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STARFISH SAPONINS, PART 23.[1] STEROIDAL GLYCOSIDES FROM THE STARFISH *HALITYLE REGULARIS*[2]

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ABSTRACT.—Eight new steroidal glycosides have been isolated from the starfish *Halityle regularis*. The first group of compounds, **1-3**, contain the 2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl disaccharide moiety, which is attached at C-24 of polyhydroxysteroid aglycones. The second group of glycosides, **4-6**, have polyhydroxylated 24-(β-hydroxyethyl) steroid aglycones with the disaccharide moieties, 2-*O*-methyl-β-D-xylopyranosyl-(1→2)-β-D-xylopyranosyl or 2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl, located at the primary hydroxyl group at C-29. Unlike the other compounds, the glycosides **7** and **8** have a sulfate group. These glycosides are accompained by two nonglycosidated polyhydroxylated sterols, i.e., (25*S*)-5α-cholestane-3β,6α,8,15β,16β,26-hexol (**10**) and (25*S*)-5α-cholestane-3β,4β,6α,8,15β,16β,26-heptol (**11**). The structures of the new metabolites were determined by interpretation of spectral data, comparison with related glycosides described previously, and chemical transformations.

Steroidal glycosides, composed of polyhydroxylated steroidal aglycone and a carbohydrate portion made up from only one or two monosaccharide units, are a growing subgroup of active compounds among the glycosides isolated from starfishes. The more common disaccharide moiety is the 2-*O*-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl residue, which is attached at C-24 of the steroid. The first representative of such compounds, the cytotoxic nodososide, was first isolated from Pacific *Protoreaster nodosus* L. (1) and later from other species (2). Further examples have been isolated from *Hacelia attenuata* Gray (3,4,5), *Patiria pectinifera* M.Tr. (6), *Acanthaster planci* L., (7) and *Oreaster reticulatus* L. (8). Continuing with our study of the biologically active metabolites from starfishes, we have been working on the polar extracts of the Pacific starfish *Halityle regularis* Fisher and have isolated several novel steroidal glycosides. In the preceding paper (9), we described two novel steroidal pentaglycoside sulfates, "asterosaponins," and in this paper, we report on the less polar components, i.e., the nonsulfated steroidal diglycosides **1-6**, the sulfated steroidal diglycosides **7** and **8**, and the polyhydroxysterols **10** and **11**. All are new compounds. Three glycosides, **1-3**, contain the 2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl disaccharide moiety, which is attached at C-24 of polyhydroxysteroid aglycones; the compounds **4-7** have polyhydroxylated 24-(β-hydroxyethyl) steroid aglycones with the disaccharide moieties located at the primary hydroxyl group at C-29; the sulfated glycoside **8** has a polyhydroxylated Δ^{22}-24-methyl-26-hydroxy steroid aglycone, with the saccharide moiety located at the primary hydroxyl group at C-26.

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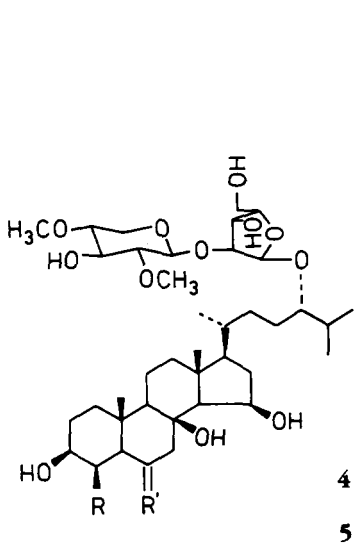
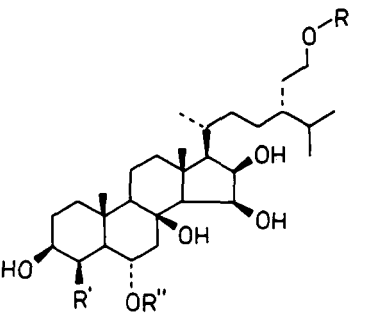
[1]For Part 22, see R. Riccio, *et al.* (9).

[2]This contribution is part of the Progetto Finalizzato "Chimica Fine e Secondaria" del CNR, ROMA. It was presented in part at the Colloque International du CNRS "Chimie des Substances Naturelles: Etat et Perspective," Gif-sur-Yvette, September 10-11, 1984.





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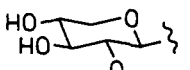
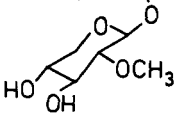
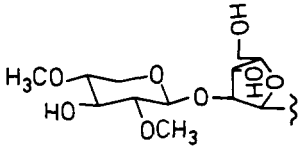
4		H	H
5		OH	H
6		OH	H
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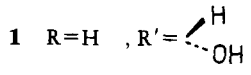
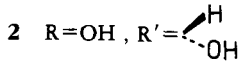
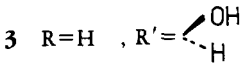
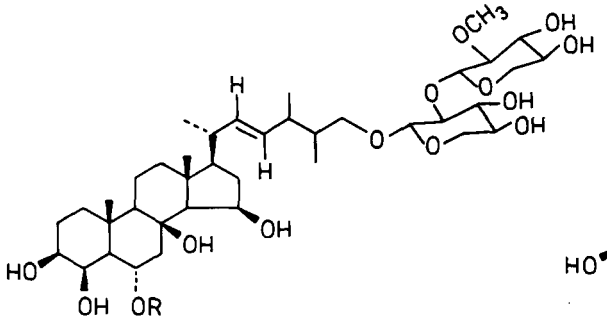
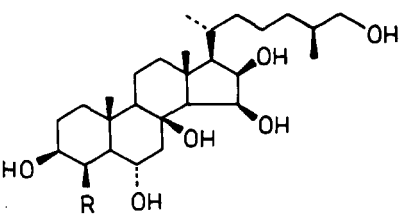








8 R=$SO_3^-Na^+$
9 R=H



10 R=H
11 R=OH

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The following instruments were used: nmr, Bruker WM-250 and WM-500; cd, Cary 61 spectropolarimeter; rotations, Perkin-Elmer model 141 polarimeter; ms, Kratos MS 50 mass spectrometer equipped with Kratos fab source or ei source; dccc, DCCC-A apparatus manufactured by Tokyo Rikakikai Co. equipped with 300 tubes; hplc, Waters Model 6000 A pump equipped with a U6K injector and a Model 401 differential refractometer detector; gc, Carlo Erba Fractovap 2900 for capillary column. The fab-mass spectra were obtained by dissolving the samples in a glycerol matrix and placing them on a probe prior to bombardment with Ar atoms of 2-6 kv energy. The *J*-modulated spin-echo experiments were performed using an evolution period of 4 ms for selective detection of quaternary carbons and 8ms for nonselective detection. Tlc was performed on silica precoated glass sheets (Merck), using kieselgel 60 F_{254}, 0.5 mm for preparative separations.

