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STARFISH SAPONINS, 52.[1] CHEMICAL CONSTITUENTS FROM THE STARFISH *ECHINASTER BRASILIENSIS*

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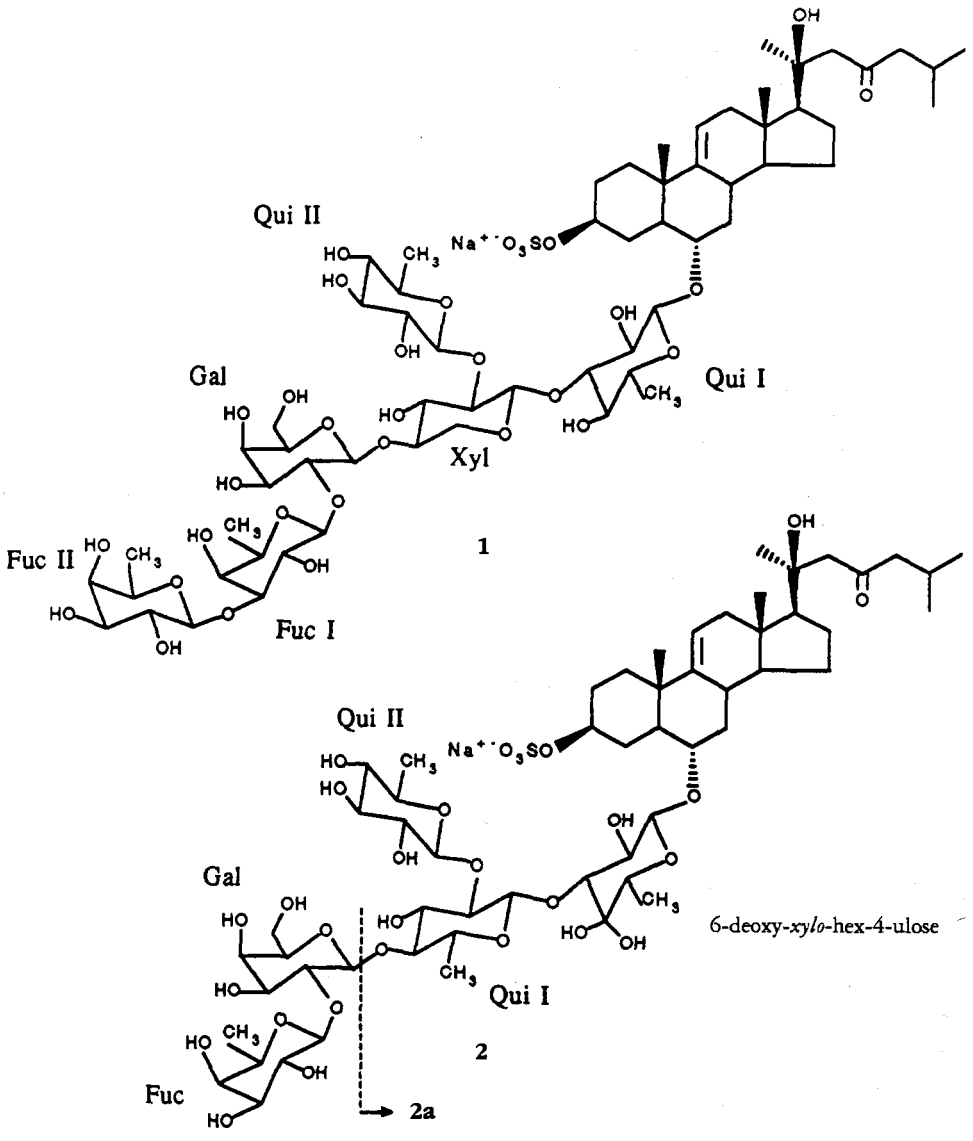
ABSTRACT.—This paper reports an analysis of the chemical constituents from the Caribbean starfish *Echinaster brasiliensis* collected at Grand Bahama Island. This species is completely devoid of cyclic steroidal glycosides, previously isolated from two species of the genus *Echinaster* in place of the more common penta- and hexa-glycoside steroidal sulfates ("asterosaponins"). Two typical "asterosaponins" and ten glycosides of polyhydroxysteroids were instead isolated in relatively large amounts from *E. brasiliensis*. The asterosaponins include the known marthasteroside A_1 [**1**] and the new brasiliensoside [**2**], while the glycosides of polyhydroxysteroids include seven new compounds (six monoglycosides and one diglycoside). The known echinasteroside A, previously found in *Echinaster sepositus* and in the related *Henricia laeviuscula* (both belonging to the family *Echinasteridae*), and laeviusculosides C and I from *H. laeviuscula* were also isolated. Most of the glycosides from *E. brasiliensis* are 3-*O*-β-xylopyranosides of Δ^4-3β,6β,8,15α,16β pentahydroxysteroid aglycones, having different side chains and sometimes a sulfate group at C-15, structural features which are typical of steroidal glycosides from starfishes of the family *Echinasteridae*. Continuing the analysis of the constituents of *E. brasiliensis*, we have also isolated a series of anthraquinones, known animal pigments found only in echinoderms and particularly in Crinoidea and in the *Echinasteridae* family of Asteroidea.

It is known that there are three types of steroid oligoglycosides in starfishes: (a) the asterosaponins, which are composed of a $\Delta^{9(11)}$-3β,6α-dioxygenated steroidal moiety with a sulfate group at C-3 and an oligosaccharide moiety made up of from five to six sugar units (occasionally three or four) attached at C-6; (b) the steroidal cyclic glycosides; and (c) several groups of polyhydroxysteroidal mono-, bi-, and recently triosides, in both sulfated and nonsulfated forms and in a recent case with phosphate conjugation (1). While the asterosaponins and the glycosides of polyhydroxysteroids have been detected in the majority of the species examined, the steroidal cyclic glycosides have been found only in two species of the genus *Echinaster* (2–4). They have a number of unusual features, but the most remarkable one is a trisaccharide chain cyclized between C-3 and C-6 of the steroidal aglycone, giving rise to a macrocyclic ring reminiscent of a crown ether.

Because of these findings and the contextual absence of asterosaponins in each of two *Echinaster* species, we attributed chemotaxonomic significance to the steroidal cyclic glycosides. This view is now questioned by the results of the present investigation of a third species of *Echinaster*, *Echinaster brasiliensis* Muller and Trochel (family Echinasteridae), collected at Grand Bahama Island, Caribbean Sea. The cyclic steroidal glycosides were completely absent from the polar extracts of this species, while two typical asterosaponins and several glycosides of polyhydroxysteroids have been isolated. The asterosaponins include the known marthasteroside A_1 [**1**] (5), and a new one, designated brasiliensoside [**2**]. Among the glycosides of polyhydroxysteroids, seven are new compounds and three, echinasteroside A [**3**], laeviusculoside C [**5**], and laeviusculoside I [**11**], are known compounds from *Echinaster sepositus* (6) and the related *Henricia laeviuscula* (7) (both belonging to the family Echinasteridae). The majority of the new glycosides of

[1]For Part 51, see M. Iorizzi, L. Minale, R. Riccio, and T. Yasumoto, *J. Nat. Prod.*, **56**, 1786 (1993).



polyhydroxysteroids are 3-*O*-β-xylopyranosides of Δ^4-3β,6β,8,15α,16β-pentahydroxysteroids with different side chains and sometimes a sulfate at C-15, closely related to those encountered in species of the family Echinasteridae (1).

