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STARFISH SAPONINS, PART 53.[1] A REINVESTIGATION OF THE POLAR STEROIDS FROM THE STARFISH *OREASTER RETICULATUS*: ISOLATION OF SIXTEEN STEROIDAL OLIGOGLYCOSIDES AND SIX POLYHYDROXYSTEROIDS

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ABSTRACT.—A reinvestigation of the starfish *Oreaster reticulatus* has led to the isolation of sixteen steroidal oligoglycosides and six polyhydroxysteroids. One steroidal monoglycoside has been identified as asterosaponin-1 [**5**], previously isolated from the same organism, and one pentaglycoside steroidal sulfate has been identified with the known ophidianoside F [**1**], previously isolated from starfish species of the family Ophidiasteridae. The novel pentaglycoside steroidal sulfates reticulatosides A [**3**] and B [**4**] represent the second examples of asterosaponins containing the 5α-cholesta-9(11)-en-3β,6α,20,22-tetraol aglycone. Of the remaining new steroidal oligoglycosides, the majority are characterized by the common (24*S*)-5α-cholesta-3β,6α,8,15α,24-pentaol aglycone, with some having a sulfate group at C-6, and differing in the sugar moiety. Compounds **11**, **12**, **15**, and **16** represent major departures from the more common structural features encountered in steroidal glycosides from starfish with the presence in **11** and **12** of a rare 5-*O*-methylgalactofuranosyl unit and in **15** and **16** of a 3-*O*-methyl-2-*O*-sulfate-xylopyranosyl unit. The latter was shown by nmr data combined with molecular dynamics calculations to exist in an 8:2 equilibrium mixture of the $^1C_4$ and $^4C_1$ conformations.

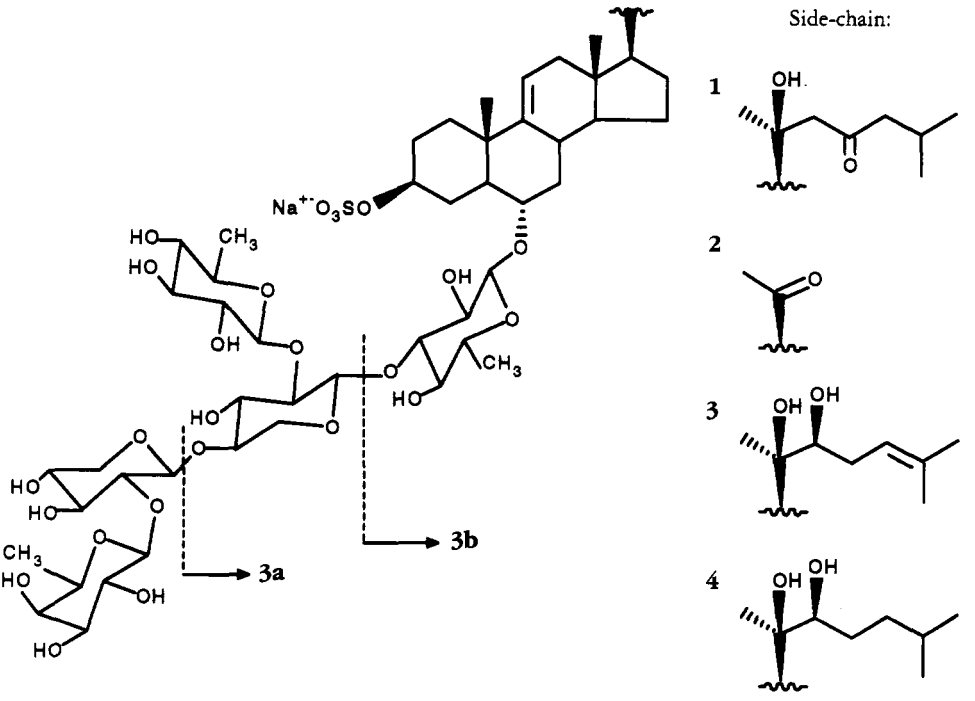
The predominant secondary metabolites of starfish are steroidal oligoglycosides and polyhydroxysteroids, which occur as complex mixtures and have usually been found in minute amounts in almost all species examined (2,3). A further illustrative example of the complexity of polar constituent mixtures in starfish is now represented by *Oreaster reticulatus* L. (family Oreasteridae, order Valvatidae), from which a mixture of twenty-two compounds has been separated into four asterosaponins [**1–4**], twelve glycosides of polyhydroxysteroids [**5–16**], and six polyhydroxysteroids [**17–22**]. The asterosaponin are the highest mol wt components, made up of a pentasaccharide chain linked at C-6 of a $\Delta^{9(11)}$-3β,6α-dihydroxysteroid 3-sulfated aglycone, while the polyhydroxysteroid glycosides are made up of a polyhydroxysteroidal aglycone and a carbohydrate portion made up from one or two monosaccharide units.

One steroidal monoglycoside has been identified as asterosaponin-1 [**5**], previously isolated from *Patiria pectinifera* (4) and again from this same organism (5). The asterosaponin ophidianoside F [**1**], and the polyhydroxysteroids **17–21** are also known compounds, with compound **1** previously isolated from *Ophidiaster ophidianus* (6) and the related *Linckia laevigata* (7). Compounds **17–19** were first obtained from *Protoreaster nodosus* (8) and then from many other related species (2), with compound **20** isolated from *Asterina pectinifera* (9), and compound **21** from *Oreaster reticulatus* (5). The isolation and structure elucidation of the remaining new compounds are described in this report.

RESULTS AND DISCUSSION

Oreaster reticulatus was collected in the Bahamas and separation and isolation of the

[1]For part 52, see Iorizzi *et al.* (1).



individual compounds from the extracts followed steps described previously (2,10). In brief, the organism was extracted with H_2O and then with Me_2CO, and all compounds were isolated from the MeOH eluate of an Amberlite XAD-2 resin through which the H_2O extracts of *O. reticulatus* were eluted and combined with the *n*-BuOH-soluble portion of the Me_2CO extracts.

Separation and purification were carried out by chromatography on Sephadex LH-60, from which the asterosaponins [**1**–**4**] were separated from the sulfated steroidal mono- and di-glycosides and sulfated polyhydroxysteroids, and from the non-sulfated constituents which were eluted as the last components, followed by dccc and hplc steps. The results of our analysis are shown in Table 1.

The known asterosaponin ophidianoside F [**1**] was identified by direct comparison (hplc, ^{1}H nmr, fabms) with an authentic sample isolated from *Ophidiaster ophidianus* (6). The fabms (*m/z* 1113 [$M_{SO_3}^-$]) and the ^{1}H- and ^{13}C-nmr spectra of compound **2** agreed in all respects with the structure of the asterone (2,11) analogue of ophidianoside F, probably formed during extraction by retroaldol cleavage of the thornasteryl A moiety of **1**.

On acid methanolysis, the novel reticulatoside A [**3**] liberated methyl xyloside, methyl fucoside, and methyl quinovoside in the proportion 2:1:2. Permethylation followed by methanolysis of the methylated material gave methyl 2,3,4-tri-*O*-methyl fucoside and methyl 2,3,4-tri-*O*-methyl quinovoside, implying that the sugar moiety of compound **3** has one branch with fucose and quinovose as terminal units. The fabms gave a molecular anion peak at *m/z* 1213 and fragments at *m/z* 1067 and 935 corresponding to the sequential loss of fucose (or quinovose) and xylose, also present in the spectrum of ophidianoside F [**1**]. Thus, reticulatoside A [**3**] is isomeric with ophidianoside F [**1**]. The ^{1}H-nmr spectrum of the intact saponin (Table 2) revealed signals due to aglycone protons identical with those observed in the analogous spectrum of protoreasteroside, the only known asterosaponin containing a 5α-cholesta-9(11),24(25)-dien-3β,6α,20,22-tetraol 3β-sulfated aglycone, isolated from *Protoreaster nodosus* and *Pentaceraster alveolatus*

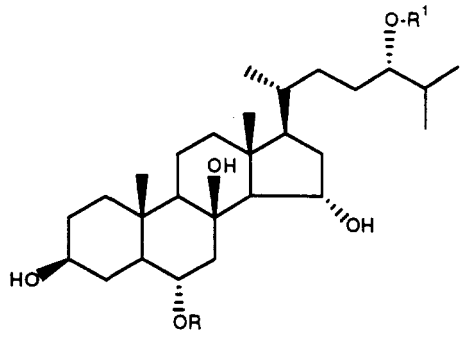
TABLE 1. Polar Steroidal Constituents Isolated from the Starfish *Oreaster reticulatus*.[a]