EXTRACTION AND ISOLATION.—The animals *Halityle regularis* Fisher (Oreasteridae), (7.5 kg) were collected in October 1983, off Nouméa, and lyophilized (2.7 kg). Animals were identified by Mr. Michel

Jangoux, Université Libre de Bruxelles, and a reference specimen of the organism is deposited at the Centre ORSTOM, New Caledonia. The extraction of the lyophilized material (2.0 kg) and the chromatography of the aqueous extracts through a column of Amberlite XAD-2 were described in a previous paper (9). The MeOH eluates from this column were combined with the *n*-BuOH extracts of the H_2O eluates (ca. 16.5 g total yield) and chromatographed in two portions on Sephadex LH-60 (4 × 80 cm; 100 g) using MeOH-H_2O (2:1) as eluent (flow rate, 10 ml/h). The eluents were collected in 7-ml fractions and monitored by tlc with *n*-BuOH-HOAc-H_2O (12:3:5) and $CHCl_3$-MeOH-H_2O (40:9:1), detection with ceric sulfate/H_2SO_4. Fractions 64-78 contained the crude "asterosaponins," which were the subject of the preceding paper. Fractions 79-97 (2 g) still contained "asterosaponins" in admixture with the diglycosides and the sulfated diglycosides, while fractions 98-145 (2.6 g) contained the crude diglycosides and the polyhydroxysteroids. These fractions (79-97 and 98-145) were separately submitted to droplet counter-current chromatography (dccc). The solvent system was $CHCl_3$-MeOH-H_2O (7:13:8) with the lower phase as the stationary phase; ascending mode; flow 10 ml/h; 3.5-ml fractions were collected and monitored by tlc (Table 1).

TABLE 1. Dccc Fractionation

Fractions No.	Total Amount (mg)	Compounds
27-42	825	"asterosaponins" +sulfated diglycosides
43-48	160	nucleosides
49-59	193	nucleosides
60-74	220	**5**
75-92	89	**2**,**3**,**4**, and **5**
93-112	60	**2** and **11**
113-126	107	**2**,**10**, and **11**
127-148	68	**1**,**2**, and **6**
149-160	36	**1**

The less polar fractions (from 60-74 and 149-160) were then submitted to hplc on a C_{18} μ-Bondapak column (30 cm × 7.8 id) with MeOH-H_2O (75:25) (flow rate 5 ml/min) to give pure compounds. The results are summarized in Table 2.

TABLE 2. Polar Steroids from *Halityle regularis* (ca. 5 kg fresh material)

Compound	Amount (mg)	Hplc Retention time (min)	Optical Rotation	Fabms (M+ Na)
Halityloside A **5**	30.8	18.1	− 3.1°	813
Halityloside B **4**	11.7	21.0	− 5.0°	797
Halityloside D **2**	18.5	14.4	−13.7°	783
Halityloside E **1**	14.4	18.0	−20.0°	767
Halityloside F **3**	6.2	24.0	−14.1°	767
Halityloside H **6**	3.0	21.6	− 5.0°	827
Steroid **10**	4.4	13.2	− 0°	
Steroid **11**	7.9	12.4	+ 6.3°	

Rotations were taken for solutions in MeOH (c ranging from 0.7 to 0.3). The mass spectra of the hexol **10** and heptol **11** were taken using eims at 70 eV; **10**, *m/z* 450 (M^+-H_2O, 20%), 432 (M^+-$2H_2O$, 100), 417 (M^+-$2H_2O$-Me, 55), 414 (M^+-$3H_2O$, 25), 303 (M^+-s.c.-$2H_2O$, 45), 285 (M^+-s.c.-$3H_2O$, 45), 267 (M^+-s.c.-$4H_2O$, 30), 260 (40), 225 (40); **11**, *m/z* 484 (M^+, 1%), 466 (M^+-H_2O, 23), 448 (M^+-$2H_2O$, 100), 433 (M^+-$2H_2O$-Me, 40), 430 (M^+-$3H_2O$, 40), 415 (M^+-$3H_2O$-Me, 23), 412 (M^+-$4H_2O$, 15), 337 (M^+-s.c.-H_2O, 11), 319 (M^+-s.c.-$2H_2O$, 32), 301 (M^+-s.c.-$3H_2O$, 35), 283 (M^+-s.c.-$4H_2O$, 30), 277 (45), 259 (32), 241 (30), 225 (34); ei hrms; **11**, 448.3183 (M^+-$2H_2O$; $C_{27}H_{44}O_5$ requires 448.3189). The ^1H- and ^{13}C-nmr spectra of each compound are in Tables 3-8.

The more polar dccc fraction 27-42 was further chromatographed on a column of Sephadex LH-20 (2 × 60 cm) using MeOH as eluent (flow rate, 9 ml/h, 3 ml fractions) to remove in the first fractions (21-26) a further amount (300 mg) of "asterosaponins" and in the last fractions (69-84) a further amount (154 mg) of nucleosides. The central fractions were then submitted to hplc on a C-18 μ-Bondapak column (30 cm × 7.8 id) with MeOH-H_2O (55:45) (flow 5 ml/min), and afforded, along with major amounts of tryptophan

TABLE 3. Assignments of ^{1}H-nmr (500 MHz, CD_3OD) Signals to 2,4-Di-*O*-methyl-β-D-xylopyranosyl-(1 2)-α-L-arabinofuranosyl Moiety of **1**, **2**, **3**, **6**, and **7** in δ (Hz)[a]

	1-H	2-H	3-H	4-H	5-H	OCH_3
arabinose	5.11 bs	4.08 d (4.0)	4.02 m	3.98 m	3.79 dd - 3.65 dd (12.5, 3.0) (12.5, 4.8)	
2,4-di-*O*-mexylose . .	4.44 d (7.6)	2.90 dd (9.0,7.6)	3.42 t 9.0	3.20 m	3.14 t - 4.02 dd (10.6) (10.6, 4.0)	3.50-3.61

[a]These data have been extracted from the spectrum of **2**. Assignments are based upon spin-decoupling techniques and comparison with spectra of nodososide (1) and similar glycosides (3,4). In all the spectra, these signals were within 0.02 ppm from those of **2**, except the arabinose signals in **6** and **7**, which were slightly shifted: 5.02 bs, 1-H; 4.08 m, 2-H and 3-H, 3.93 m, 4-H.

and "asterosaponins," two peaks with longer retention times, i.e., 14.3 min (7.8 mg) and 18 min (9.8 mg). The peak eluted after 14.3 min contained halityloside I (**8**), fabms *m/z* 883 (M+Na), while the peak eluted after 18 min was still a mixture of two compounds. The major one could be identified as halityloside H, 6-*O*-sulfate (**7**).