Continuing the analysis of the constituents of *E. brasiliensis*, we have also isolated a series of anthraquinones, which are known pigments among the animals only in echinoderms and in particular in the class Crinoidea and in the family Echinasteridae (8) of the class Asteroidea. This finding reinforces the view of Stonik and Elyakov (8) of the taxonomically significant distribution of anthraquinoid pigments in the Asteroidea.

RESULTS AND DISCUSSION

Separation and isolation of the individual compounds from the aqueous extracts followed the steps described previously (1,9). In brief, all compounds were isolated from the MeOH eluate of an Amberlite XAD-2 resin through which the H_2O extracts of *E. brasiliensis* were passed. Further separation and purification were done by chromatography on Sephadex LH-60, by which the asterosaponins were separated from the

polyhydroxysteroid mono- and diglycosides and from the anthraquinone pigments eluted as last components, followed by dccc and hplc steps. The results of our analysis are shown in Table 1.

ASTEROSAPONINS.—The major saponin, marthasteroside A_1 [**1**], was identified by direct comparison (hplc, ^{1}H nmr, fabms) with an authentic sample isolated from *Marthasterias glacialis* (5).

The new brasiliensoside [**2**] gave molecular-anion peaks at *m/z* 1273 (hydrated form) and *m/z* 1255 (keto form) in the fab mass spectrum (negative ion mode) and showed two spots on SiO_2 tlc. These data are in agreement with the presence in its oligosaccharide chain of a keto sugar easily converted into its hydrate form, such as the ovarian asterosaponin I from *Asterias* genus (10–13), containing the 6-deoxy-*xylo*-hex-4-ulose unit directly attached to the aglycone. In confirmation, the ^{13}C-nmr spectrum (Table 2), measured in pyridine-d_5/H_2O, showed a signal for a fully substituted carbon at 92.2 ppm assigned to the gem-diol carbon, and a resonance for a methyl carbon at an unusual high field, δ_C 12.9 ppm, due to its β position (C-5) relative to the gem-diol function (C-4). These signals as well the signals at 104.2 (C-1), 72.6 (C-2), 90.3 (C-3), and 73.3 (C-5) ppm are identical with those observed in the spectrum of ovarian asterosaponin I (=forbeside C) and assigned to the 6-deoxy-*xylo*-hex-4-ulose unit (hydrate form) glycosidated at C-3. On solvolysis with pyridine and dioxane, compound **2** gave thornasterol A [i.e., (20*S*)-3β,6α,20-trihydroxy-5α-cholest-9(11)-en-23-one] because of the lability of glycopyranosiduloses in alkaline media, which results in release of the substituents (14) (e.g., at C-1 and C-3), thus confirming the direct attachment of the keto sugar to the aglycone. ^{1}H-nmr [five anomeric proton signals at δ 4.58, 4.52, 4.45 (5H in total), each doublet with J=7.5 Hz] and ^{13}C-nmr spectra [five anomeric carbon signals at δ_C 106.7, 105.2, 104.2, 103.1, and 102.1 ppm] indicated that all sugars are in the pyranose form with β-oriented glycosidic linkages. The ^{13}C-nmr spectrum of **2** also

TABLE 1. Compounds Isolated from the Starfish *Echinaster brasiliensis* (540 g fresh).

Compound	Amount (mg)	Rotation [α]D	Hplc[a] eluent MeOH-H_2O
Marthasteroside A_1 [**1**]	30	+3.6°	45:55[b]
Brasiliensoside [**2**]	23	+3.9°	45:55[b]
Echinasteroside A [**3**]	2.0		55:45
Desulfated-22(23)-dihydroechinasteroside A [**4**]	1.8	−17.5°	75:25
Laeviusculoside C [**5**]	3.5	+10.8°	55:45
Desulfated-echinasteroside B [**6**]	2.0	−16.6°	75:25
Echinasteroside C [**7**]	1.5	−15.0°	75:25
Echinasteroside D [**8**] / Echinasteroside E [**9**]	4.0	−7.5°	55:45
Echinasteroside F [**10**]	1.5	−1.2°	50:50
Laeviusculoside I [**11**]	25.0	−6.6°	75:25[b]
Echinasteroside G [**12**]	4.5	−9.3°	75:25
15	1.8		75:25
16	2.3		75:25
17	4.0		75:25
18	1.3		75:25
19	2.1		75:25

[a]C_{18} μ-Bondapak column (30 cm×3.9 mm i.d.); flow rate 2 ml/min.
[b]C_{18} μ-Bondapak column (30 cm×7.8 mm i.d.); flow rate 5 ml/min.
[c]Resistant to attempt at separation.

TABLE 2. Assignments of ^{13}C-nmr Signals (pyridine-d_5 + one drop of H_2O) of the Sugar Moiety of Brasiliensoside [**2**].[a]

Sugar carbon	Z[b]	Qui I	Qui II	Gal	Fuc
1	104.2	103.1	105.2	102.1	106.2
2	72.6	82.8	75.0	81.9	71.2
3	90.3	74.3	76.3	74.5	74.4
4	92.2	84.3	76.2	68.8	73.0
5	73.3	71.8	73.3	75.6	71.2
6	12.9	17.6	17.6	61.3	16.4

[a]Assignments based on comparison with the known compounds forbeside C (11) and thornasteroside A (15).

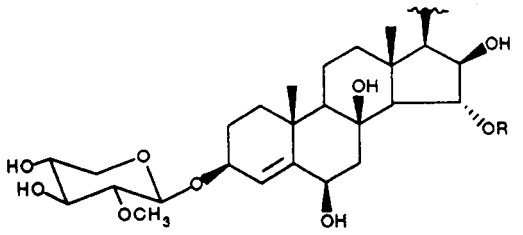
[b]Z=6-deoxy-*xylo*-hex-4-ulose, hydrate form.