Compound	Amount (mg)	[α]D	Reference	Hplc[b] eluent (MeOH-H_2O)
Asterosaponins				
Ophidianoside F [**1**]	3.0		Riccio *et al.* (6)	1:1
Asterone analogue [**2**]	15.0			45:55
Reticulatoside A [**3**]	6.0	−7.9°		1:1
Reticulatoside B [**4**]	4.2	−6.6°		
Glycosides of polyhydroxysteroids				
Asterosaponin P-1 [**5**]	2.5		Kicha *et al.* (4)	1:1
Oreasteroside A [**6**]	0.5	+20°		7:3
Oreasteroside B [**7**]	5.8	−15°		7:3
Oreasteroside C [**8**]	4.4	−7.5°		7:3
Oreasteroside D [**9**]	3.5	−15.3°		7:3
Oreasteroside E [**10**]	1.0	−16.6°		7:3
Oreasteroside F [**11**]	2.3	+10°		7:3
Oreasteroside G [**12**]	18.0	+13.8°		1:1
Oreasteroside H [**13**][c]	1.5	+5.0°		1:1
Oreasteroside I [**14**]	3.3	+10°		1:1
Oreasterosides J and K [**15**+**16**]	2.0			1:1
Polyhydroxysteroids				
17	4.5		Riccio *et al.* (8)	7:3
18	25.0		Riccio *et al.* (8)	7:3
19	7.0		Riccio *et al.* (8)	7:3
20	1.5		Higuchi *et al.* (9)	75:25
21	5.0		Segura de Correa *et al.* (5)	1:1
22	1.5	+12.5°		1:1

[a]From 4.5 kg fresh wt.
[b]C_{18} μ-Bondapak column (30 cm×7.8 mm i.d.); flow rate 5 ml/min.

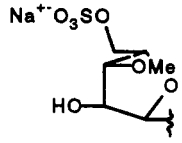
(12). Particularly characteristic were the resonances of the 18- and 21-methyl protons at δ 0.85 and 1.25, respectively, shifted relative to those observed in the spectrum of the corresponding thornasterol A-containing saponin [**1**], δ 0.81 and 1.37, respectively. In the ^{13}C-nmr spectrum of **3** the aglycone carbon signals (Table 2) were superimposable on those of protoreasteroside (12), while the sugar carbon signals (Table 2) matched very closely those of ophidianoside F [**1**]. The 20*R*,22*S*-configuration in the aglycone of **3** was based on the same arguments used before with protoreasteroside (12), i.e., the chemical shift of the C-21 methyl proton signal of the intact saponin in pyridine-d_5 (δ 1.60) and of the 20,22-d_6-acetonide (δ 1.41) of the monoquinovoside **3b**, in comparison with the reported data for 5α-cholesta-3β,20,22-triol models (13). The monoquinovoside was obtained from **3** along with the trisaccharide **3a**, made up by only quinovose (×2) and xylose, on enzymatic hydrolysis with *Charonia lampas* glycosidase mixture. The structure of the trisaccharide moiety of **3a**, which was determined by spectral comparison with prosapogenol qui$\overset{1\to2}{\rightarrow}xyl\overset{1\to3}{\rightarrow}$qui-aglycone derived from, e.g., patirioside A (14), gave further support to the sequence and the structure of the saccharide chain in **3**. Thus, the novel reticulatoside A can be defined as (20*R*,22*S*)-20,22-dihydroxy-6α-*O*-β-D-fucopyranosyl-(1→2)-β-D-xylopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl-5α-cholest-9(11)-en-3β-yl sodium sulfate [**3**].

Reticulatoside B [**4**] is related to **3**, being its 24,25-dihydro- derivative, as shown by fabms, *m/z* 1215 [$M_{SO_3}^-$], and its ^{1}H- and ^{13}C-nmr spectra (see Tables 2 and 3).

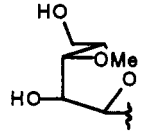


Sugar chain R^1=

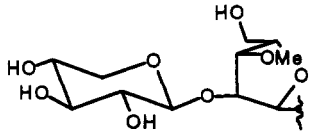
5 R=H



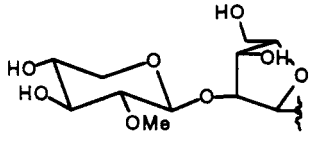
6 R=H



7 R=H

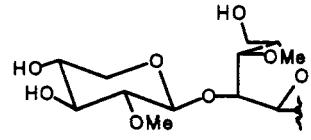


8 R=H

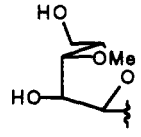


Sugar chain R^1=

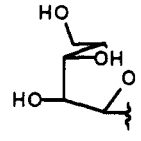
9 R=H



13 R=$SO_3^-Na^+$



14 R=$SO_3^-Na^+$



Identification of the known polyhydroxysteroid asterosaponin-1 [**5**] was achieved by direct comparison (fabms, ^{1}H and ^{13}C nmr) with an authentic sample (5).

The ^{1}H-nmr data of the oreasterosides [**6–9**] as shown in Table 4, demonstrated conclusively that they have the same 5α-cholestane-3β,6α,8,15α,24*S*-pentaol aglycone as in **5** but a different sugar unit. Thus, it was only necessary to settle the structure of the saccharide moieties. The fabms spectrum of the very minor component orestearoside A [**6**], *m/z* 597 $[M-H]^-$, 451 $[M-H-146]^-$, along with the presence of a methoxy singlet at δ 3.45 in the ^{1}H-nmr spectrum, indicated that **6** contained a methoxylated pentose (146 mass units) moiety. A detailed analysis of the ^{1}H-nmr spectrum and comparison with the desulfated asterosaponin-1 [**5**] (5) established the (24*S*)-24-*O*-α-(3-*O*-methyl-L-arabinofuranosyl)-5α-cholestane-3β,6α,8,15α-tetraol structure (15). The 24*S*-configuration was assigned by analogy with asterosaponin P-1, whose stereochemistry was recently confirmed by X-ray crystallographic analysis of its desulfated derivative (15).

The fabms of oreasteroside B [**7**] gave a quasi-molecular ion at *m/z* 729 $[M-H]^-$, accompanied by fragments at *m/z* 597 and 451 corresponding to the consecutive loss of a pentose (132 mass units) and of an *O*-methyl pentose (146 mass units) residue, thus indicating the sequence. Examination of the ^{1}H-nmr spectral data of **7**, aided by

TABLE 2. ^{1}H- and ^{13}C-Nmr Data for the Steroidal Aglycone of the Asterosaponins, Reticulatosides A [**3**] and B [**4**].

Position	Compound					
	3			**4**		
	^{1}H		^{13}C	^{1}H		^{13}C
	(CD_3OD)	(C_5D_5N)	(C_5D_5N)	(CD_3OD)	(C_5D_5N)	(C_5D_5N)
1			35.4			35.2
2			28.6			27.8
3	4.22 m		78.0	4.22 m		77.3
4			30.0			30.5
5			48.5			48.4
6			79.6			79.2
7			40.6			40.6
8			34.8			34.6
9			144.5			144.7
10			37.8			37.5
11	5.37 br d (5.5)		116.5	5.37 br d (5.5)		116.1
12			42.1			42.0
13			41.1			40.9
14			53.5			53.3
15			22.6			22.3
16			24.8			24.6
17			54.5			54.4
18	0.84 s	0.94 s	13.1	0.84 s	0.93 s	12.9
19	1.03 s	1.10 s	18.8	1.03 s	1.10 s	18.6
20			76.8			76.2
21	1.25 s	1.60 s	20.4	1.23 s	1.58 s	20.5
22			77.6			77.0
23			29.8			27.8
24	5.28 t (6.7)		123.4			36.5
25			130.8			
26	1.66 s	1.57 s	25.7	0.94 d (6.8)	0.84 d (6.8)	21.7
27	1.76 s	1.66 s	17.6	0.94 d	0.86 d	22.0

decoupling experiments, showed signals for an α-arabinofuranosyl and a β-xylopyranosyl residue, and one methoxyl group. The upfield shift of H-3′ to δ_H 3.73 m (δ 4.00 in the arabinofuranosides) suggested the location of the methoxyl group at C-3′ of the arabinose residue. In our experience, a downfield shift for the signal of the anomeric proton of the 3-*O*-methyl arabinofuranosyl unit to δ_H 5.17 is indicative of the location of the β-xylopyranosyl unit at C-2′ of an arabinose attached at C-24 of the aglycone (2).