METHANOLYSIS OF HALITYLOSIDE A (**5**) AND D (**2**):—*Sugar analysis*.—A solution of halityloside A (**5**) (5 mg) in anhydrous 2M-HCl in MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 10 h. After being cooled, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2.

A small portion of the residue was trimethylsilylated with trisil Z (Pierce Chemical Co.), 15 min at room temperature. Gc analysis at 140° using a 25 m column of SE-30 (hydrogen carrier, flow 10 ml/min) gave gc peaks which co-eluted with those of silylated methyl xylosides and methyl 2-*O*-methylxylosides.

The remainder of the above residue in dry pyridine (1 ml) was treated with *p*-bromobenzoyl chloride (80 mg) and a few mg of 4-dimethylaminopyridine. The mixture was stirred overnight at 60° under N_2, chilled H_2O was added to the solution, and after 30 min, the solution was extracted with $CHCl_3$. The extract was washed successively with saturated aqueous $NaHCO_3$ and H_2O. After evaporation of the solvent, the residue was separated by preparative tlc, using 30% Et_2O in hexane as solvent, to give two major compounds; methyl 2,3,4-tri-*O*-(*p*-bromobenzoyl)-α-D-xylopyranoside: Rf = 0.53; ^{1}H nmr δ ($CDCl_3$) 7.90-7.45 (12H, m, Ar-H), 6.08 (1H, t, *J*= 10.5 Hz, 3-H), 5.39 (1H, ddd, *J*=10.5, 10.5, 6.0, 4-H), 5.23 (1H, dd, *J*=10.5, 4.0 Hz, 2-H), 5.13 (1H, d, *J*=4.0 Hz, 1-H), 4.07 (1H, dd, *J*=10.5, 6.0 Hz, 5-He), 3.83 (1H, t, *J*=10.5, 5-Ha), 3.66 (OCH_3); cd ($CHCl_3$): 236/253, Δε +1.5/−0.3, A=−1.8 (calcd. A=0) (10); and methyl 2-*O*-methyl-3,4-di-*O*-(*p*-bromobenzoyl)-α-D-xylopyranoside: Rf=0.18; ^{1}H nmr δ ($CDCl_3$) 7.90-7.50 (8H,m, Ar-H), 5.83 (1H, t, *J*=10.5 Hz,3-H), 5.23 (1H, ddd, *J*=10.5, 10.5, 6.0 Hz, 4-H), 4.97 (1H, d, *J*=4.0 Hz, 1-H), 3.98 (1H, dd, *J*=10.5, 6.0 Hz, 5-He), 3.74 (1H, t, *J*=10.5 Hz, 5-Ha), 3.56 (1H, dd, *J*=10.5, 4.0 Hz, 2-H), 3.51-3.46 (2- OCH_3); cd ($CHCl_3$): 236/253, Δε+17.4/−35.9, A=−53.3 (calcd. A=−62) (10). The cd amplitudes were derived without weighing the samples; the concentrations of solutions submitted to cd measurements were estimated by uv absorption at 246 nm using the following standard ε values: dibenzoate, 38,200: tribenzoate, 57,200 (10).

A solution of halityloside D (**2**) (7.8 mg) was methanolyzed as before and a small portion of the residue used for a gc analysis. After trimethylsilylation, the gc peaks co-eluted with those of silylated methylarabinosides; 25 m SE-30 column, 140°. The major part was *p*-bromobenzoylated as before. Purification of the benzoate mixture gave two major compounds; methyl 2,3,4-tri-*O*-(*p*-bromobenzoyl)-β-L-arabinopyranoside: Rf = O.46; ^{1}H nmr δ ($CDCl_3$) 7.95, 7.84, 7.67, 7.64, 7.53, and 7.44 (each 2H d, *J*=9.0 Hz, Ar-H), 5.88 (1H, dd, *J*=10.5, 3.0 Hz, 3-H), 5.73 (1H, br s, 4-H), 5.65 (1H, dd, *J*=10.5, 3.0, Hz, 2-H), 5.20 (1H d, *J*=3.0 Hz, 1-H), 4.17-3.94 (each 1H br d, *J*=12.0 Hz, 5-H_2), 3.48 (OCH_3); cd ($CHCl_3$) 236/253, Δε −33.5/+99.3, A = +132.8 (calc. A +140) (10); and methyl 2,4-di-*O*-methyl-3-*O*-(*p*-bromobenzoyl)-α-D-xylopyranoside: Rf = 0.12; ^{1}H nmr δ ($CDCl_3$) 7.98 and 7.60 (each 2H, d, *J*=9.0, Ar-H), 5.55 (1H, t, *J*=10.0, 3-H), 4.88 (1H, d, *J*=4.0 Hz, 1-H), 3.82 (1H, dd, *J*=10.5, 5.5 Hz, 5-He), 3.65-3.50 (2H, m, 5-Ha and 4-H), 3.40 (1H, dd, *J*=10.0, 4.0 Hz, 2-H), 3.46, 3.40, and 3.36 (OCH_3).

ACETONIDE FORMATION FROM HALITYLOSIDES A (**5**), B (**4**), D (**2**), AND STEROIDS (**10**) AND (**11**).—Each of the above compounds (2.5-4.5 mg) in dry Me_2CO (0.5 ml) containing *p*-TsOH (1 mg) was stirred overnight at room temperature. The mixture was neutralized with $BaCO_3$, centrifuged, and the supernatant evaporated to dryness. The acetonides were then purified by chromatography through a Pasteur pipette filled with a slurry of silica gel, using 5% MeOH in $CHCl_3$ and increasing MeOH content to 10%. The acetonides were eluted with 10% MeOH in $CHCl_3$. The results from each compound are given below.

From halityloside A (**5**).—3,4-15,16-Bis-Acetonide, which was further purified by hplc on a C_{18} μ-Bondapak column (30 cm × 4 mm i.d.) with MeOH-H_2O (80:20) (flow 2 ml/min; elution time 16 min); fabms *m/z* 893 (M+Na); ^{1}H nmr δ (CD_3OD) 0.89, 0.92, 0.99 (each 3H, d, J=7.0, 6.5, and 6.2 Hz, 26-, 27-, and 21-H_3), 1.26 (3H, s, 19-H_3), 1.39 (3H, s, 18-H_3), 1.34, 1.36, 1.52, and 1.53 [each 3H, s, O-C(CH_3)$_2$-O], 4.03 (1H, m, 3α-H), 4.61 (1H, dd, J=5, 3 Hz, 4-H), 4.74 (2H, m, 15- and 16-H).