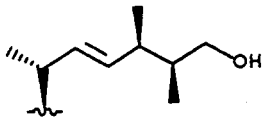
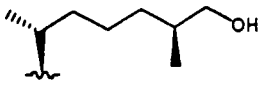
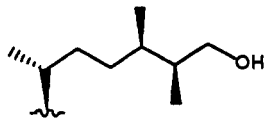
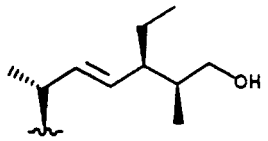
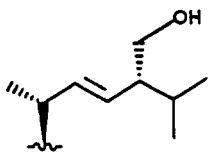
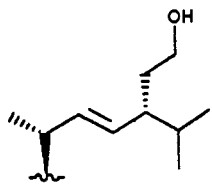
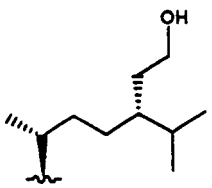
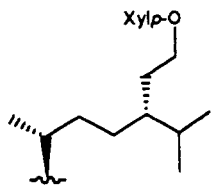
confirmed the presence of the common thornasterol A aglycone with the sulfate at C-3 and the oligosaccharide chain at C-6. On acid methanolysis. brasiliensoside [**2**] gave methyl fucosides, methyl quinovosides, and methyl galactosides in the ratio 1:2:1, and permethylation analysis determined that fucose and quinovose were the terminal monosaccharides. The structure of the oligosaccharide chain was then derived by ^{13}C-nmr spectroscopy (Table 2) and comparison with known compounds. The sugar carbon signals assigned to the Qui-(1→2)-Qui(1→3)-*xylo*-hexulose moiety match very closely those assigned to the same trisaccharide portion of ovarian asterosaponin I (11). The remaining sugar signals were almost identical with those assigned to the terminal disaccharide moiety Fuc-(1→2)-Gal-(1→4)- found in many asterosaponins (15). To confirm the sequence, brasiliensoside [**2**] was submitted to enzymatic hydrolysis with a glycosidase mixture from *Charonia lampas* giving the triglycoside **2a** (fabms *m/z* 965 $[MSO_3]^-$), made up from quinovose (×2) and 6-deoxy-*xylo*-hex-4-ulose, whose ^{1}H-nmr spectrum was virtually identical with that of forbeside G (16). The structure of the new brasiliensoside [**2**] thus differs from the ovarian asterosaponin I for an "in chain" fucose replaced in **2** by a galactose.

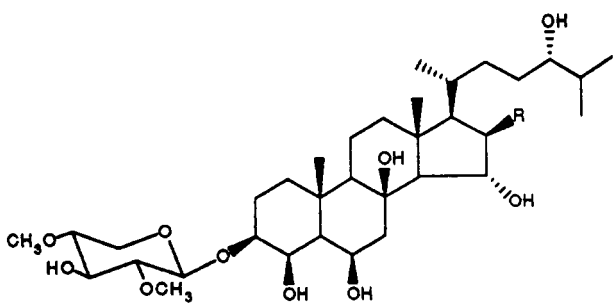
GLYCOSIDES OF POLYHYDROXYSTEROIDS.—Identification of the known echinasteroside A [**3**], laeviusculoside C [**5**], and laeviusculoside I [**11**] was achieved by direct comparison (fabms, ^{1}H nmr, hplc) with authentic samples (6,7). Likewise, compound **6**, corresponding to the desulfated echinasteroside B, was identified by direct comparison with an authentic sample as obtained by solvolysis in dioxane/pyridine of the sulfated natural product (6).

Spectral data (^{1}H nmr and ^{13}C nmr) indicated that compounds **8–10** possessed nuclei identical with those of the previous known 3-*O*-β-(2′-*O*-methyl)xylopyranosides **3** and **5**, while **4** and **7** were their 15-desulfated derivatives. Thus it was only necessary to settle the structure and the stereochemistry of their side chains.

Compounds **4** and **7** gave ions in fabms (negative ion mode) at *m/z* 625 and *m/z* 611 $[M-H]^-$, respectively. In their ^{1}H-nmr spectra the signals for H-15 and H-16 were observed at δ 4.18 (dd, J=11.2, 2.5 Hz) and 3.98 (dd, J=7.5, 2.5 Hz), shifted upfield relative to the 15-*O*-sulfated analogues, δ_H 4.80 dd and 4.38 dd. The spectrum of **4** showed also signals for three secondary methyls at δ 0.96, 0.84, and 0.82 (all d's J=7 Hz) and one-CH_2OH group, δ_H 3.49 dd (J=10.5, 6.5 Hz)–3.38 (partially obscured by the MeOH signal), indicative of a 24-methyl-26-hydroxylated side chain. Model 24-methyl-26-hydroxysteroids with all possible configurations at C-24 and C-25 have been synthesized, and it has been shown that threo and erythro isomers can be easily differentiated by ^{1}H-nmr chemical shifts of C-26, C-27, and C-28 protons (17). In the



side chain	R	side chain	R
	$SO_3^-Na^+$		H
3		**7**	
	H		$SO_3^-Na^+$
4		**8**	
	$SO_3^-Na^+$		$SO_3^-Na^+$
5		**9**	
	H		$SO_3^-Na^+$
6		**10**	



11 R=H
12 R=OH

spectrum of the natural **4** the signals at δ 0.84 and 0.82 (Me-27 and -28) and a 3.49 dd –3.38 (26-H_2) match closely those observed in the spectra of the threo model pair 24*R*,25*S*/24*S*,25*R* (17) (Figure 1). Assignment of absolute configuration was achieved by the ^{1}H-nmr spectral data of the 26-*O*-(+)-MTPA ester of **4** [MTPA=α-methoxy-α-(trifluoromethyl)phenylacetic acid, Mosher's reagent (18). The term (+) or (−)-MTPA ester refers to an ester obtained using acid chloride prepared from *R*-(+) or *S*-(−) acid, respectively.] The pattern of the 26-methylene proton signal, a broad doublet (J=6 Hz) at δ 4.25, was a firm indication of the 25*S* absolute configuration and hence of the *R* configuration at C-24. In the case of a 25*R* configuration, the 26-methylene protons of the (+)-MTPA ester derivative are expected to resonate as two well separated double doublets (e.g., in the model 24*S*,25*R* they have been observed at δ 4.14–4.38) (17).

The ^{1}H-nmr spectrum of compound **7** showed signals for two secondary methyls at δ_H 0.94 and 0.97 (d's, J=7 Hz) and one -CH_2OH group, δ_H 3.46 dd (J=10.5, 6.0 Hz)–3.30 (partially obscured by MeOH), indicating a 26-hydroxylated side chain, a functionality very common among polyhydroxysteroids from starfishes (1). Its ^{13}C-nmr spectrum (Table 3) gave support to this assignment. The 25*S* configuration is proposed on the basis of the 26-methylene proton signals resonating as a 2H doublet at δ 4.21 in the ^{1}H-nmr spectrum of the 26-*O*-(+)-MTPA ester and as two well separated double doublets at 4.06 (J=11, 7 Hz) and 4.25 (J=11, 5.5 Hz) in that of the 26-*O*-(−)-MTPA ester (1,19).