TABLE 3. ^{13}C-Nmr Data for the Saccharide Moiety of the Asterosaponins Reticulatosides A [**3**] and B [**4**] in C_5D_5N and One Drop of H_2O.[a]

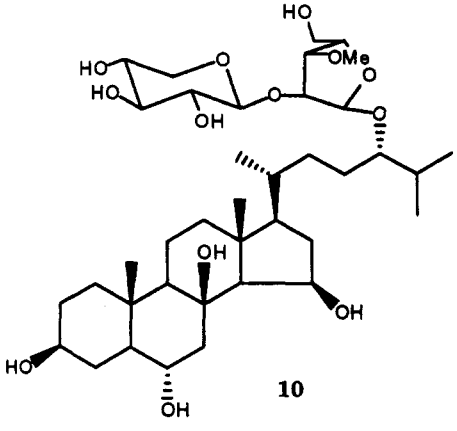
Sugar carbon	Qui I	Xyl I	Qui II	Xyl II	Fuc
1	105.1	103.4	103.5	100.9	105.3
2	73.3	82.4	75.2	83.0	71.4
3	88.3	74.0	76.3	75.9	74.2
4	73.9	76.5	75.4	69.6	73.8
5	71.8	65.6	72.7	65.6	71.5
6	17.6		18.0		16.5

[a]Data taken from the spectrum of **3**.

TABLE 4. Nmr Data for the Steroidal Aglycones of Glycosides **6–14** in CD_3OD.

Position	Compound								
	6–9[a]	**7–9**[b]	**10**	**11**		**12**		**13–14**[c]	**14**
	^1H	^{13}C	^1H	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1		39.4			39.5		39.5		39.5
2		31.3			31.4		31.3		31.9
3	3.50 m	72.0	3.51 m	3.50 m	72.2	3.50 m	72.0	3.50 m	72.1
4		32.2			32.4		32.7		32.7
5		53.5			53.7		51.7		52.3
6	3.68 dt (3.5, 9.0)	67.5	3.68 dt	3.65 dt	67.7	4.45 dt (3.5, 9)	77.0	44.5 dt	77.0
7	2.41 dd (12.5, 3.5)	50.0	2.40 dd	2.44 dd	49.0	2.73 dd	47.6	2.70 dd	49.0
8		75.8			76.0		75.8		76.0
9		57.2			57.4		57.2		57.3
10		37.7			37.9		38.4		37.4
11		19.5			19.4		19.4		19.6
12		42.8			43.2		43.1		42.9
13		45.3			45.4		45.4		45.5
14		67.0			64.5		64.5		66.9
15	4.23 dt (10, 3.5)	69.7	4.44 t (5)	4.08 dd (11, 2.5)	80.8	4.10 dd (11, 2.5)	80.5	4.26 dt	69.6
16		41.7		4.01 dd (8, 2.5)	82.9	4.02 dd (8, 2.5)	82.9		41.5
17		55.8			60.5		60.4		55.9
18	1.00 s	15.3	1.02 s	1.14 s	17.0	1.16 s	17.0	1.00 s	15.4
19	1.05 s	14.1	1.30 s	1.05 s	14.0	1.11 s	14.3	1.11 s	14.2
20		36.2			31.0		31.0		36.6
21	0.92 d (7)	18.8	0.96 d (7)	0.96 d (7)	18.5	0.96 d (7)	18.5	0.93 d (7)	19.0
22		32.8			35.0		34.8		32.8
23		28.1			28.6		28.6		28.9
24		83.8			42.6		42.5		84.9
25		31.1			30.8		30.6		31.9
26	0.92 d (7)	18.3	0.92 d	0.88 d	19.2	0.88 d	19.0	0.93 d	18.4
27	0.92 d	17.8	0.93 d	0.90 d	19.9	0.90 d (7)	20.0	0.93 d	18.3
28					31.7		31.7		
29					68.0	3.76 m	68.1		

[a]At 500 MHz; data taken from the spectrum of **7**.
[b]At 125 MHz; data taken from the spectrum of **7**.
[c]Data taken from the spectrum of **14**.



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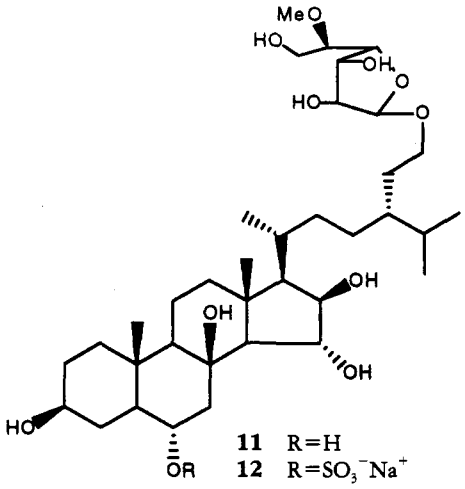
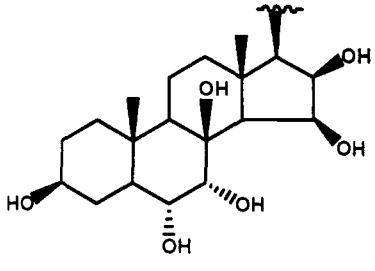
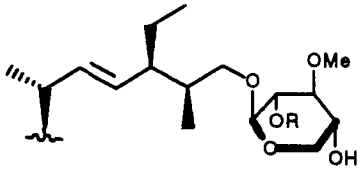
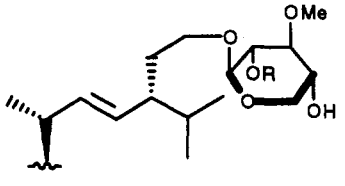


11 R=H
12 R=$SO_3^-Na^+$





15 R=$SO_3^-Na^+$ 15a R=H
stereochemistry at C-24 and C-25 is relative



16 R=$SO_3^-Na^+$ 16a R=H

In this latter connection we have noted that the resonance of the anomeric proton appears at δ 4.95–4.97 ppm in the ^{1}H-nmr spectra of the many steroidal 24-*O*-α-arabinofuranosides isolated from starfish, while it is shifted to δ 5.07–5.11 ppm in β-D-xylopyranosyl(1→2)-α-arabinofuranosides (2,17). Analysis of the ^{13}C-nmr spectrum (Table 5) confirmed the location of the methoxyl group at C-3′ and also indicated the attachment of the β-xylopyranosyl unit at C-2′ of arabinose [δ_C, C-1′ 107.8, C-2′ 90.6, C-3′ 87.0, C-4′ 82.8 ppm; *cf.* δ_C 109.4, 83.8, 78.6, and 85.3 ppm in steroidal 24-*O*-α-arabinofuranosides (16)]. Furthermore, the glycosidation shift observed for C-24 [δ_C 83.8 ppm in **7** vs. 76.4 ppm in its aglycone (9)] confirmed the attachment of the disaccharide moiety at C-24 of the aglycone. The 24*S* configuration was proposed on the basis of the chemical shifts of the side-chain carbons, identical with those of asterosaponin P-1 [**5**] (5,15).

Oreasteroside C [**8**] is isomeric with **7**, with its fabms giving $[M-H]^-$ at *m/z* 729 with fragments at *m/z* 583 $[M-H-146]^-$ and 451 $[M-H-146-132]^-$, corresponding to the consecutive loss of a methoxylated pentose followed by a pentose. The ^{1}H-nmr

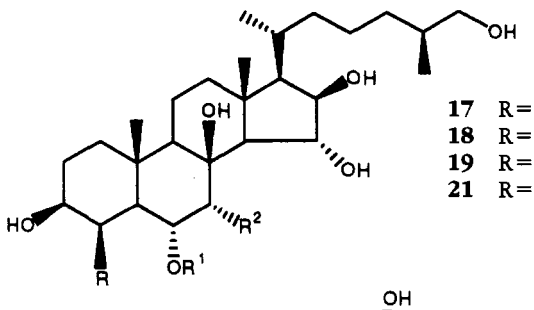
TABLE 5. Nmr Data for the Saccharide Portions of Glycosides **6–14** in CD_3OD.