From halityloside B (**4**).—15,16-Acetonide; fabms *m/z* 837 (M+Na); ^{1}H nmr δ (CD_3OD) 0.88, 0.92 and 0.99 (each 3H, d, J=7.0, 6.6, 6.3 Hz, 26-, 27-, and 21-H_3), 1.03 (3H, s, 19-H_3), 1.39 (3H, s, 18-H_3), 1.35 and 1.52 [each 3H, s, O-C(CH_3)$_2$-O], 4.74 (m, 15 and 16-H).

From halityloside D (**2**).—3,4-Acetonide; fabms *m/z* 823 (M+Na); ^{1}H nmr δ (CD_3OD) 0.92, 0.94, and 0.96 (each 3H, d, J=7.0, 6.6, and 6.1 Hz, 27-, 26-, and 21-H_3), 1.25 (3H, s, 19-H_3), 1.30 (3H, s, 18-H_3), 1.34 and 1.52 [3H each, s, O-C(CH_3)$_2$-O], 4.60 (1H, dd, J=5, 3 Hz, 4-H).

From hexol (**10**).—15,16-Acetonide, eims *m/z* 493 (M^+−Me); ^{1}H nmr δ (CD_3OD) 0.95 and 0.97 (6H, two doublets, J=6.5 and 6.8 Hz, 21- and 27-H_3), 1.03 (3H, s, 19-H_3), 1.39 (3H, s, 18-H_3), 1.34 and 1.51 [3H each, s, O-C(CH_3)$_2$-O], 4.74 (m, 15 and 16-H).

From heptol (**11**).—3,4-15,16-Bis-Acetonide; eims *m/z* 549 (M^+−Me) ^{1}H nmr δ (CD_3OD) 0.95 and 0.97 (6H, two doublets, J=7.0 and 7.2 Hz, 21- and 27-H_3), 1.26 (3H, s, 19-H_3), 1.39 (3H, s, 18-H_3), 1.35 and 1.52 [6H each, s, O-C(CH_3)$_2$-O], 4.03 (1H, m, 3α-H), 4.61 (1H, dd, J=5.5 and 3.0 Hz, 4-H), 4.70 (1H, t, J=5.5 Hz, 15- or 16-H, 4.74 (1H, m, 16- or 15-H).

SOLVOLYSIS OF HALITYLOSIDE I (**8**) GIVING **9**.—A solution of **8** (7.8 mg) in dioxane (0.2 ml) and pyridine (0.2 ml) was heated at 120° for 2 h in a stoppered reaction vial. After the solution had cooled, H_2O (2 ml) was added, and the solution was extracted with *n*-BuOH (3 × 1 ml). The combined extracts were washed with H_2O and evaporated to dryness under reduced pressure. The residue was purified by hplc on a C_{18} μ-Bondapak column (30 cm × 7.8 mm id) with MeOH-H_2O (75:25) (flow 5 ml/min) to give 5.2 mg of **9**; elution time: 12 min; fabms *m/z* 781 (M+Na); ^{1}H- and ^{13}C-nmr in Tables 5-8.

TABLE 4. Assignments of ^{13}C-nmr (62.9 MHz, CD_3OD) Signals to 2,4-Di-*O*-methyl-β-D-xylopyranosyl-(1↦2)-α-L-arabinofuranosyl moiety of **1**, **2**, **3**, and **6** in ppm[a]

	C-1	C-2	C-3	C-4	C-5	OCH_3
arabinose	108.0	92.4	77.9	84.2	62.8	
2,4-di-*O*-mexylose . .	105.1	84.9	76.6	81.0	64.4	61.0-59.0

[a]These data have been extracted from the spectrum of **2**. In the spectra of **1** and **3**, these signals were within ±0.1 ppm of those of **2**, while in the spectrum of **6**, deviations were observed for C-2 of arabinose (91.4 ppm) and C-1 of xylose (104.6 ppm). Assignments have been made by comparing the spectra with those of methyl β-D-xylopyranoside and methyl-α-L-arabinoside (11), using the assignments reported for nodososide (1) and selective decoupling, which allowed C-4 of arabinose and C-2 of oxylose to be distinguished. Moltiplicities of each signal were determined by *J*-modulated spin-echo technique.

SOLVOLYSIS OF GLYCOSIDE SULFATE (**7**) GIVING **6**.—A solution of the hplc peak eluted after 18 min (conditions given above) (9.8 mg) in dioxane-pyridine was treated as above. The residue was further purified by tlc in $CHCl_3$-MeOH-H_2O (80:18:2) to furnish halityloside H (**6**, 2.5 mg).

RESULTS AND DISCUSSION

Separation of the polar extracts from *H. regularis* was made by using the following successive chromatographic steps: a) recovery of the polar material from the aqueous extracts on a column of Amberlite XAD-2, b) chromatography of the MeOH eluate on column of Sephadex LH-60 to separate the more polar sulfated "asterosaponins" from the less polar steroids, c) dccc to fractionate the less polar components, and d) hplc on a preparative C_{18} μ-Bondapak column.

This procedure afforded six novel glycosides, halityloside A (**5**), B (**4**), D (**2**), E (**1**), F (**3**), and H (**6**), isolated in that order, and two nonglycosidated polyhydroxysteroids **10** and **11**. Compounds **1-3** are characterized by having 24-hydroxysteroid aglycones, while compounds **4-6** have 24-(β-hydroxyethyl) steroid aglycones. We also isolated a

third group of steroidal diglycosides which have a sulfate group: halityloside H, 6-*O*-sulfate (**7**) and halityloside I (**8**).

THE 24-HYDROXYSTEROIDAL GLYCOSIDES.—*Halityloside D.*—(24*S*)-24-*O*-[2,4-Di-*O*-methyl-β-D-xylopyranosyl-(1↦2)-α-L-arabinofuranosyl]-5α-cholestane-3β,4β,6α,8,15β, 24-hexol (**2**) is the major component of this group. An examination of its spectral data (^{1}H and ^{13}C nmr; Tables 3 and 4) indicated that **2** contains one α-arabinofuranosyl, one β-xylopyranosyl unit, and two methoxyl groups. Acid methanolysis yielded methyl arabinoside (gc) and a second methyl glycoside. Benzoylation of the reaction mixture with *p*-bromobenzoyl chloride and pyridine followed by tlc-SiO_2 separation gave methyl 2,3, 4-tri-*O*-(*p*-bromobenzoyl)-β-L-arabinopyrano side, and methyl 2,4-di-*O*-methyl-3-*O*-(*p*-bromobenzoyl-α-D-xylopyranoside, identified by their ^{1}H nmr. The sign of the exciton-split cd curve 236/253, $\Delta\epsilon$ −33.5/+99.3, A +132.8, (calcd. A +140), for the first compound established the L-configuration of arabinose (10); we assumed the common D-configuration for the xylose by analogy with the many D-xylosides isolated from this and other starfishes (1,3,4,7).