Compounds **8** and **9** were isolated as a mixture resistant to attempts of separation by reversed-phase hplc, even after removal of the sulfate at C-15 by solvolysis in dioxane/pyridine. Because of the limited amounts available (4.0 mg), we decided to pursue the structure determination on the mixture. The fabms (negative ion mode) gave only one molecular anion peak at *m/z* 717 $[MSO_3]^-$. The ^{1}H-nmr spectrum contained signals for the 2-*O*-methylxylopyranosyl unit and for the Δ^4-3β,6β,8,16β-tetrahydroxy-15α-sulfoxy tetracyclic steroid nucleus. In addition, the spectrum contained an eight-line pattern centered at δ 5.22 (dd, J=15, 7.5) and 5.48 (dd, J=15, 7.2) which could be assigned to the Δ^{22}-trans protons. In the hydroxymethylene proton region a multiplet

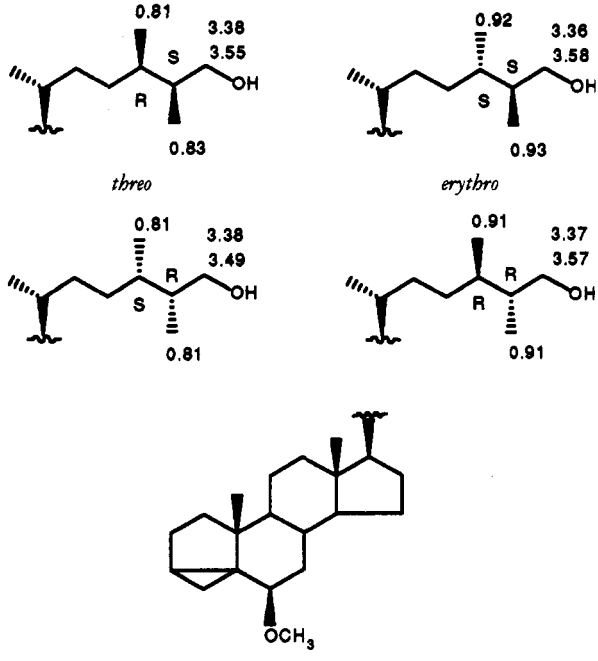


FIGURE 1. ^{1}H nmr data of model 24-methyl-26-hydroxysteroids.

TABLE 3. Assignments of nmr Signals (CD_3OD) to the Side Chain of Compounds **4**, **7**–**10** [*J* (Hz) values are shown in parentheses].

Position	Compound								
	4	**7**[a]		**8**[b]		**9**[b]		**10**[c]	
	^1H	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
20			30.7	2.60 m	34.3	2.60 m	34.6		31.8
21	0.96 d (7)	0.97 d (7)	18.4	1.06 d (7)	20.3	1.06 d (7)	20.3	0.96 d (7)	18.5
22			37.0	5.48 dd (15, 7.2)	138.8	5.47 dd (15, 7.2)	139.2		34.9
23			24.8	5.22 dd (15, 7.5)	130.8	5.22 dd (15, 7.5)	133.7		28.9
24			34.9		49.0		47.5		42.1
25			37.1		41.2		33.6		30.7
26	3.49 dd (10.5, 6.5)	3.46 dd (10.5, 6)	68.4	3.30 dd	66.6	0.87 d (7)	19.5	0.87 d (7)	19.1
	3.38 dd	3.32 dd		3.65 dd					
27	0.84 d (7)	0.94 d (7)	17.3	0.95 d (6.8)	15.6	0.91 d (7)	21.2	0.90 d (7)	20.1
28	0.82 d (7)				25.8		36.3		30.6
29				0.87 t (7)	12.5	3.62 m	61.5		70.0

[a]Other signals: H-3 (4.23 br t), H-4 (5.67 br s), H-6 (4.34 br t), H-7 (2.60 dd, J=15, 3 Hz), H-15 (4.18 dd, J=11.2, 2.5 Hz), H-16 (3.98 dd, J=7.5, 2.5 Hz), H_3-18 (1.16 s), H_3-19 (1.40 s). C-1 (39.7), C-2 (27.9), C-3 (77.4), C-4 (126.3), C-5 (148.5), C-6 (76.1), C-7 (45.1), C-8 (76.4), C-9 (57.8), C-10 (37.7), C-11 (19.6), C-12 (43.0), C-13 (44.1), C-14 (63.6), C-15 (80.9), C-16 (82.8), C-17 (60.6), C-18 (16.7), C-19 (22.6).

[b]Signals taken from the spectrum of their mixture.

[c]Other signals: H-3 (4.27 br t), H-4 (5.67 br s), H-6 (4.33 t, J=3 Hz), H-7 (2.69 dd, J=15, 3 Hz), H-15 (4.80 dd, J=11, 2.5 Hz, H-16 (4.38 dd, J=7.5, 2.5 Hz), H_3-18 (1.22 s), H_3-19 (1.40 s). C-1 (39.7), C-2 (28.0), C-3 (77.5), C-4 (126.3), C-5 (148.5), C-6 (76.0), C-7 (45.0), C-8 (76.4), C-9 (57.6), C-10 (37.2), C-11 (19.6), C-12 (42.8), C-13 (43.6), C-14 (61.8), C-15 (87.5), C-16 (80.2), C-17 (60.7), C-18 (16.9), C-19 (22.7).

at δ 3.62 and two double doublets at δ 3.65 (J=10.5, 6.5) and 3.30 (partially obscured by the MeOH signal) were suggestive for Δ^{22E}-24-(β-hydroxyethyl) and Δ^{22E}-24-ethyl-26-hydroxy side chains, taking into account the mol wt of the two isomers.

In the methyl region of the spectrum we observed two doublets at δ 0.87 and 0.91, each integrating for less than 3H, assigned to H_3-26 and H_3-27 of compound **9**, and one doublet at δ 0.95 and a triplet at 0.87, each integrating for less than 3H, assigned to H_3-27 and H_3-29 of compound **8**. Model Δ^{22}-24-ethyl-26-hydroxysteroids have been synthesized, and their ^{1}H nmr and ^{13}C nmr measured (20). With these models in our hands it was easy to analyze the ^{13}C-nmr data taken on the mixture of **8** and **9**. The signals for the side chain carbons are reported in Table 3. The ^{1}H- and ^{13}C-nmr spectra of the synthetic pair **13a** (24*R*,25*S*)/**14a** (24*S*,25*R*) are significantly different from those of the pair **13b** (24*R*,25*R*)/**14b** (24*S*,25*S*); major differences relate to the chemical shifts of the C-26 and C-27 protons and of the C-27 and C-28 carbons (20) (Figure 2). Our values, δ_H 0.95 (H_3-27), 3.30–3.65 (H_2-26) and δ_C 15.6 (C-27) and 25.8 (C-28) ppm are very close to those of the pair **13a**/**14a**. We also showed that differentiation within each pair can be derived from the chemical shifts of the 26-methylene protons of the (+)- and (−)-MTPA derivatives. More proximate signals in the spectrum of the (+)-MTPA ester point to the 25*S* configuration, while the inverse behavior is an indication of the 25*R* configuration. Thus we treated the desulfated mixture of **8** and **9** with (+)-MTPA chloride and measured the spectrum of the reaction mixture showing two double doublets at δ 4.10 and 4.31 for the (Me)CH-CH_2-O-MTPA protons, in agreement with a 25*S* (**13a**) configuration. The 26-protons resonances are expected to be more separated (δ_H 4.04–4.38) in the case of 25*R* (**14a**) configuration. Thus we assign the 24*R*,25*S* configuration to the isomer **8**; the 24*S* configuration is only suggested for the isomer **9** by analogy with echinasteroside B [**6**] (15-$OSO_3^-Na^+$) isolated from *E. sepositus* (6) and the many 24-(β-hydroxyethyl) steroids isolated from starfishes (1).