Position	Compound									
	6, 13[a]	**7, 10**[b]		**8**	**9**		**11, 12**[c]		**14**	
	3-*O*-Me-α-arabinofuranosyl	3-*O*-Me-α-arabinofuranosyl		α-arabinofuranosyl	3-*O*-Me-α-arabinofuranosyl		5-*O*-Me-β-galactofuranosyl		α-arabinofuranosyl	
	^1H	^1H	^{13}C	^1H	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1′	4.97 d (1.5)	5.17 d (1.5)	107.8	5.11 d (1.5)	5.17 d (1.5)	108.0	4.85 d (1.5)	109.3	4.95 d (1.5)	109.5
2′	4.08 dd (1.5, 4)	4.08 dd (1.5, 4)	90.6	4.07 dd (1.5, 4)	4.08 dd (1.5, 4)	91.2	3.96 m	82.7	4.00 dd (1.5, 4)	83.8
3′	3.57 dd (6, 4)	3.73 m	87.0	4.04 m	3.73 m	87.5	3.96 m	78.9	3.86 dd (6.5, 4)	78.9
4′	4.06 m	4.02 m	82.8	4.00 m	4.01 m	82.7	3.96 m	83.8	4.04 m	85.4
5′	3.68 dd (11.2, 5.2)	3.68 dd (11.2, 4.2)	62.7	3.81 dd (11.2, 3)	3.68 dd (11.2, 4.2)	62.8	3.44 m	83.6	3.66 dd (12.5, 5)	63.0
	3.74 dd (11.2, 3)	3.78 dd (11.2, 3)		3.66 dd (11.2, 4.2)	3.77 dd (11.2, 3)				3.78 dd (12.5, 3.5)	
6′							3.74 d (6) 2H	62.4		
OMe	3.45 s	3.45 s	58.3		3.47 s	58.4	3.57 s	59.6		
		β-xylopyranosyl		2-*O*-Me-β-xylopyranosyl	2-*O*-Me-β-xylopyranosyl					
1″		4.34 d (7)	105.5	4.44 d (7.5)	4.40 d	105.7				
2″		3.21 dd (7, 9)	74.7	2.89 dd (7.5, 9.5)	2.88 dd	84.0				
3″		3.40 m	77.7	3.34 t (9.5)	3.34 t	77.5				
4″		3.50 m	71.0	3.50 m	3.51 m	71.1				
5″		3.19 t (10.6)	66.9	3.19 t (10)	3.18 t	67.0				
		3.85 dd (10.6, 4)		3.83 dd (10, 5.2)	3.83 dd					
OMe				3.61 s	3.61 s	61.2				

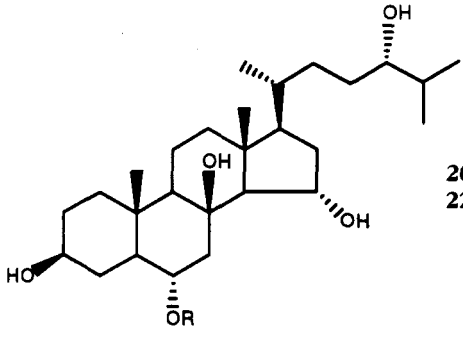
[a]Proton signals taken from the spectrum of **6**

[b]Proton signals taken from the spectrum of **7**.

[c]Data taken from the spectrum of **12**.



17 R=H; R^1=H; R^2=H
18 R=H; R^1=H; R^2=OH
19 R=OH; R^1=H; R^2=OH
21 R=OH; R^1=$SO_3^-Na^+$; R^2=OH



20 R=H
22 R=$SO_3^-Na^+$

spectrum of **8** also showed signals for an α-arabinofuranosyl and a β-xylopyranosyl unit, both determined by decoupling, and one methoxyl group. An upfield shift of H-2″ to δ_H 2.89 dd (7.5 and 9.5 Hz) was diagnostic of the presence of a 2-*O*-methylxylopyranosyl unit (2). An accurate analysis of the chemical shift values and comparison with nodososide, a steroidal glycoside containing the 2-*O*-methyl-β-D-xylopyranosyl(1→2)-α-L-arabinofuranosyl moiety (2), previously isolated from *Protoreaster nodosus* (17) and then found in many glycosides from starfish, established the structure of the sugar moiety and indicated its location at C-24 in **8**. The 24*S* configuration was suggested by analogy with **7** and the many steroidal 24-*O*-arabinosides isolated from starfish (2).

The fabms of oreasteroside D [**9**] gave a quasi-molecular ion at *m/z* 743 $[M-H]^-$ accompanied by fragments at *m/z* 597 and 451, corresponding to the consecutive loss of two methoxylated pentose units. Examination of the ^{1}H-nmr spectral data (Table 5) showed signals for a 2-*O*-methyl-β-xylopyranosyl ($\delta_{H\text{-}2'}$ 2.88 dd, -OCH_3 3.61 s) and for a 3-*O*-methyl-α-arabinofuranosyl ($\delta_{H\text{-}3'}$ 3.73 m, -OCH_3 3.47 s) unit. The resonance of the anomeric proton of the arabinose moiety at δ 5.17 (d, J=1.5 Hz) was again suggestive of the location of the disaccharide moiety, made up by a 2-*O*-methyl-β-D-xylopyranosyl unit linked at C-2 of a 3-*O*-methyl-α-L-arabinofuranosyl unit, at C-24. The ^{13}C-nmr spectrum confirmed the sequence and the structure of the disaccharide moiety [glycosidation shift observed for C-2 of arabinose, δ_C 91.2 vs. 83.8 ppm in the steroid 24-*O*-α-arabinosides (16)] and indicated its attachment at C-24 of the aglycone [δ_C 84.8 vs. 76.4 ppm in its aglycone (9)]. The 24*S* configuration was proposed on the basis of the same arguments used for **7**.

Oreasteroside E [**10**], fabms *m/z* 729 $[M-H]^-$, 597 $[M-H-132]^-$, 451 $[M-H-132-146]^-$, is isomeric with **7**, differing only in the stereochemistry of the 15-hydroxyl group. The 15β-hydroxy stereochemistry in **10** was suggested by the broad triplet at δ 4.44 (J=5 Hz), replacing the double triplet at δ 4.23 (J=10 and 3.5 Hz), the shape of which is indicative of a 15β-hydroxy group (2,18), and by the downfield shift of the C-18 methyl signal to δ 1.30 s (δ 1.00 in the 15α-hydroxy analogue **7**), and was supported by comparison with many published data on starfish-derived 15β-hydroxy steroids (19–21).

Oreasteroside F [**11**] showed an intense quasi-molecular ion at *m/z* 671 $[M-H]^-$ and a fragment at *m/z* 495 in the fabms, which corresponds to the loss of a methylated hexose unit. The ^{13}C- (Table 5) and ^1H-nmr resonances for the sugar residue match those of the 5-*O*-methyl-β-galactofuranosyl residue found in indicoside A, a steroidal glycoside isolated from the starfish *Astropecten indicus* (22). To support this assignment further, oreasteroside F [**11**] was acetylated to give a hexaacetate, *m/z* 923 $[M-H]^-$, the ^1H-nmr spectrum of which showed seven resolved signals for the sugar protons with chemical shifts and coupling constants identical with those observed in the spectrum of the acetylated indicoside A (22). Thus, the glycosyl residue accounts for 175 mass units leaving 495 mass units for the aglycone. This corresponds to a C-29-saturated sterol, mol wt 496, with six hydroxyl groups (four secondary, one primary, and one tertiary; ^{13}C DEPT measurements). Examination of the spectral data (Table 4) and comparison with those of the many polyhydroxysteroids and glycosides of polyhydroxysteroids isolated in our laboratory, indicated that **11** contains a (24*R*)-24-ethyl-5α-cholestane-3β,6α,8,15α,16β,29-hexaol aglycone, already found in miniatoside A, a glycoside from *Patiria miniata* (23). We note that the 3β,6α,8,15α,16β-hydroxylation pattern is common among polyhydroxysteroids from starfish (2), and the (24*R*)-24-ethyl-5α-cholestane-3β,6α,8,15α,16β,29-hexaol has been isolated, as its 29-sulfated derivative, from *Poraster superbus* (24). A ^{13}C-nmr spectrum clarified that the monosaccharide moiety is attached at C-29 of the steroidal part in **11** [$\delta_{C_{29}}$ 68.0 ppm vs. 62.2 in the model 29-hydroxysteroids (25)], and the difference of 0.7 ppm between the C-26 and C-27 carbon signals in the ^{13}C-nmr spectrum of **11** was suggestive of the 24*R* configuration (25).