The glycosidation shift observed in **2** for the C-2 of the arabinofuranose (92.4 ppm; Table 4) established the location of the terminal 2,4-di-*O*-methylxylose at 2-OH of the arabinose moiety. By subtracting those signals due to the carbons of the disaccharide moiety (Table 4) from the ^{13}C-nmr signals of **2**, the remaining signals (Table 6) were founded to be identical to those assigned to the aglycone carbons of attenuatoside B-1, 24-*O*-[2-*O*-methyl-β-D-xylopyranosyl-(1↦2)-α-L-arabinofuranosyl]-5α-cholestane-3β,4β,6α,8,15β,24-hexol, isolated from the starfish *Hacelia attenuata* (4). Thus, halityloside D (**2**) is related to attenuatoside B-1 by a methyl group at 4-OH of the 2-*O*-methylxylose unit. The resonances associated with the hydrogen atoms of the steroidal moiety also have chemical shift values (Table 5) essentially identical in both spectra, and the Fabms spectrum of **2** showed a molecular ion species at *m/z* 783 (M+Na), fourteen mass units shifted relative to attenuatoside B-1 (*m/z* 769). The major support for the location of a secondary hydroxy group at C-15β is found in a ^{13}C-nmr spectrum where the signal for C-20 is observed at 36.4 ppm. (Note that in 16β-hydroxysteroids this signal is upfield shifted to ca. 31.0 ppm.) Further, the 3β,4β-di-OH assignment was confirmed by the formation of an acetonide from **2**. Recently we have assigned the 24*S*-configuration to nodososide, 24-*O*-[2-*O*-methyl-β-D-xylopyranosyl-(1↦2)-α-L-arabinofuranosyl]-5α-cholestane-3β,5,6β,8,15α,24-hexol (15), and we now propose the same 24-*S* configuration for **2** and related **1** and **3** because the chemical shifts of the signals assigned to the side chain carbons are virtually identical to those of the corresponding signals for nodososide (Table 6). The common 20*R* configuration proposed for this and the other steroids described in this paper is based on the chemical shifts of the 21-methyl protons (ca. 0.95 in compounds with saturated side chain and 1.04 in the Δ^{22}-compound **9**) (16).

Halityloside E (**1**).—Compound **1** is the 4-deoxy derivative of halityloside D (**2**) and is related to attenuatoside A-1, a glycoside from *H. attenuata* (3) by introduction of a methyl group at the 4-OH of the 2-*O*-methylxylose unit.

Halityloside F.—(24*S*)-24-*O*-[2,4-Di-*O*-methyl-β-D-xylopyranosyl-(1↦2)-α-L-arabinfuranosyl]-5α-cholestane-3β,6β,8,15β,24-pentol (**3**), is epimeric with halityloside E (**1**) and differs from **1** in its stereochemistry at C-6, which in **3** is 6β-OH. The ^{1}H-nmr spectrum showed the 19-methyl singlet downfield shifted to 1.21 and an apparent quartet at 3.88 ppm with J=3 Hz, characteristic of an equatorial proton coupled with three other protons (6β- or 11β-hydroxy group). The signal at 19.9 ppm (CH by *J*-modulated spin-echo) in the ^{13}C-nmr spectrum was assigned to C-11 and eliminated the possibility of a hydroxyl group located there. The ^{13}C-nmr spectrum

TABLE 5. ^{1}H-nmr data (500 MHz, CD_3OD) for Steroidal Hydrogens in **1-11** in δ (Hz)

H at C	1	2	3	4[a]	5[a]	6[a]	7[b]	8[b]	9	10	11
3	3.55 m	—[c]	3.65 m	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	3.62 m	3.5 m
4		4.29 br s			4.29 br s	4.29 br s	4.33 br s	4.33 br s	4.29 br s		4.29 br s
6	3.74 ddd (10.5,10.5,4)	4.20 ddd (10.5,10.5,4)	3.88 q (3)	3.74 ddd (10.5,10.5,5)	4.22 ddd (10.5,10.5,4)	4.22 ddd (10.5,10.5,4)	4.95 ddd	4.95 ddd	4.21 ddd (10.5,10.5,4)	3.74 ddd (10.5,10.5,4)	4.22 ddd (10.5,10.5,4)
7	2.40 dd[d] (12,3.5)	2.48 dd[d] (12,4)	2.42 dd (14,3)	2.42 dd[d] (12,3.5)	2.50 dd[d] (12.5,4)	2.50 dd[d] (12.5,4)	2.73 dd[d]	2.72 dd[d]	2.48 dd[d] (12,4)	2.42 dd[d] (12.5,4)	2.49 dd[d] (12.5,4)
15	4.45 m	4.45 m	4.45 m	4.42 dd (5.6,6.5)	4.42 dd (5.6,6.6)	4.41 dd (5.6,6.6)			4.45 m	4.40 dd (5.6,6.7)	4.40 dd (5.6,6.8)
16	2.40 m[d]	2.40 ddd[d] (15,12,5)	2.39 m[d]	4.25 t (6.5)	4.25 dd (6.6,7.0)	4.25 t (6.5)			2.27 m[d]	4.25 t (6.5)	4.25 t (6.5)
24	—[e]	—[e]	—[e]								
28									0.96 d (6.8)		
18	1.30 s	1.29 s	1.30 s	1.28 s	1.27 s	1.27 s			1.32 s	1.27 s	1.27 s
19	1.02 s	1.19 s	1.21 s	1.02 s	1.19 s	1.19 s	1.27 s	1.27 s	1.19 s	1.02 s	1.19 s
21	0.96 d (6.3)	0.96 d (6.3)	0.97 d (6.6)	0.98 d (6.6)	0.99 d (6.3)	0.99 d (6.3)			1.04 d (6.5)	0.98 d (6.5)	0.98 d (6.5)
26	0.94 d (6.4)	0.94 d (6.5)	0.94 d (6.6)	0.91 d (6.8)	0.91 d (6.8)	0.91 d (6.8)			3.4-3.55 m	3.46 dd[d] (10,5)	3.46 dd[d] (10,5)
27	0.93 d (6.5)	0.93 d (6.5)	0.93 d (6.6)	0.88 d (6.8)	0.88 d (6.9)	0.88 d (6.8)			0.94 d (6.5)	0.95 d (6.5)	0.94 d (6.5)

[a]In the 24-(β-hydroxyethyl)-steroids, **4-7**, the 29-hydroxymethylene protons are confused in the region δ 3.4-3.6

[b]**7** is the 6-*O*-sulfated derivative of **6** and **8** is the 6-*O*-sulfated derivative of **9**; reported only signals which are shifted relative to their desulfated analogue.