Echinasteroside F [**10**] is a further example of those diglycosides in which the two

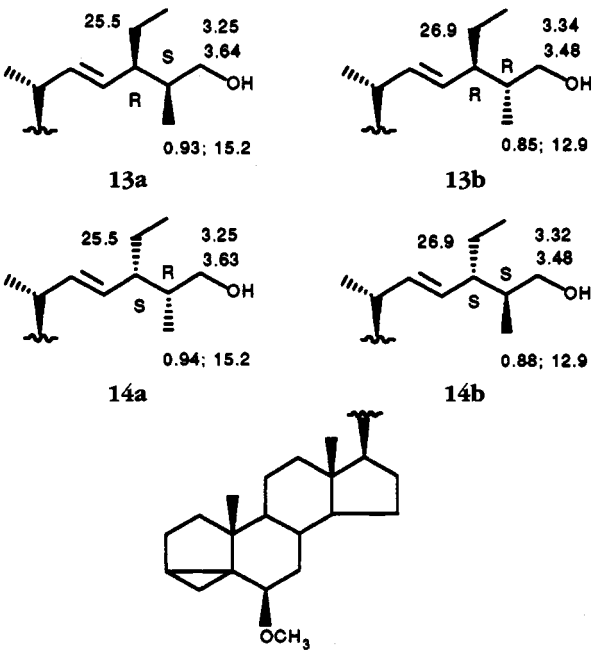


FIGURE 2. ^{1}H nmr data of model Δ^{22}-24-ethyl-26-hydroxysteroids.

monosaccharides are not linked to each other. The fabms (negative ion mode) gave a molecular anion peak at *m/z* 851 $[MSO_3]^-$. Examination of the spectral data (^{1}H nmr and ^{13}C nmr, Tables 3 and 4) indicated that **10** contained the same 24-ethyl-5α-cholest-4(5)-ene-3β,6β,8,15α,16β,29-hexaol 15-sulfated aglycone already found in echinasteroside B [**6**] ($R=SO_3^-Na^+$) (6). A detailed ^{1}H-nmr analysis using COSY experiments also identified the presence of two monosaccharide residues as 2-*O*-methyl-β-xylopyranosyl and β-xylopyranosyl units, and the assignments were achieved (Table 4). ^{13}C-nmr data indicated C-3 and C-29 as sites of glycosidation (in **10**, C-29): 70.0 ppm, C-28 30.6 ppm; in echinasteroside B C-29 62.3 and C-28: 34.8 ppm) (6). A larger downfield shift has been constantly observed for the anomeric proton signal in steroid 3-*O*-xylopyranosides (δ_H 4.44–4.40) than in steroid 29-*O*-xylopyranosides (δ_H 4.25–4.29) (1,21,22), and on this basis the relative positions of the monosaccharides in **10** have been determined (i.e., 2-*O*-methyl-β-xylopyranosyl, δ_H anomeric 4.46, at C-3 and β-xylopyranosyl, δ_H anomeric 4.23, at C-29).

Echinasteroside G [**12**] is related to the known laeviusculoside I [**11**] (7), co-occurring in the same organism, by introduction of an additional hydroxy group at the position 16β in the steroid aglycone. The fabms (negative ion mode) showed a quasi-molecular ion peak at *m/z* 643 $[M-H]^-$, sixteen mass units shifted relative to **11** (*m/z* 625), accompanied by a peak at *m/z* 483 corresponding to the loss of a dimethoxylated pentose unit. An analysis of the ^{1}H- and ^{13}C-nmr spectra of **12** (Table 5) and comparison with **11** and the many polyhydroxysteroids and steroid glycosides isolated in our laboratory [e.g., thromidioside: 3β-*O*-(2-*O*-methyl-β-D-xylopyranosyl)-24-methyl-5α-cholest-24(28)-ene-3β,4β,6β,8,15α,16β,26-heptaol (23)] allowed a 5α-cholestane-3β,4β,6β,8,15α,16β,24-heptaol structure to be established for the aglycone of **12**. The ^{13}C nmr indicated C-3 as the site of glycosidation and also confirmed the structure of the dimethoxylated pentose unit as a 2,4-di-*O*-methyl-β-xylopyranose, a common monosaccharide unit among glycosides from starfishes. The 24*S* configuration is suggested by comparison of the isopropyl methyl signals in the ^{1}H-nmr spectra of the 24-(+)-MTPA and 24-(−)-MTPA ester derivatives, at higher field in the 24-(+)-MTPA ester (δ 0.80 and 0.82 d's) than in the 24-(−)-MTPA (δ 0.89 and 0.90 d's) (1,13).

ANTHRAQUINONE PIGMENTS.—The last eluted fractions from the Sephadex LH-60 column, containing mainly coloring matter, were further purified by reversed-phase hplc to yield five pigments, the known compounds **15–19**, all of which are derivatives

TABLE 4. Assignments of nmr Signals (CD_3OD) of the Carbohydrate Moiety of Compounds **4**, **7–10** [*J* (Hz) values are shown in parentheses].

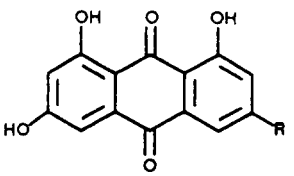
	Compound					
	4, 7, 8, 9[a]		**10**			
	2-*O*-Methyl-β-xylopyranosyl		2-*O*-Methyl-β-xylopyranosyl		β-Xylopyranosyl	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1′	4.45 d (7.5)	104.6	4.46 d	104.9	4.23 d (7.5)	105.2
2′	2.85 dd (7.5, 8.5)	84.9	2.84 dd	85.0	3.18 dd (7.5, 9)	75.0
3′	3.30[b]	77.4	3.30[b]	77.5	3.36[b]	77.9
4′	3.50 m	71.2	3.50 m	71.4	3.51 m	71.5
5′	3.20 dd (11.5, 9.5)	66.8	3.20 dd	66.8	3.24 dd (11.5, 9.5)	67.0
	3.85 dd (11.5, 5)		3.85 dd		3.87 dd (11.5, 5)	
OMe	3.61 s	61.2	3.62 s	61.2		

[a]Signals extracted from echinasteroside C [**7**].
[b]Under solvent signal.