Oreasterosides G–H [**12–13**] are the 6-sulfated derivatives of **11** and **6**, respectively. Their fabms gave molecular anion peaks [$M_{SO_3}^-$] at *m/z* 751 and 677, shifted eighty mass units relative to **11** and **6**, respectively. Their ^1H-nmr spectra were very similar to those of **11** and **6**, except that the signals for H-6, H-7, and CH_3-19, which were shifted downfield to δ_H 4.45 dt (δ_H 3.65 in **11** and 3.68 in **6**), 2.73 dd in **12** and 2.70 dd in **13** (δ_H 2.44 in **11** and 2.41 in **6**), and 1.11 s (δ_H 1.05 in both **11** and **6**). These data indicated that the sulfate group was at C-6 in both **11** and **6**. For compound **12**, this was supported by ^{13}C-nmr signals for C-6 shifted downfield to δ_C 77.0 (δ_C 67.7 in **11**) and for C-5 and C-7 shifted upfield to δ_C 51.7 (δ_C 53.7 in **11**) and δ_C 47.6 (δ_C 49.0 in **11**), respectively. Solvolysis of **12** using dioxane/pyridine afforded oreasteroside E [**11**], and similar treatment of **13** gave oreasteroside A [**6**].

The novel oreasteroside I [**14**], fabms *m/z* 663 [$M_{SO_3}^-$], is the 24-*O*-α-arabinofuranoside of the previously mentioned (24*S*)-cholesta-3β,6α,8,15α,24-pentaol 6-sulfated aglycone and was identified through its ^1H- and ^{13}C-nmr spectra (Tables 4 and 5). The ^{13}C-nmr spectrum confirmed the position of the sulfate at C-6 and of the arabinose moiety at C-24 of the aglycone and also indicated the *S* configuration at C-24, based on the same arguments used for **7** and **9**. On solvolysis using dioxane/pyridine, **14** was converted into the known (24*S*)-24-*O*-(α-L-arabinofuranosyl)-5α-cholestane-3β,6α,8,15α,24-pentaol (5).

Oreasterosides J and K [**15**, **16**] were isolated as a mixture resistant to separation by reversed-phase hplc, even after removal of the sulfate group at C-2 of the xylosyl residue. Because of the limited amounts available (2 mg) we decided to pursue structure determination on the mixture. The fabms gave only one anion peak at *m/z* 735 [$M_{SO_3}^-$]. The ^1H-nmr spectrum (Table 6) contained signals for the 3β,6α,7α,8,15β,16β-hexahydroxy tetracyclic steroidal nucleus, already found in coscinasteroside A, a steroidal glycoside from *Coscinasterias tenuispina* (10), and in 5α-cholestane-3β,6α,7α,8,15β,16β,26-heptaol first isolated from *Pycnopodia helianthoides* (26), and later found in other species (20,27). The hydroxylation pattern of the tetracyclic steroidal nucleus was supported by the ^{13}C-nmr data (Table 6) and comparison with the already-

TABLE 6. Nmr Data for Oreasterosides J [**15**] and K [**16**] in CD_3OD.[a]

Position	Compound							
	15 (minor)		**16** (major)		**15a**		**16a**	
Aglycone[b] side-chain	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
20	2.66 m	34.6	2.66 m	34.6	2.66 m	34.7	2.66 m	34.6
21	1.09 d (7)	20.4	1.09 d (7)	20.3	1.10 d	20.4	1.10 d	20.3
22	5.53 dd (15, 7.5)	139.4	5.58 dd	139.9	5.53 dd	139.4	5.58 dd	139.8
23	5.25 dd (15, 7.2)	131.7	5.25 dd	130.6	5.26 dd	131.7	5.25 dd	130.6
24		50.2		47.2		49.7		47.2
25		39.1		33.4		39.1		33.2
26	3.28 dd (10, 6) 3.78 dd (10, 6.5)	73.6	0.92 d (6.8)	21.2	3.26 dd 3.82 dd	74.7	0.92 d	21.3
27	1.00 d (6.8)	15.8	0.88 d	19.5	1.00 d	15.9	0.88 d	19.5
28		25.6		33.6		25.7		33.8
29	0.89 t (7)	12.5	3.76 m	68.9	0.89 t	12.6	3.75 m	69.9

Position	**15**		**16**		**15a–16a**	
Sugar	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	4.70 d (3.7)	101.7	4.71 d (3.7)	101.8	4.20 d (7)	105.4
2	4.26 dd (3.7, 5.0)	76.3	4.28 dd (3.7, 5.0)	76.7	3.22 dd (7, 9.3)	74.6
3	3.42 t (5.0)	82.0	3.40 t (5.0)	82.5	3.05 t (9.3)	87.6
4	3.64 m	68.6	3.64 m	68.7	3.53 m	70.9
5	3.95 dd (10.2, 3.7)	63.4	3.97 dd (10.2, 3.7)	63.7	3.20 t (10)	66.8
	3.34 dd[c]		3.34 dd[c]		3.85 dd	
OMe	3.57 s	59.1	3.58 s	59.4	3.65 s	61.0

[a]Signals taken from the spectrum of the mixture.

[b]Other signals: δ_H H-3 (3.50, m), H-6 (3.85, dd, J=11.5 and 2.5 Hz), H-7 (3.91, d, J=2.5 Hz), H-15 (4.51, dd, J=7.0 and 5.5 Hz), H-16 (4.21, t, J=7 Hz), H_3-18 (1.31, s), H_3-19 (1.02 s); δ_C C-1 (39.1), C-2 (31.5), C-3 (72.3), C-4 (32.1), C-5 (44.3), C-6 (69.4), C-7 (76.5), C-8 (79.3), C-9 (50.3), C-10 (37.5), C-11 (19.1), C-12 (43.0), C-13 (44.3), C-14 (55.2), C-15 (71.3), C-16 (73.9), C-17 (63.6), C-18 (17.8), C-19 (13.9).

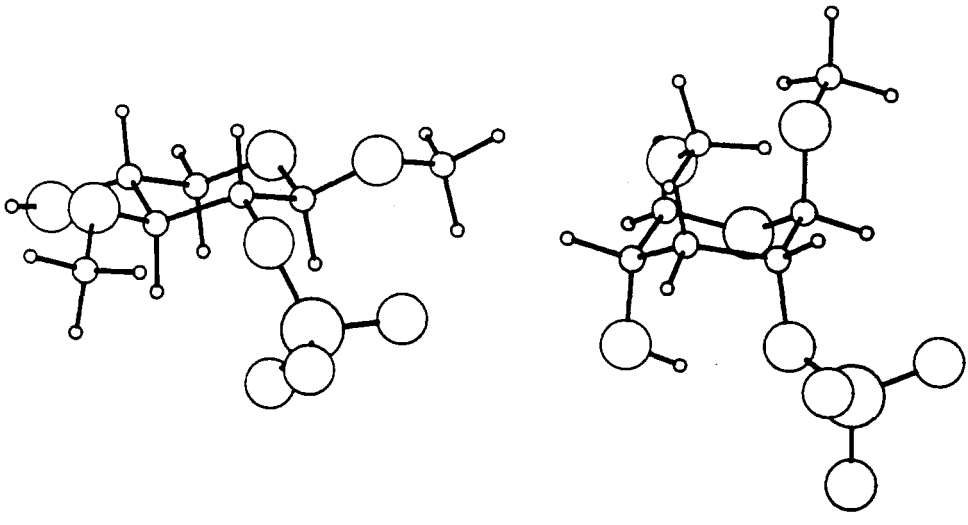
[c]Under solvent signals.

known polyhydroxylated steroid (26). In addition, the ^1H-nmr spectrum contained two partially overlapping eight-line patterns centered at δ 5.58 (major; dd, J=15 and 7.5 Hz) −5.25 (J=15 and 7.2 Hz) and 5.53 (minor; dd, J=15 and 7.5 Hz) −5.25 (J=15 and 7.2 Hz), which could be assigned to the Δ^{22}-*trans* protons of two isomeric steroids differing in their side-chains. In the hydroxymethylene proton region a multiplet at δ 3.76 and two double doublets at δ 3.78 (J=10 and 6 Hz), partially overlapped with the multiplet at δ 3.76, and δ 3.28 (J=10 and 6.5 Hz) were suggestive of Δ^{22E}-24-(β-oxygenated ethyl) and Δ^{22E}-24-ethyl-26-oxygenated side-chains. In the methyl region of the spectrum we observed two doublets at δ 0.88 and 0.92 assigned to Me-26 and Me-27 of compound **16** and one doublet at δ 1.00 and one triplet (0.89, t), overlapping with the doublet at δ 0.88, assigned to the Me-27 and Me-29 of compound **15**. Model Δ^{22}-24-ethyl-26-hydroxysteroids have been synthesized (28), and several steroid 29-*O*-glycosides of the 29-hydroxystigmastane series have been isolated (2,29,30) and their ^1H- and ^{13}C-nmr spectra measured. Taking into account these models, it was easy to analyze the ^{13}C-nmr data of the mixture of **15** and **16**, which also established the attachment of the sugar moiety at C-26 of **15** and at C-29 of **16**. The signals for the side-chain carbons are reported in Table 6. In the ^1H-nmr spectrum of the mixture of **15** and **16**, we also observed a set of signals clearly assignable to the sugar moiety, which appeared as double signals. In the ^{13}C-nmr spectrum all the signals for the sugar moiety were also doubled (Table 6) and were assignable to the presence of two isomers **15** and **16** which differ in the side-chain of the steroidal aglycones, but have an identical sugar