[c]Signal confused in the δ 3.6-3.5 region. In spectra of 3β,4β-dihydroxyderivative, **2**,**5**,**6**, and **9**, the 3-H signal was detected by decoupling.

[d]The other 7- and 16-proton are confused in the high field region of the spectrum; the other 26-proton resonates under solvent signal.

[e]Signal under solvent signal.

TABLE 6. ^{13}C-nmr shifts (62.9 MHz, CD_3OD) of steroid carbons in halitylosides **1-6** and in compounds **9** and **11** in ppm[a]

Carbon atoms	1	2	3	N[b]	4	5	6	9	11
1	39.5	39.8	41.4	34.4	39.5	39.6	39.6	39.8	39.7
2	31.5	26.3	31.7	30.9	31.5	26.2	26.3	26.2	26.2
3	72.2	73.7	72.4	68.2	72.2	73.6	73.7	73.7	73.7
4	32.4	69.2	36.4	41.6	32.4	69.1	69.2	69.2	69.2
5	54.0	57.4	49.8	76.5	54.0	57.2	57.4	57.4	57.4
6	67.7	64.8	74.8	77.9	67.7	64.7	64.8	64.8	64.8
7	—[c]	49.8	44.3	41.1	49.8	—[c]	—[c]	49.5	—[c]
8	77.3	77.5	79.4	77.2	77.3	77.1	77.2	77.5	77.2
9	57.5	58.2	57.1	48.7	57.6	58.5	58.6	58.6	58.6
10	38.0	38.2	36.8	39.2	38.0	38.1	38.0	38.2	38.3
11	19.7	19.1	19.9	19.7	19.5	18.9	18.9	19.2	18.9
12	43.5	43.4	43.3	42.9	43.6	43.4	43.6	43.3	43.6
13	44.4	44.5	44.4	45.5	44.6	44.5	44.7	44.3	44.6
14	62.7	62.9	61.9	66.5	61.3	61.2	61.4	63.0	61.4
15	71.2	71.3	71.4	70.1	71.2[d]	71.2[d]	71.4[d]	71.2	71.3
16	42.5	42.5	42.4	40.4	72.9[d]	72.8[d]	72.9[d]	43.5	72.8
17	58.1	58.6	58.1	55.9	63.1	62.9	62.9	57.9	63.2
18	16.5	16.5	16.6	15.4	17.9	17.9	17.9	16.7	17.9
19	14.1	16.9	15.7	18.0	14.1	16.9	16.9	16.9	16.9
20	36.4	36.4	36.4	36.3	31.4[e]	31.3[e]	31.4[e]	40.0[f]	31.0
21	19.1	19.2	19.1	19.0	18.6	18.6	18.5	21.1	18.4
22	33.0	33.0	33.0	32.8	34.8	34.7	34.8	137.2	37.1
23	28.8	28.9	28.9	28.6	28.7	28.6	28.8	133.9	24.8
24	84.7	84.8	84.8	84.5	42.4	42.1	42.6	40.8	35.0
25	31.6	31.7	31.7	31.5	30.8[e]	30.6[e]	30.6[e]	39.6[f]	37.0
26	18.3	18.3	18.2	18.2	19.2	19.1	19.1	74.0	68.6
27	18.3	18.4	18.3	18.3	20.0	20.0	20.0	17.3	17.3
28	=	=	=	=	32.0	31.8	31.8	14.5	=
29	=	=	=	=	69.7	69.7	68.1	=	=

[a]The signals were assigned using the *J*-modulated spin-echo technique (positive signals for C and CH_2 groups and negative ones for CH and CH_3), selective decoupling for C-24 assignment in **1-3** and **N**, and hydroxyl substituent parameter found in steroids (12,13), by chemical shift comparison with known polyhydroxysteroids (3,4,5,14) and by comparison from compound to compound.

[b]**N** is nodososide, *i.e.* (24*S*)-24-*O*-[2-*O*-methyl-β-xylopyranosyl-(1↦2)-α-L-arabinofuranosyl]-5α-cholestane-3β,5,6β,8,15α,24-hexol; the spectrum reported in reference 1 was run in d_5-pyridine.

[c]Signals under solvent signals (ca. 50 ppm);

[d,e,f]Assignments may reversed within the same column.

(Table 6) and comparison with model 5α-cholestane-3β,6β-diol (17) and the previous glycosides **1** and **2** confirmed the proposed formulation for the aglycone and indicated C-24 to be the site of glycosidation. We note that the calculations of the chemical shift value of the 18-protons by using the additive substituent parameters from the Zürcher's compilations (18) led to a value of 1.14 ppm, which is far from the observed one (δ 1.30) in **1**, **2**, **3**, **9**. In the above compounds, the contributions of the proximate 8β- and 15β-hydroxyl groups may be modified by steric effects and skeletal deformations, thereby negating the approach based on independent contributions by the structural units.

THE 24-(β-HYDROXYETHYL) STEROIDAL GLYCOSIDES.—*Halityloside A*.—(24*R*)-29-*O*-[2-*O*-Methyl-β-D-xylopyranosyl-(1↦2)-β-D-xylopyranosyl]-24-ethyl-5α-cholestane-3β,4β,6α,8,15β,16β,29-heptol (**5**) is the major glycoside component of the mixture. Acid methanolysis yielded methyl xyloside and methyl 2-*O*-methylxyloside

(gc). Benzoylation with *p*-bromobenzoyl chloride and pyridine of the reaction mixture followed by tlc-SiO_2 separation gave methyl 2,3,4-tri-*O*-(*p*-bromobenzoyl)-α-D-xylopranoside, and methyl 2-*O*-methyl-3,4-di-*O*-(*p*-bromobenzoyl)-α-D-xylopyranoside, identified by ^{1}H nmr. The cd curve, 236/253, Δε +17.4/−35.9, A −53.3 (calcd. A = −62) (10) of the second structure establish the D-configuration of 2-*O*-methylxylose; in the case of a 1,2,3 *eee* tribenzoate system (first structure), the calculated "A value" is zero, and we have observed a very small split cd curve with a negative "A value." The shifts of the anomeric carbons by ^{13}C-nmr (Table 8) and the coupling constants of the anomeric protons at δ 4.74 (J=7.1 Hz) and 4.40 (J=7.4 Hz) by ^{1}H nmr (Table 7) have suggested that the glycosidic linkages in **5** are β. The presence of two low-field signals at 84.7 and 81.5 ppm in the ^{13}C-nmr spectrum (Table 8) suggests the sequence and the interglycosidic linkage of the disaccharide moiety since the lower-field signal is due to C-2 of the 2-*O*-methylxylose, and the second one is assignable to the glycosidated carbon-2 of the xylosyl unit (19). The fabms confirmed the sequence 2-*O*-methylxylosyl-xylosylaglycone from the sequential loss of 146 (2-*O*-methylxylose) and 132 (xylose) mass units from M+H and water losses, i.e. *m/z* 591 (M+H −146−3H_2O) and 459 (M+H −278−3H_2O).

