of **8** + **10** the peak observed at δ 3.73 compared well with that of the 24*R* isomer.

The mixture of **8** + **10** inhibited growth of the pathogenic fungus *C. cucumerinum* (active at 1 μg). Compound **10** was active at a level of 3 μg, thus showing **8** to be active at less than 1 μg.

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LITERATURE CITED

1. C. Pathirana and R.J. Andersen, *J. Am. Chem. Soc.*, **108**, 8288 (1986).
2. L. Minale, R. Riccio, C. Pizza, and F. Zollo, in: "Natural Products and Biological Activities," a NAITO Foundation Symposium. Ed. by H. Imura, T. Goto, T. Murachi, and T. Nakajima, University of Tokyo Press and Elsevier Science Publishers, New York, 1986, pp. 59–73.
3. M.C. Dart and H.B. Henbest, *J. Chem. Soc.*, 3563 (1960).
4. R. Riccio, M.V. D'Auria, M. Iorizzi, L. Minale, D. Laurent, and D. Duhet, *Gazz. Chim. Ital.*, **115**, 405 (1985).
5. A.L. Homans and A. Fuchs, *J. Chromatogr.*, **51**, 327 (1970).

6. G.D. Ruggieri and R.F. Nigrelli, *Am. Zool.*, **6**, 592 (1966).
7. R. Riccio, M. Iorizzi, L. Minale, Y. Oshima, and T. Yasumoto, *J. Chem. Soc., Perkin Trans. 1*, 1337 (1988).
8. K. Bock and C. Pederson, in: "Advances in Carbohydrate Chemistry and Biochemistry." Ed. by R.S. Tipson and D. Horton, Academic Press, New York, 1983, Vol. 41, pp. 27–66.
9. R. Riccio, L. Minale, S. Pagonis, C. Pizza, F. Zollo, and J. Pusset, *Tetrahedron*, **38**, 3615 (1982).
10. R. Higuchi, Y. Noguchi, T. Komori, and T. Sasaki, *Liebigs Ann. Chem.*, 1185 (1988).
11. M. Iorizzi, L. Minale, and R. Riccio, *Gazz. Chim. Ital.*, in press.
12. V.A. Stonik and G.B. Elyakov, in: "Bioorganic Marine Chemistry." Ed. by P.J. Scheuer, Springer-Verlag, Berlin, Heidelberg, 1988, Vol. 2, pp. 43–86.
13. R. Riccio, L. Minale, S. Bano, and V.U. Ahmad, *Tetrahedron Lett.*, **28**, 2291 (1987).
14. C. Pizza, P. Pezzullo, L. Minale, E. Breitmaier, J. Pusset, and P. Tirard, *J. Chem. Res., Synop.*, 76 (1985); *J. Chem. Res., Miniprint*, 969 (1985).
15. J.W. Blunt and J.B. Stothers, *Org. Magn. Reson.*, **9**, 439 (1977).
16. M. Iorizzi, L. Minale, R. Riccio, M. Debray, and J.L. Menou, *J. Nat. Prod.*, **49**, 67 (1986).
17. J.A. Dale and H.S. Mosher, *J. Am. Chem. Soc.*, **95**, 512 (1973).
18. K. Tachibana, M. Sakaitani, and K. Nakanishi, *Tetrahedron*, **41**, 1027 (1985).
19. R. Riccio, M. Iorizzi, O. Squillace-Greco, L. Minale, D. Laurent, and Y. Barbin, *Gazz. Chim. Ital.*, **114**, 143 (1984).
20. F. Zollo, E. Finamore, and L. Minale, *J. Nat. Prod.*, **50**, 794 (1987).
21. R. Riccio, O. Squillace-Greco, L. Minale, S. La Barre, and D. Laurent, *J. Nat. Prod.*, **51**, 1003 (1988).
22. M.V. D'Auria, M. Iorizzi, L. Minale, R. Riccio, and E. Uriarte, *J. Nat. Prod.*, **53**, 94 (1990).

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