moiety attached to the side-chain. The ^{13}C-nmr spectrum assisted by DEPT measurements showed methoxyl carbon signals at δ 59.4 (59.1), one methylene carbon at δ 63.7 (63.4), and four methine carbons at 101.8 (101.7), 76.7 (76.3), 82.5 (82.0), and 68.7 (68.6) ppm, indicative of a methylated sulfated pentose moiety. Comparison of the above data with those reported for methyl pentopyranosides (31), taking into account the known sulfation and methylation shifts (32), suggested a 3-*O*-methyl-2-*O*-sulfated-β-xylopyranoside as the most probable structure for the sugar moiety of **15** and **16**. Interpretation of the 2D-COSY spectrum established the *J* connectivity of the sugar protons starting from the H-1 protons at δ 4.71 d–4.70 d through H-2 at δ 4.28 dd–4.26 dd, H-3 at δ 3.40 t–3.42 t, H-4 at δ 3.64 m and H_2-5 at δ 3.97 dd–3.95 dd, and δ 3.34 overlapped with the MeOH signal. The methoxyl signals at δ 3.57 s–3.58 s were assigned to C-3, whereas the signals for the H-2 protons shifted downfield to δ 4.28–4.26 supported the location of a sulfate at C-2. The coupling constants of the methine proton signals were strongly deviated from the expected values for a xylopyranosyl structure. On solvolysis in dioxane/pyridine, **15–16** afforded a mixture of a less polar components **15a–16a**, still resistant to separation, which showed in the fabms a quasi-molecular ion at *m/z* 655 $[M-H]^-$ corresponding to the desulfated derivatives **15a** and **16a**. The 500 MHz ^{1}H-nmr spectrum of this desulfated mixture (Table 6) showed signals for a 3-*O*-methyl-pyranose unit with the expected coupling constant values. The ^{13}C-nmr data (Table 6) were also consistent with a 3-*O*-methyl-β-xylopyranosyl structure. The deviation of the coupling constant values of the methine protons of the sugar ring of **15** and **16** from those usually observed for a β-xylopyranosyl moiety, prompted us to investigate the effect of a sulfoxy substituent at C-2 on the conformation of a xylopyranosyl residue. A conformational analysis on the model methyl-3-*O*-methyl-2-*O*-sulfate-β-xylopyranoside was carried out by molecular dynamics and mechanics calculations (see Experimental) (33). The results of this analysis showed that the $^4C_1$ and $^1C_4$ conformations had a force field energy difference of -0.77 kcal^{-1}, with $^1C_4$ being the lowest energy conformer. Thus at 25°, in accordance with the Boltzmann distribution, methyl 3-*O*-methyl-2-*O*-sulfate-β-xylopyranoside exists as an equilibrium mixture of two conformers, with 80% of the molecules having the $^1C_4$ conformation and 20% having the $^4C_1$ conformation. In methyl β-xylopyranoside the $^4C_1$ conformation can be estimated to be more stable than the $^1C_4$ one by 2.9 kcal/mole^{-1}. For our model an unfavorable steric interaction between the bulky sulfoxy and the anomeric oxygen could account for the lower energy of the $^1C_4$ conformation compared with the $^4C_1$ conformation. The good agreement between the coupling constants measured in the nmr spectrum of **15** and **16** and the weighted average of the coupling constants, calculated using a modified Karplus equation (34) of the $^1C_4$ (80%) and $^4C_1$ (20%) conformations of the methyl 3-*O*-methyl-2-*O*-sulfated-β-xylopyranoside, supports our analysis (Figure 1).

The 24*R* configuration is suggested for compound **16** by analogy with the many 24-(β-hydroxyethyl) steroids isolated from starfish, and is supported by the chemical shift difference ($\Delta\delta = 0.04$ ppm) between the C-26 and C-27 methyl signals, which is close to that reported for the Δ^{22},(24*R*)-24-β-hydroxyethyl)steroid models ($\Delta\delta$ 0.05 ppm) and differs from that of the 24*S* isomer ($\Delta\delta$ 0.02 ppm) (25). The ^{13}C-nmr chemical shifts for C-27 and C-28 and the ^{1}H-nmr chemical shifts for H_2-26 and H_3-27 are diagnostic for the relative stereochemistry at C-24 and C-25 in Δ^{22}-24-ethyl-26-hydroxysteroids (28). Our values for compound **15**, δ_H 1.00 and 3.28–3.78 and δ_C 15.8 and 25.6, are close to the pair 24*R*/25*S*, 24*S*/25*R*. Insufficient amounts of sample did not allow determination of the absolute configuration, which required acid hydrolysis followed by ^{1}H-nmr analysis of the 26-*O*-(+)-*R*-MTPA and 26-*O*-(−)-*S*-MTPA esters (28).

The known polyhydroxysteroids **17–21** listed in Table 1 were identified by direct



$^4C_1$ $^1C_4$

Coupling constants calculated for the $^4C_1$ and $^1C_4$ conformations of methyl 3-*O*-methyl-2-*O*-sulfate-β-xylopyranoside

	$^4C_1$			$^1C_4$	
	dih	*J* in Hz		dih	*J* in Hz
1–2	+173.0°	7.96	1–2	+65.3°	2.51
2–3	−174.1°	8.98	2–3	−68.2°	3.17
3–4	+175.9°	9.16	3–4	+69.4°	3.00
4–5_a	−58.9°	4.64	4–5_a	+53.8°	0.93
4–5_b	−177.8°	10.21	4–5_b	−64.3°	1.82

Coupling constants observed experimentally in **15–16** and calculated for an 8:2 equilibrium mixture of the two conformers $^1C_4$ and $^4C_1$ of methyl 3-*O*-methyl-2-*O*-sulfate-β-xylopyranoside.

	^{J}H-1–H-2	^{J}H-2–H-3	^{J}H-3–H-4	^{J}H-4–H-5a	^{J}H-4–H-5b
Exptl.	3.7	5.0	5.0	nd	3.7
Calcd.	3.6	4.4	4.2	1.7	3.5

nd=not detected because of the overlapping of the signals.

FIGURE 1. Conformational analysis of the methyl 3-*O*-methyl-2-*O*-sulfate-β-xylopyranoside unit in **15** and **16** by molecular dynamics and mechanics calculations.

comparison with authentic samples (5,8,9). The only polyhydroxysteroid not previously encountered is 5α-cholestane-3β,6α,8,15α,24-pentaol 6-sulfate [**22**], now also found as the aglycone of oreasterosides G and I [**12, 14**]. The fabms gave a molecular anion peak at *m/z* 531 [$M_{SO_3}^-$]; solvolysis in a dioxane/pyridine mixture afforded (24*S*)-5α-cholestane-3β,6α,8,15α,24-pentaol [**20**], fabms, *m/z* 451 [M−H]$^-$, previously described from *Asterina pectinifera* (9). The sulfate group was located at C-6 by comparing the ^{1}H-nmr data of **22** with those of its desulfated analogue. The sulfation at C-6 resulted in the downfield shift of H-6 (δ 4.26 vs. 3.65), H-7β (δ 2.70 vs. 2.40) and CH_3-19 (δ 1.11 vs. 1.05).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra, Bruker WM-250 (^{1}H at 250 MHz, ^{13}C at 62.9 MHz), and Bruker AMX-500 (^{1}H at 500 MHz, ^{13}C at 125 MHz) δ (ppm), *J* in Hz, spectra referenced to CHD_2OD signal at 3.34 ppm and to the central carbon CD_3OD signal at 49.0 ppm; mass spectra, VG ZAB mass spectrometer equipped with fab source [in glycerolthioglycerol (3:1) matrix; Xe atoms of 2-6 kV]; optical rotations, Perkin-Elmer model 241 polarimeter; glc, Carlo Erba Fractovap 2900 for capillary column (OV-101, 25 m, 158°, He carrier, flow 2 ml/min^{-1}); reversed-phase hplc, C_{18} μ-Bondapak column (30

cm×7.8 mm i.d.; flow rate 5 ml/min^{-1}) and C_{18} μ-Bondapak column (30 cm×3.9 mm i.d.; flow rate 2 ml/min^{-1}), Waters Model 6000 A pump equipped with U6K injector and a differential refractometer, model 401; dccc, DCC-A apparatus manufactured by Tokyo Rikakikai Co. equipped with 250 tubes and Buchi apparatus equipped with 300 tubes; molecular mechanics and dynamics calculations were carried out on a SGI Personal Iris 35G computer.