TABLE 7. ^{1}H-nmr (500 MHz, CD_3OD) Signals of 2-*O*-Methyl-β-D-xylopyranosyl-(1↦2)-β-D-xylopyranosyl Moiety in Compounds **4**, **5**, and **9**[a]

Chemical shifts (ppm)	protons (no.)	Multiplicities (Hz)	Assignment
4.74	1	d, (7.1)	1-H
4.40	1	d, (7.4)	1-H
3.92	1	dd, (11.9, 4.8)	5-He
3.86	1	dd, (8.0, 4.8)	5-He
3.65	3	s	OCH_3
3.38	1	t, (8.7)	3-H
3.20	1	dd, (11.9, 9.5)	5-Ha
2.97	1	dd, (8.7, 7.1)	2-H

[a]The remaining sugar protons gave complex overlapping signals in the region δ 3.4-3.6.

The fabms showed a major molecular ion species at *m/z* 813 (M+Na) corresponding to a molecular weight of 790 dalton. The glycosyl residue accounts for 279 m.u. leaving 511 m.u. for the aglycone. This corresponds to a C_{29} saturated sterol, m.w. 512, with seven hydroxyl groups (five secondary, one primary and one tertiary; J-modulated spin-echo ^{13}C nmr). 24-(β-Hydroxyethyl)-cholestane aglycones were already found in 29-*O*-xylosides (attenuatosides) isolated from the starfish *H. attenuata* (5), and in agreement with such a structure the ^{1}H nmr of **5** showed three methyl doublets at δ 0.99 (21-H) and 0.91-0.88 (26- and 27-H), and two methyl singlets at δ 1.27 and 1.19 (Table 5). The ^{13}C-nmr spectrum (Table 6) confirmed the presence of a 24-(β-hydroxyethyl) side-chain for the aglycone and suggested C-29 to be the site of glycosidation as the chemical shifts of the signals assigned to the side-chain carbons are virtually identical to those of the corresponding signals for attenuatoside S-I (5). At 500 MHz, the ^{1}H-nmr spectrum showed four isolated signals for the hydroxymethine protons of the aglycone (the signal for the 3-H is obscured in the region δ 3.4-3.6): namely, an apparent double triplet (J=4.0 and 10.5 Hz) due to 6β-H shifted downfield to δ 4.22 by the presence of the 4β-hydroxy group, one br singlet at δ 4.28 due to 4α-H, and two doublets of doublets at δ 4.25 (1H, J=6.6, 7.0 Hz) and 4.42 (1H, J=5.6, 6.6 Hz) coupled

TABLE 8. Assignments of ^{13}C-nmr (62.9 MHz, CD_3OD) Signals to 2-*O*-Methyl-β-D-xylopyranosyl-(1→2)-β-D-xylopyranosyl Moiety of Compounds **4**, **5**, and **9** in ppm[a]

	C-1	C-2	C-3	C-4	C-5	OCH_3
xylose	103.6	81.5	76.9	71.3	66.5	=
2-*O*-mexylose	104.4	84.7	77.7	71.3	66.6	60.7

These data have been extracted from the spectrum of **5**, Assignments were suggested by using the assignments reported for the 2-*O*-methyl-β-D-xylopranosyl carbons of nosodoside (1) and related glycosides (3,4) and for methyl β-D-xylopyranosyl-(1→2)-β-D-xylopyranoside (19).

to each other. These latter two signals led to a structural element with vicinal, secondary hydroxy groups both adjacent to a carbon bearing one proton. With the tertiary hydroxy group at C-8, there was only one way such a fragment could occur in our steroid i.e., 15, 16. The ^{13}C-nmr spectrum of **5** (Table 6) confirmed the 3β,4β,6α,8-hydroxylation pattern (compare the spectra of compounds **2** and **5**) and also suggested the 15β,16β-dihydroxy stereochemistry. The 15α,16β- stereochemistry was readily eliminated as the chemical shifts for C-15 and C-16 of 15α,16β-dihydroxy steroids previously isolated from the starfishes (14) are far (ca. 80-83 ppm) from our values. The 15α,16α-stereochemistry could be eliminated by the downfield shift of the 18-methyl protons in the ^{1}H-nmr spectrum, and the downfield shift of carbon 18 in the ^{13}C-nmr spectrum. If **2** is used as a starting structure (15β-OH) and ^{13}C-nmr values are calculated for the compound with an additional 16α-hydroxy group, using the substituent effects that have been published for hydroxy steroids (12), the calculated C-14: 60.5, C-15: 83.2, C-16: 93.8, C-17: 69.9 ppm and experimental data are significantly different. Introduction of a 16β-hydroxy group in **2** is expected to cause a large deviation from the additivity for C-15 and C-16 (ranging from −10 to −15 ppm) because of the very strong steric interactions between the vicinal *cis*-hydroxy groups (13). In agreement with this, the signals for C-15 and C-16 71.2 and 72.8 ppm are considerably shifted upfield from the predicted positions C-15: 83.2 and C-16: 93.6 ppm employing the additivity parameters (12). We note that C-16 is shifted upfield further by the vicinal side-chain at C-17. The *cis*-stereochemistry of the vicinal 1,2-diol groups at C-15 and C-16 were confirmed by the formation of a bis-acetonide from **5**.

The 24*R* configuration is compatible with ^{1}H- and ^{13}C-nmr data but is not rigorously established. In the 220 MHz spectrum of 29-hydroxyclionaterol (24*R*), the C-26 and C-27 methyl protons appear as a triplet at δ 0.84 because coincidental overlap of the low-field arm of one doublet (δ *0.83*) with the high-field arm of the other (δ *0.86*). On the other hand, the C-26 and C-27 methyl protons appear as two overlapping doublets at 0.84 and 0.85 in the spectrum of 29-hydroxysitosterol (24*S*) (20). In our spectrum, the isopropyl methyl protons appear as two doublets separated by 0.03 ppm as in the case of 24*R*-isomer. Very recently, the ^{13}C-nmr spectra of the (24*R*)- and (24*S*)-24-ethyl-5α,-cholest-7-ene-3β,29-diol were measured, and significant differences were obtained for the resonances of the isopropyl methyl carbons. In the (24*S*)-isomer, the 26- and 27-carbons appear as very close signals at 19.2 and 19.3 ppm, while in the (24*R*)-isomer they are separated by ca. 1 ppm (18.6-19.7 ppm).[3] Again our values (19.1-20.0) compared better with those of the 24*R*-isomer.