TABLE 5. Assignments of nmr Signals (CD_3OD) of Glycosides **11** and **12** [*J* (Hz) values are shown in parentheses].

Position	Compound			
	11		**12**	
	^1H	^{13}C	^1H	^{13}C
1		41.0		40.9
2		25.3		25.2
3	3.69 m	81.0	3.69 m	80.6
4	4.30 br s	74.6	4.30 br s	74.6
5		50.5		50.5
6	4.30 br s	76.1	4.30 br s	76.1
7	2.44 dd (14.5, 3.2)	45.2	2.48 dd (14.5, 3.2)	45.2
8		76.5		76.5
9		57.7		57.7
10		36.8		36.9
11		19.4		19.1
12		42.8		43.0
13		45.4		45.1
14		66.5		63.7
15	4.32 dt (10, 3)	70.1	4.04 dd (11, 2.5)	81.0
16		41.6	4.18 dd (8, 2.5)	83.0
17		56.0		60.9
18	0.99 s	15.4	1.15 s	16.6
19	1.48 s	18.7	1.48 s	18.6
20		36.3		31.1
21	0.94 d (7)	19.1	0.97 d (7)	18.6
22		33.6		33.6
23		31.6		31.8
24	3.24 m	78.2	3.25 m	78.5
25		34.6		34.7
26	0.92 d (7)	17.6	0.95 d (7)	17.6
27	0.94 d (7)	19.6	0.91 d (7)	19.3

of 1,6,8-trihydroxy-9,10-anthraquinone. Their structures were assigned on the basis of their mass, uv, and ^1H-nmr spectral data and comparison with the literature data. Crinemodin [**15**] is one of the most common crinoid pigments (24) and has also been found in the starfishes *Echinaster echinophorus* (25) and *H. laeviuscula* (26), both belonging to the family Echinasteridae. Isorhodoptilometrin [**17**] and ω-rhodoptilometrin [**18**], both with a hydroxylated C_3 side chain, were also previously found in *E. echinophorus* (25,27) and *H. laeviuscula* (26). Rhodoptolometrin [**16**] was isolated from the crinoid



15 R=-$CH_2CH_2CH_3$
16 R=-CHOH-CH_2CH_3
17 R=-CH_2CHOH-CH_3
18 R=-$CH_2CH_2CH_2OH$
19 R=-CH_2CHOH-$CH_2CH_2CH_3$

Comathus bennetti (28). The C_5 hydroxylated side chain pigment **19** was previously found in the crinoid *C. bennetti* (28) and in the starfish *E. echinophorus* (25).

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 glycerol-thioglycerol (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, 156°, helium 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 i.d.; flow rate 2 ml·min^{-1}), Waters Model 6000 A pump equipped with U6Kinjector 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.

EXTRACTION AND ISOLATION.—The animals *E. brasiliensis* were collected at Grand Bahama Island in July 1990, and identified by Professor M. Jangoux, Université Libre de Bruxelles; a voucher specimen is preserved there. The animals (540 g) were chopped and soaked in H_2O (twice, 2 liters for 8 h each); the aqueous extracts were decanted and passed through a column of Amberlite XAD-2 (600 g). This column was washed with distilled H_2O and eluted with MeOH (7 liters) to give, after removal of the solvent, a glassy material (2.3 g). The solid mass was extracted with Me_2CO (2.5 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 1.4 g of a glassy material, which was combined with the above MeOH eluate from Amberlite XAD-2 column and chromatographed on a column of Sephadex LH-60 (4×80 cm) with MeOH-H_2O (2:1) as eluent. Fractions (7.5 ml) were collected and analyzed by tlc on SiO_2 with MeOH-HOAc-H_2O (12:3:5) and $CHCl_3$-MEOH-H_2O (80:18:2).

Fractions 16-27 contained the crude asterosaponins (327 mg); fractions 28–57 (340 mg) were a mixture of sulfated glycosides and glycosides of polyhydroxysteroids; fractions 58–110 (77.8 mg) contained almost exclusively a mixture of lipids, and fractions 111–230 contained a mixture of anthraquinone pigments.

Fractionation of asterosaponins (327 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 12 ml/h; 6-ml fractions were collected and analyzed by tlc on SiO_2 with *n*-BuOH–HOAc–H_2O (12:3:5)] to give four main fractions. Fractions 21–26 (46 mg) and 27–31 (32.3 mg) contained a mixture of the marthasteroside A_1 [**1**] and brasiliensoside [**2**], fractions 32–37 (98 mg) contained brasiliensoside [**2**], and fractions 38–57 contained almost exclusively the sulfated diglycoside **10**. 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.

Fractions 28–57 (340 mg) from LH-60 were a complex mixture of steroid glycosides and polyhydroxysteroids along with a minor amount of sulfated glycosides. Fractionation was pursued by dccc using $CHCl_3$-MeOH-H_2O (7:13:8) (ascending mode, the lower phase was the 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 1–10 (35.8 mg) mainly contained sulfated compounds; fractions 11–40 (45 mg) were constituted of uv visible components (nucleosides); and the last fractions 40–120 after purification by hplc on a C_{18} column (30 cm×3.9 mm i.d.) with MeOH-H_2O (75:25) gave pure nonsulfated glycosides **4**, **6**, **7**, **11**, and **12** as shown in Table 1.

Hplc with MeOH-H_2O (55:45) of the more polar fractions 1–10 (35.8 mg) gave pure sulfated glycosides **3**, **5**, **8**, and **9**.

Fractions 111–230 eluted from LH-60 column contained a mixture of anthraquinone pigments, which were further purified by hplc on a C_{18} column with MeOH-H_2O (75:25) (flow rate 1.2 ml·min^{-1}) to give pure **15–19**.

OTHER PHYSICAL DATA.—*Brasiliensoside* [**2**].—Rotation data see Table 1; fabms see text; ^{1}H nmr in CD_3OD δ_H (aglycone) 0.81 (3H, s, Me-18), 0.93 and 0.94 (each 3H, d, *J*=6.5 Hz, Me-26 and -27), 1.02 (3H, s, Me-19), 1.37 (3H, s, Me-21), 2.40 (2H, d, *J*=6.5 Hz, H_2-24), 2.62 (2H, ABq, *J*=15 Hz, H_2-22), 4.22 (1H, m, H-3α), 5.37 (1H, br d, *J*=5.5 Hz, H-11); δ_H (sugars) 1.26, 1.28, 1.41, 1.49 (each 3H, d, *J* ranging from 6.5 to 7.0 Hz, Me-5 of 6-deoxy-*xylo*-hex-4-ulose, quinovose, and fucose), 4.45 (2H), 4.52 (2H), 4.58 (1H) (each d, *J*=7.5 Hz, anomeric-H's); ^{13}C nmr (pyridine-d_5/H_2O) δ_C (aglycone) C-1 (35.3), C-2 (28.7), C-3 (77.1), C-4 (30.1), C-5 (48.6), C-6 (79.3), C-7 (40.7), C-8 (34.6), C-9 (144.9), C-10 (37.7), C-11 (116.0), C-12 (41.7), C-13 (40.9), C-14 (53.3), C-15 (22.6), C-16 (24.5), C-17 (58.8), C-18 (13.0), C-19 (18.7), C-20 (73.3), C-21 (26.3), C-22 (54.6), C-23 (211.6), C-24 (53.3), C-25 (23.7), C-26 (22.0), C-27 (21.9); δ_C (sugars) see Table 2.