ANIMAL MATERIAL.—*Oreaster reticulatus* L. was collected at Grand Bahama Island, Bahamas, in July 1990. Reference specimens are preserved at the Dipartimento di Chimica delle Sostanze Naturali, Università Federico II, Napoli.

EXTRACTION AND ISOLATION.—The animals (4.5 kg) were chopped and soaked in H_2O (three times, 2 liters for 6 h); the aqueous extracts were decanted and passed through a column of the Amberlite XAD-2 (1 kg). This column was washed with distilled H_2O and eluted with MeOH (6.5 liters) to give, after removal of the solvent, a glassy material (3.0 g). The residual solid mass was extracted with Me_2CO (5 liters, three times) and the Me_2CO extracts were combined, evaporated under vacuum, and partitioned between H_2O and Et_2O. The aqueous residue was then extracted with *n*-BuOH. Evaporation of the *n*-BuOH extracts afforded 5 g of a glassy material that was combined with the above MeOH eluate from the Amberlite XAD-2 column, and chromatographed on a column of a Sephadex LH-60 (4×80 cm) with MeOH-H_2O (2:1) as eluent. Fractions (6 ml) were collected and analyzed by tlc on SiO_2 using MeOH-HOAc-H_2O (12:3:5) and $CHCl_3$-MeOH-H_2O (80:18:2).

Fractions 76–102 contained the crude asterosaponins (930 mg), with fractions 103–128 (750 mg), being a mixture of sulfated glycosides and glycosides of polyhydroxysteroids, and fractions 129–178 (1.20 g) containing a mixture of glycosides of polyhydroxysteroids and of polyhydroxysteroids.

Fractionation of the asterosaponins (930 mg) was pursued by dccc using *n*-BuOH-Me_2CO-H_2O (3:1:5) [descending mode; the upper phase was used as the stationary phase; flow rate 10 ml/h, 5 ml fractions were collected and analyzed by tlc on SiO_2 with *n*-BuOH-HOAc-H_2O (12:3:5)] to give three main fractions. Fractions 55–67 (84 mg) contained almost exclusively the asterone derivative **2**, fractions 68–106 (79.4 mg) contained a mixture of reticulatoside A [**3**] and the known ophidianoside F [**1**], and the last-eluted fractions 106–160 (34.5 mg) contained reticulatoside B [**4**]. All fractions were then separated by hplc on a C_{18} μ-Bondapak column (30 cm×7.8 mm i.d.) with eluents as shown in Table 1, to give the pure isolates.

Fractions 103–128 (750 mg) from the Sephadex LH-60 column were a complex mixture of sulfated glycosides and sulfated polyhydroxysteroids. Fractionation was pursued by dccc using *n*-BuOH-Me_2CO-H_2O (3:1:5) (ascending mode; the lower phase was used as the stationary phase; flow rate 12 ml/h, 6-ml fractions were collected). All fractions were then purified by hplc on a C_{18} μ-Bondapak column with MeOH-H_2O (1:1) as eluent, to give the pure compounds **5**, **12**, **13**, **14**, **21**, and **22**, along with a mixture of **15** and **16**.

Fractions 129–178 (1.200 g) from the Sephadex LH-60 column were a mixture of steroidal glycosides and polyhydroxysteroids. Fractionation was pursued by dccc using $CHCl_3$-MeOH-H_2O (7:13:8) (ascending mode; lower phase was stationary phase); fractions (5 ml each) were collected and monitored by tlc on SiO_2 with $CHCl_3$-MeOH-H_2O (80:18:2).

Fractions 14–32 (340 mg) were constituted by uv visible components (nucleosides) and the fractions 33–141 after purification by hplc on a C_{18} column (30 cm×7.8 mm i.d.) gave pure glycosides and the polyhydroxysteroids **6**, **7–11**, **17**, and **18–20**.

Asterone analogue [**2**].—^{1}H nmr (CD_3OD) δ_H (aglycone) 0.57 (3H, s, H_3-18), 1.02 (3H, s, H_3-19), 2.16 (3H, s, H_3-21), 4.22 (1H, m, H-3), 5.46 (1H, br d, H-11); δ_H (sugars) identical to those reported for reticulatoside A [**3**]; ^{13}C nmr (pyridine-d_5) δ_C (aglycone) C-1 (35.6), C-2 (29.3), C-3 (77.7), C-4 (30.7), C-5 (49.1), C-6 (79.9), C-7 (41.3), C-8 (36.9), C-9 (146.2), C-10 (38.4), C-11 (116.0), C-12 (42.6), C-13 (40.6), C-14 (53.7), C-15 (23.2), C-16 (25.5), C-17 (63.4), C-18 (13.1), C-19 (19.2), C-20 (209.2), C-21 (31.1); δ_C (sugars) identical with those reported for reticulatoside A [**3**] (Table 3).

Reticulatoside A [**3**].—[α]D, see Table 1; fabms, see text; ^{1}H and ^{13}C nmr of aglycone in Table 2; ^{1}H nmr (CD_3OD) δ_H (sugars) 1.29 (3H, d, J=6.2 Hz, H_3-5 of quin I), 1.31 (3H, d, J=5.9 Hz, H_3-5 of fuc), 1.39 (3H, d, J=5.9 Hz, H_3-5 of quin II), 3.95 (1H, dd, J=11.3 and 4.6 Hz, He-5 of xylose II), 4.13 (1H, dd, J=12 and 4 Hz, He-5 of xylose I), 4.43 (2H, d, anomeric-Hs), 4.57 (3H, d, anomeric-Hs); ^{13}C-nmr data of sugar portion shown in Table 3.

Reticulatoside B [**4**].—[α]D, see Table 1; fabms, see text; ^{1}H- and ^{13}C-nmr data of aglycone shown in Table 2; ^{1}H nmr (CD_3OD) δ_H (sugars) 1.30, 1.31, 1.39 (each d, J=6.5 Hz, H_3-quinovose and fucose), 3.94 (1H, dd, J=11.1 and 4.6 Hz, He-5 of xylose II), 4.13 (1H, dd, J=12 and 4.0 Hz, He-5 of xylose I), 4.42 (2H, d, anomeric-Hs), 4.57 (3H, d, anomeric-Hs); ^{13}C-nmr data of sugar portion shown in Table 3.

Oreasteroside A [**6**].—[α]D, see Table 1; fabms, see text; ^{1}H- and ^{13}C-nmr data shown in Tables 4 and 5.

Oreasteroside D [**9**].—[α]D, see Table 1; fabms, see text; ^{1}H- and ^{13}C-nmr data shown in Tables 4 and 5; ^{13}C nmr (CD_3OD) δ_C (aglycone) C-1 (39.6), C-2 (31.5), C-3 (72.1), C-4 (32.3), C-5 (53.6), C-6 (67.6), C-7 (50.2), C-8 (76.0), C-9 (57.4), C-10 (37.8), C-11 (19.6), C-12 (42.9), C-13 (45.5), C-14 (67.2), C-15 (69.8), C-16 (41.8), C-17 (55.9), C-18 (15.4), C-19 (14.2), C-20 (36.3), C-21 (19.0), C-22 (33.0), C-23 (28.3), C-24 (84.8), C-25 (31.3), C-26 (18.4), C-27 (18.0).

(24S)-5α-Cholestane-3β,6α,8,15α,24-pentaol 6-sulfate [**22**].—[α]D, see Table 1; fabms, see text; ^{1}H nmr (CD_3OD) δ_H 0.92 (3H, d, J=6.8 Hz, H_3-21), 0.95 (6H, d, J=6.8 Hz, H_3-26 and -27), 1.01 (3H, s, H_3-18), 1.11 (3H, s, H_3-19), 2.70 (1H, dd, J=12.5 and 3.5 Hz, H-7), 3.50 (1H, m, H-3), 4.26 (1H, dt, J=3.5 and 10 Hz, H-15), 4.46 (1H, dt, J=10 and 3.5 Hz, H-6).