Halityloside B (**4**).—This compound is closely related to **5**, but lacks the 4β-hydroxy group. The structure was derived from nmr spectroscopy and comparison with **5**. The fabms gave a molecular ion species at *m/z* 797, shifted sixteen mass units relative

[3]M. Anastasia, P. Allevi, P. Ciuffreda (Dipartimento di Chimica e Biochimica Medica, Universitá di Milano), and R. Riccio, unpublished results.

to halityloside A (**5**), *m/z* 813. On treatment with Me_2CO/TsOH, it gave a monoacetonide.

Halityloside H (**6**).—The ^{13}C-nmr signals for the aglycone carbons (Table 5) were virtually identical with those of halityloside A (**5**). The sugar carbon signals were superimposable on those of compounds **1** - **3**, apart from minor deviations for C-2 of arabinose and C-1 of xylose. A small deviation for the chemical shift of carbon-29 on passing from **5** to **6** was also observed. Fabms gave a molecular ion at *m/z* 827 (M+Na).

THE SULFATED GLYCOSIDES.—*Halityloside H, 6-O-sulfate* (**7**).—The fabms gave a molecular ion at *m/z* 929 (M+Na), where M is the molecular weight of the sodium salt. The ^{1}H-nmr spectrum was very similiar to that of halityloside H (**6**), expect the signals for 6-H, 7-H, and 19-H were shifted downfield to δ 4.95 (4.22 in **6**), 2.73 (2.50 in **6**), and 1.32 (1.19 in **6**) ppm, respectively. These data placed the sulfate group at C-6 in **6**. On solvolysis using dioxane-pyridine mixture (21), it afforded halityloside H (**6**).

Halityloside I (**8**).—The fabms gave a molecular ion at *m/z* 883. Solvolysis of **8** afforded the desulfated derivative **9**, which gave molecular ion at *m/z* 781 (M+Na). This established that **8** is the sodium salt of a sulfated molecule. An examination of ^{1}H- and ^{13}C-nmr spectra of **9** immediately suggested the presence of a 2-*O*-methyl-β-xylopyranosyl (1↦2)-β-xylopyranosyl unit; the ^{13}C-nmr spectrum also established the 3β,4β,6α,8,15β-hydroxylation pattern, as the signals for the carbons 1-19 were virtually identical with those of the corresponding signals of **2** (Table 6). At 500 MHz, the ^{1}H-nmr spectrum of **9** contained an eight-line pattern centered at δ 5.28 with J=15 (coupling 22-23-H *trans*), 7.5 and 4.5 Hz, which could be assigned to the Δ^{22}-*trans* protons. In the methyl region, a doublet at δ 1.04 (J=6.5 Hz) was in agreement with the expected value for the C-21 methyl group (20*R*-configuration) (16). The protons at C-20 and C-24 in allylic positions have been shifted downfield and overlap at δ 2.18. Irradiation of this multiplet in a double resonance experiment did collapse the methyl doublets at 1.04 (21-H) and 0.96 (28-H) ppm to singlets and also simplified the olefinic signal at 5.28. Several overlapping signals around 1.65 ppm were next irradiated. The methyl doublet at δ0.94 (27-H) collapsed to a singlet and the multiplets centered at δ 3.40 and 3.55 ppm [−C(26)H_2O−] were simplified. These experiments indicated a -CH(CH_3)-CH=CH-CH(CH_3)-(CH(CH_3)-CH_2O-side chain. The location of the glycosyl residue at the primary hydroxy group at C-26 followed from the ^{13}C-nmr data, which excluded C-3, C-4, C-6, C-8, and C-15 as sites of glycosidation. Further, the ^{13}C-nmr chemical shift values of the side chain carbons were in agreement with ^{13}C-nmr data of a Δ^{22}, 24-methyl-26-hydroxysteroid C-22: 136.2, C-23: 134.1, C-24: 40.4, C-25: 42.2, C-26: 66.9, C-27: 17.5, C-28: 14.5 ppm isolated from the starfish *Echinaster sepositus* (22), and the glycosidation shifts that have been reported for aglycones with primary hydroxy groups (23). In **8**, the sulfate group was located at C-6, as the downfield shifts of 6-H, 7-H, and 19-H in ^{1}H nmr relative to the desulfated analogue **9** (Table 5).

THE POLYHYDROXYSTEROIDS: **10** AND **11**.—*(25S)-5α-Cholestane-3β,4β,6α,8,15β,16β,26-heptol* (**11**).—The eims showed a small molecular ion at *m/z* 484 corresponding to a fully saturated cholestane-heptol. The fragmentation pattern with ions for stepwise water loss, *m/z* 466, 448.3138 ($C_{27}H_{44}O_5$ requires 448.3189), 430, 412, and with ions corresponding to the loss of a hydroxylated C-8 side chain together with one, two, three, and four molecules of water (*m/z* 337, 319, 301, and 283) and with ions at *m/z* 241 and 225 (cleavage of the 12,13 and 8,14 bonds) closely resembled that observed in the spectrum of 5α-cholestane-3β,4β,6α,8,15α,16β,26-heptol isolated from the starfish *Protoreaster nodosus* (24). The cholestane- heptol from *H. regularis* differs from the above sterol only in its stereochemistry at C-15, which in **11** is 15β-OH.

The ^{13}C-nmr signals for the side chain carbons were identical in both spectra, while the signals for the carbons 1-19 in the ^{13}C-nmr spectrum of **11** were identical with those of the corresponding carbons of **5**. On treatment with Me_2CO and TsOH, it gave a bis-acetonide. The 25*S* configuration was recently assigned to 5α-cholestane-3β,6α,8,15α,16β,26-hexol (15). We assume the same 25*S* configuration for this sterol because the shifts of the signals assigned to side chain carbons by ^{13}C-nmr are identical in both compounds (14).

(25S)-5α-Cholestane-3β,6α,8,15β,16β,26-hexol (**10**).—This minor steroid is related to **11**. It lacks the 4β-hydroxy group, and its structure was derived from ^{1}H-nmr spectroscopy, eims (Experimental), and comparison with **11**. On treatment with Me_2CO and TsOH, it gave a monoacetonide.

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