Echinasteroside G [**12**].—Rotation data see Table 1; fabms see text; ^{1}H and ^{13}C nmr of the steroid aglycone see Table 5; ^{1}H nmr (CD_3OD) δ_H (sugar) 4.47 (1H, d, J=7.5 Hz, H′-1), 2.95 (1H, dd, J=9.0, 7.5 Hz, H′-2), 3.48 (1H, t, J=9.0 Hz, H′-3), 3.20 (1H, m, H′-4), 3.19 (1H, t, J=10.5, H′-5), 4.04 (1H, dd, overlapped with H-15, H′-5), 3.50, and 3.65 (each 3H, s, -OMe); ^{13}C nmr (CD_3OD) δ_C C-1 (102.5), C-2 (84.7), C-3 (76.7), C-4 (80.5), C-5 (64.2), -OCH_3 61.0 and 59.0.

Crinemodin [**15**].—Uv λ max (MeOH) 254 (14,500), 265 (14,700), 291 (14,000), 304 sh, 440 nm; fabms (negative ion mode) *m/z* $[M-H]^-$ 297; ^{1}H nmr (CD_3OD) δ_H 1.02 (3H, t, J=7 Hz), 1.73 (2H, m), 2.71 (2H, t, J=7.5 Hz), 6.57, 7.12, 7.20, 7.60 (each 1H, s).

Rhodoptilometrin [**16**].—Uv λ max (MeOH) 253 (14,600), 266 (13,900), 271 (13,800), 437 (7,800) nm; fabms (negative ion mode) *m/z* $[M-H]^-$ 313; ^{1}H nmr (CD_3OD) δ_H 1.00 (1H, t, J=7.5 Hz), 1.82 (2H, quint, J=7.5 Hz), 4.66 (1H, t, J=7 Hz), 6.56, 7.20, 7.32, 7.80 (each 1H, s).

Isorhodoptilometrin [**17**].—Uv λ max (MeOH) 253 (14,600), 267 (13,700), 437 (7900), 458 (7600), nm; fabms (negative ion mode) *m/z* $[M-H]^-$ 313; ^{1}H nmr (CD_3OD) δ_H 1.25 (1H, d, J=7 Hz), 2.81 (2H, d), 4.05 (1H, m), 6.52, 7.15, 7.20, 7.61 (each 1H, s).

ω-*Rhodoptilometrin* [**18**].—Uv λ max (MeOH) 252 (14,600), 267 (13,800), 291 (14,600), 437 (8,600), 455 (7600) nm; fabms (negative ion mode) *m/z* $[M-H]^-$ 313; ^{1}H nmr (CD_3OD) δ_H 1.92 (2H, m), 2.82 (2H, t), 3.65 (2H, t, J=6.2 Hz), 6.56, 7.14, 7.18, 7.62 (each 1H, s).

1,6,8-Trihydroxy-3-(2-hydroxypentyl)anthraquinone [**19**].—Uv λ max (MeOH) 253 (17,000), 267 (18,300), 290 (11,300), 441 (19,800), 461 sh nm; fabms (negative ion mode) *m/z* $[M-H]^-$ 341; ^{1}H nmr (CD_3OD) δ_H 0.98 (1H, t, J=6.8 Hz), 2.77 and 2.90 (each 1H, dd), 3.88 (1H, m), 6.58, 7.19, 7.22, 7.68 (each 1H, s).

METHANOLYSIS OF BRASILIENSOSIDE [**2**]: SUGAR ANALYSIS.—A solution of the glycoside **2** (1 mg) in anhydrous 2 M HCl in MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 8 h. After having been cooled, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2. The residue was trimethylsilylated with TRISIL Z (Pierce Chemical Co.) for 15 min at room temperature. Glc analysis (OV-101 capillary column, 25 m, 156°, He carrier, flow 2 $ml \cdot min^{-1}$) gave peaks that co-eluted with those of the methyl quinovosides, methyl fucosides, and methyl galactosides standards in the ratio 2:1:1.

METHYLATION OF BRASILIENSOSIDE FOLLOWED BY METHANOLYSIS: TERMINAL SUGARS.—Brasiliensoside [**2**] (4 mg) in 1.0 ml dry DMF was added under N_2 to a stirred mixture of NaH (70 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 by 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 (OV-101, 25 m, 98°, helium carrier, flow 10 ml/min). Glc peaks co-eluted with those of methyl-2,3,4-tri-*O*-methylfucoside and methyl-2,3,4-tri-*O*-methylquinovoside standards.

ENZYMATIC HYDROLYSIS OF BRASILIENSOSIDE [**2**].—Saponin **2** (4 mg) in 1 ml of citrate buffer (pH 4.5) was incubated with 6 mg of glycosidase mixture of *Ch. lampas* (Seikagaku Kogyo) at 37° for 90 min. The reaction was followed by tlc in 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 MeOH eluate was evaporated and purified by hplc on a C_{18} column (30 cm×3.8 mm i.d.) with MeOH-H_2O (52:48) to give the trisaccharide **2a**: fabms (negative ion mode) *m/z* $[MSO_3]^-$ 965; ^{1}H nmr (CD_3OD) δ_H (aglycone) identical with those reported for natural **2**; δ_H (sugars) 1.26, 1.28, and 1.31 (each 3H, d, J ranging from 6.5 to 7 Hz, Me-5 of 6-deoxy-*xylo*-hex-4-ulose and quinovose), 4.45, 4.50, and 4.57 (each d, J=7.5 Hz, anomeric-H's); ^{1}H nmr (pyridine-d_5) δ_H (sugars) 1.61, 1.70, and 1.76 (each 3H, d, J ranging from 6.5 to 7 Hz, Me-5 of 6-deoxy-*xylo*-hex-4-ulose and quinovose), 5.00, 5.06, and 5.10 (each d, J=7.5 Hz, anomeric-H's).