METHANOLYSIS OF RETICULATOSIDES A [**3**] AND B [**4**]: SUGAR ANALYSIS.—A solution of glycosides **3** and **4** (1 mg) in anhydrous 2 M HCl in MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 8 h. On cooling, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, with the supernatant evaporated to dryness under N_2. The residue was trimethylsilylated with Trisil Z (Pierce Chemical Co.) for 15 min at room temperature. Glc analysis (OV-101 capillary column, 25 m, 158°, He carrier, flow 2 ml/min^{-1}) gave peaks that co-eluted with those of the methyl xyloside, methyl fucoside, and methyl quinovoside standards in the ratio 2:1:2.

METHYLATION OF RETICULATOSIDE B [**4**] FOLLOWED BY METHANOLYSIS: TERMINAL SUGARS.—A solution of **4** (3 mg) in 1.0 ml of dry DMF was added under N_2 to a stirred mixture of NaH (65 mg) in dry DMF (1.0 ml). The mixture was stirred for 2 h, and MeI (0.4 ml) was added. The reaction mixture was kept for 4 h at room temperature. The excess of NaH was destroyed with a few drops of MeOH, and, after addition of H_2O, the mixture was extracted with $CHCl_3$. The organic layer was washed with H_2O and evaporated under vacuum. The residue in anhydrous 2 M HCl in MeOH (0.3 ml) was heated at 80° in a stoppered reaction vial for 8 h. After cooling, the mixture was concentrated under a steam of N_2 and was used for glc analysis (SE-30, 25 m capillary column; 95°, He carrier, flow rate 10 ml/min^{-1}). Glc peaks co-eluted with those of methyl-2,3,4-tri-*O*-methyl fucoside and methyl-2,3,4-tri-*O*-methyl quinovoside standards.

ENZYMATIC HYDROLYSIS OF RETICULATOSIDE A [**3**].—Saponin **3** (3 mg) in 1 ml of citrate buffer (pH 4.5) was incubated with 7.5 mg of a glycosidase mixture of *Charonia lampas* at 37° for 4 days. The reaction was followed by tlc on SiO_2 in *n*-BuOH-HOAc-H_2O (12:3:5). After the disappearance of the starting material, the reaction mixture was passed through a C_{18} Sep-Pak cartridge which was washed with H_2O, and eluted with MeOH. The mixture was purified by hplc (C_{18} μ-Bondapak column) with MeOH-H_2O (52:48) to give two peaks containing the monosaccharide **3b** and the trisaccharide **3a**.

^{1}H nmr (CD_3OD) of **3a**: δ_H (aglycone) in Table 5; δ_H (sugars) 1.29 (3H, d, J=6.2 Hz, H_3-5 of quinovose I), 1.39 (3H, d, J=5.9 Hz, H_3-5 of quinovose II), 3.92 (1H, dd, J=11.3 and 4.6 Hz, He-5 of xylose), 4.42 (1H, d, J=7.1 Hz, H-1 of xylose), 4.53 and 4.55 (1H each, d, J=7.5 Hz, anomeric-Hs of quinovose I and II); fabms (negative ion) m/z 935 (M^-).

^{1}H nmr (CD_3OD) of **3b**: δ_H (aglycone) identical to those reported for **3a**; δ_H (quinovose) 4.31 (1H, d, J=7.0 Hz, H-1′), 3.30 (2H, H-3′ and H-5′, overlapped and under solvent signal), 3.19 (1H, t, J=7 and 9.5 Hz, H-2′), 3.01 (1H, t, J=9.0 Hz, H-4′), 1.29 (3H, d, J=6.2 Hz, H_3-5′); fabms (negative ion) m/z 657 (M^-).

ACETONIDE FORMATION FROM **3b**.—Monosaccharide **3b** (0.6 mg) in dry Me_2CO-d_6 (0.5 ml) containing *p*-TsOH (0.3 mg) was stirred at room temperature for 20 h. The mixture was neutralized with $BaCO_3$, centrifuged, and the supernatant evaporated to dryness under reduced pressure to give the corresponding 20,22-acetonide. ^{1}H nmr (CD_3OD) δ_H 0.78 (3H, s, H_3-18), 1.02 (3H, s, H_3-19), 1.32 (6H, s, O-C(CH_3)$_2$-O, 20,22-acetonide), 1.41 (3H, s, H_3-21), 1.68 and 1.75 (3H each, s, H_3-26 and -27), 3.50 (1H, m, H-3α), 3.59 (1H, ddd, J=10.5, 10.5, and 3.5 Hz, H-6), 3.87 (1H, t, H-22), 5.20 (1H, t, J=6.7 Hz, H-24), 5.34 (1H, br t, H-11); δ_H (sugar) identical to those reported for **3b**.

SOLVOLYSIS OF COMPOUNDS **12–16** AND **22**.—Solutions (1.5 mg each) of **12–16** and **22** in pyridine (0.1 ml) and dioxane (0.1 ml) were heated at 130° for 2 h in a stoppered reaction vial. After the solutions were cooled, H_2O (1 ml) was added to each mixture, and each was extracted with *n*-BuOH (3×1 ml). The combined extracts were evaporated to dryness under reduced pressure. Each residue was purified by hplc [C_{18} μ-Bondapak column (30 cm×3.9 mm i.d., flow rate 2 ml/min^{-1}) with MeOH-H_2O (75:25) as eluent] to give the desulfated materials.

Glycoside **12** desulfated: fabms (negative ion) m/z 671 $(M-H)^-$, 475 $[(M-H)-176]^-$; ^{1}H-nmr data identical to those reported for glycoside **11**; glycoside **13** desulfated: fabms (negative ion) m/z 597 $(M-H)^-$, ^{1}H-nmr data identical to those reported for **6**; glycoside **14** desulfated: fabms (negative ion) m/z 583 $(M-H)^-$, ^{1}H nmr (CD_3OD) δ_H (aglycone) 0.94 (6H, d, J=7 Hz, H_3-26 and -27), 1.00 (1H, s, H_3-18), 1.11 (1H, s, H_3-19), 2.40 (1H, dd, J=12.5 and 3.5 Hz, H-7), 3.65 (1H, dt, H-6), 3.50 (1H, m, H-3α), δ_H (sugar)

data identical to those reported for **14**; glycosides desulfated **15a** and **16a**: fabms in the text; ^{1}H- and ^{13}C-nmr data shown in Table 6.

ACETYLATION OF GLYCOSIDE **11**.—Compound **11** (2 mg) was acetylated in pyridine and Ac_2O and stirred at room temperature for 8 h. The solution was extracted with $CHCl_3$ (two times) and the combined extracts were evaporated to afford the 3,6,15,2′,3′,6′-hexaacetate. Fabms (negative ion) *m/z* 923 $(M-H)^-$, 797 (*m/z* 923–126), 755 (*m/z* 797–42), 713 (*m/z* 755–42), 671 (*m/z* 713–42); ^{1}H nmr ($CDCl_3$) δ_H (aglycone) 0.81 and 0.84 (3H each, d, J=7 Hz, H_3-26 and -27), 0.90 (3H, d, J=7 Hz, H_3-21), 1.09 (3H, s, H_3-19), 1.19 (3H, s, H_3-18), 2.03, 2.04, 2.05, 2.09, 2.11, and 2.12 (3H each, s, CH_3-C=O), 3.98 (1H, dd, J=7 and 2.5 Hz, H-16), 4.71 (1H, dd, J=11 and 2.5 Hz, H-15), 4.68 (1H, m, H-3α), 4.93 (1H, dt, J=3.5 and 9 Hz, H-6); δ_H (sugar) 3.68 (1H, dt, H-5′), 4.10 (1H, dd, J=6 and 3 Hz, H-4′), 4.23 (1H, dd, J=11.7 and 6.3 Hz, H-6′), 4.33 (1H, dd, J=11.7 and 6.3 Hz, H-6″), 5.01 (1H, s, H-1′), 5.03 (1H, d, J=1.3 Hz, H-2′), 5.20 (1H, dd, J=6 and 1.3 Hz, H-3′).

MOLECULAR MECHANICS AND DYNAMICS CALCULATIONS.—These techniques were carried out using the force field CHARMM (QUANTA 3.3 software package). Two minimum energy conformations, $^{4}C_1$ and $^{1}C_4$ of 3-*O*-methyl-2-*O*-sulfate-β-xylopyranoside, were obtained performing a High Temperature Molecular Dynamic Simulation (HTMDS) followed by Energy Minimization. Thus, 500 conformations of 3-*O*-methyl-2-*O*-sulfate-β-xylopyranoside were achieved by means of a molecular dynamics simulation for 50 ps at 1000 K, using the Verlet algorithm. All the conformations were then subjected to an energy minimization (400 steps, conjugate gradient algorithm). An r-dependent dielectric factor was used in all calculations.

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