SOLVOLYSIS OF BRASILIENSOSIDE [**2**]: THORNASTEROL A.—A solution of compound **2** (5 mg) in a mixture of dioxane (1 ml) and pyridine (1 ml) was heated at 120° for 2 h in a stoppered reaction vial. After the solution had cooled, the solvent was removed under reduced pressure and the residue was partitioned between H_2O (5 ml) and *n*-BuOH (5 ml). The extraction was repeated three times, and the combined extracts were washed with H_2O and evaporated to dryness under reduced pressure. The residue was purified by hplc (C_{18} μ-Bondapak column) with MeOH-H_2O (65:35) to give the major peak, which was identified as (20*S*)-3β,6α,20-trihydroxy-5α-cholest-9(11)-en-23-one (thornasterol A) by ^{1}H nmr and ms.

SOLVOLYSIS OF GLYCOSIDES **8**, **9**, AND **10**.—A solution of **8** and **9** (1.5 mg) in pyridine (0.1 ml each) and dioxane (0.1 ml each) was heated at 130° for 2 h in a stoppered reaction vial. The residue was evaporated to dryness and purified by hplc (C_{18} μ-Bondapak column 30 cm×3.8 mm i.d.) with MeOH-H_2O (75:25) as eluent, to give only one peak (desulfated mixture of **8** and **9**) fabms (negative ion mode) *m/z* $[M-H]^-$ 637; ^1H nmr δ_H (aglycone) 0.87, 0.92, 0.94 (each d, J=7 Hz), 0.87 (t, J=7 Hz), 1.07 (d, J=7 Hz, Me-21), 1.19 (s, Me-18), 1.40 (s, Me-19), 2.60 (dd, J=15, 3 Hz, H-7), 3.65–3.30 (each dd, H_2-26), 3.94 (dd, J=7.5, 2.5 Hz, H-16), 4.19 (dd, J=15, 3 Hz, H-15), 4.22 (br t, H-3α), 4.34 (t, J=3, H-6), 5.30 and 5.47 (dd, J=15, 7.5 and 15, 7.2 Hz, H-22 and H-23), 5.45 and 5.30 (dd, J=15, 7.5 and 15, 7.2, H-22 and H-23), 5.67 (br s, H-4).

Similarly the glycoside **10** was desulfated: fabms (negative ion mode) *m/z* $[M-H]^-$ 771 (100%), $[M-132]^-$ 639 (20); ^1H nmr δ_H (aglycone) 0.87 (3H, d, J=6.8 Hz, Me-26 or -27), 0.90 (3H, d, J=6.8 Hz, Me-26 or -27), 0.96 (3H, d, J=6.8 Hz, Me-21), 1.16 (3H, s, Me-18), 1.40 (3H, s, Me-19), 2.61 (1H, dd, J=15, 3 Hz, H-7), 4.00 (1H, dd, J=7.5, 2.5 Hz, H-16), 4.18 (1H, dd, J=11, 2.5 Hz, H-15), 4.22 (1H, br t, H-3α), 4.34 (1H, t, J=3 Hz, H-6), 5.67 (1H, br s, H-4); δ_H (sugars) data identical with those reported for the natural echinasteroside F [**10**].

MTPA ESTERS OF GLYCOSIDES **4** AND **7**.—Compound **4** (1 mg) was esterified with fresh distilled (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (4 μl) in dry pyridine (0.10 ml) for 1 h at room temperature to give after removal of the solvent, the 26-(+)-MTPA ester: ^1H nmr (CD_3OD) significant signal at δ 4.25 (2H, br d, H_2-26). Echinasteroside C [**7**] (0.7 mg) was esterified with (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride as above to give the 26-(+)-MTPA ester: ^1H nmr (CD_3OD) significant signal at δ 4.21 (2H, d, J=6 Hz, H_2-26). The 26-(−)-MTPA ester of **7** (0.7 mg) was prepared using (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride fresh distilled in an identical manner: ^1H nmr (CD_3OD) gave significant signals at δ 4.06 (1H, dd, J=11, 7 Hz) and 4.25 (1H, dd, J=11, 5.5 Hz) for H_2-26.

MTPA ESTER OF GLYCOSIDES **8** AND **9**.—The desulfated mixture of **8** and **9** (2 mg) was esterified with (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride as above to give the 26-(+)-MTPA ester. ^1H nmr (CD_3OD) gave significant signals at δ 4.10 (1H, dd) and 4.31 (1H, dd) for H_2-26).

MTPA ESTER OF GLYCOSIDES **11** AND **12**.—Compounds **11** (2 mg) and **12** (2 mg) were esterified with (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride as above to give the 3′,24-di-(+)-MTPA esters. Compound **11**: ^1H nmr ($CDCl_3$) δ_H 0.80 and 0.82 (each 3H, d, J=7 Hz, Me-26 and -27), 0.81 (3H, s, Me-18), 0.86 (3H, d, J=7 Hz, Me-21), 1.20 (3H, s, Me-19), 3.03 (1H, dd, J=7.5, 9.5 Hz, H-2′), 3.27 and 3.38 (each 3H, s, -OMe), 4.10 (1H, br s, H-6), 4.14 (H-4 and H-15), 4.45 (1H, d, J=7.5 Hz, H-1′), 4.92 (1H, m, H-24), 5.21 (1H, t, J=8.5 Hz, H-3′). 3′-15,24-tri-(−)-MTPA esters: ^1H nmr ($CDCl_3$) δ_H 0.83 (3H, d, J=7 Hz, Me-21), 0.89 and 0.90 (each 3H, d, J=7 Hz, Me-26, and -27), 0.96 (3H, s, Me-18), 1.14 (3H, s, Me-19), 3.15 (1H, dd, J=7.5, 9.5 Hz, H-2′), 3.26 and 3.40 (3H, s, -OMe), 4.06 (1H, dd, H-5′), 4.13 (1H, br s, H-4), 4.90 (1H, m, H-24), 5.20 (1H, t, J=8.5 Hz, H-3′), 5.26 (1H, dt, H-15). Compound **12**: 3′,24-di-(+)-MTPA ester: ^1H nmr ($CDCl_3$) δ_H 0.81 and 0.83 (each 3H, d, J=7 Hz, Me-26 and -27), 0.89 (3H, d, J=7 Hz, Me-21), 3.02 (1H, dd, J=7.5, 9.5 Hz, H-2′), 4.46 (1H, d, J=7 Hz, H-1′), 4.93 (1H, m, H-24), 5.19 (1H, t, J=8.5 Hz, H-3′). 3′-24-di-(−)-MTPA ester: ^1H nmr ($CDCl_3$) δ_H 0.83 (3H, d, J=7 Hz, Me-21), 0.87 and 0.89 (each 3H, d, J=7 Hz, Me-26 and -27), 3.15 (1H, dd, J=7.5, 9.5 Hz, H-2′), 4.48 (1H, d, J=7 Hz, H-1′), 4.90 (1H, m, H-24), 5.20 (1H, t, J=8.5 Hz, H-5′).

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