Novel Marine Polyhydroxylated Steroids from the Starfish *Myxoderma platyacanthum*

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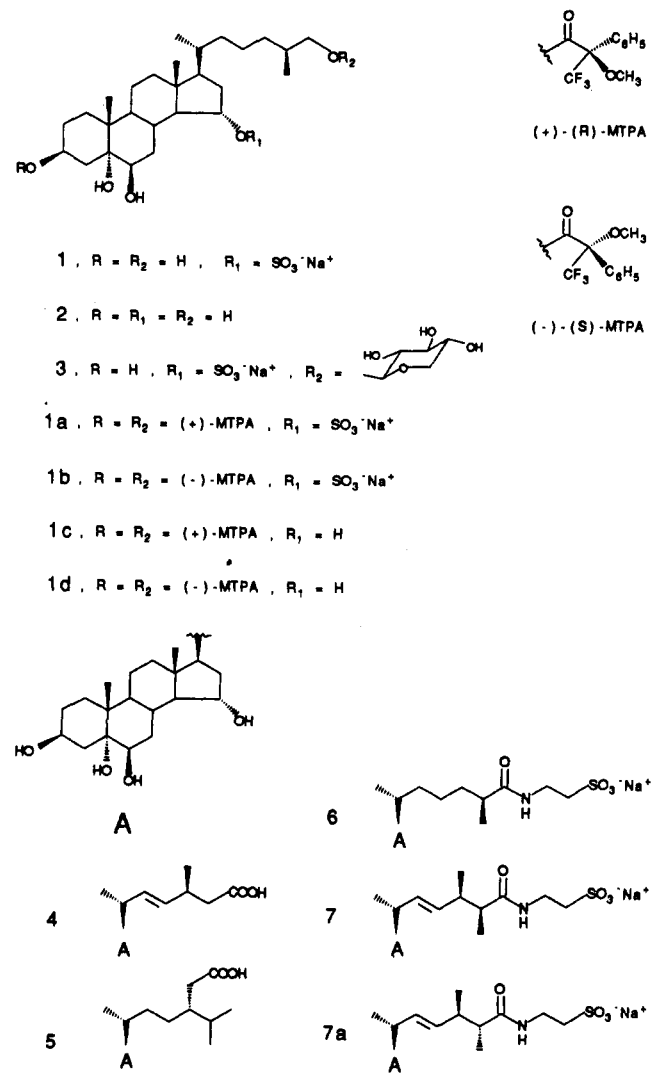
Nine polyhydroxy steroids and two steroid glycosides have been isolated from the starfish *Myxoderma platyacanthum*. The novel compounds **1–7** possess the same 3β,5α,6β,15α-tetrol nucleus (15-sulfated in **1** and **3**) but differ in the side chains. Two levels of oxidation (CH_2OH and CO_2H) at the side chain methyls were encountered in this class of steroids; in **6** and **7** the 26-carboxylic acid function is found as amide derivative of taurine. The novel compound **8** is an asterosaponin assigned as myxodermoside A, consisting of a $\Delta^{9(11)}$-3β,6α-dihydroxysteroidal moiety, a tetrasaccharide portion attached at C-6, and a sulfate group at C-3. Steroids **9–11** have known structures. The general structure of the novel compounds was determined from spectral data (^1H and ^{13}C NMR and FAB MS), and the stereochemistry of the side chains was determined by correlating the respective spectral data with those of synthetic models of known configuration. The stereoselective synthesis of the steroid **4** side chain (Δ^{22E}, 27-nor-24-methyl-26-carboxy) is also described.

Steroidal oligoglycosides and polyhydroxy steroids are the predominant metabolites of starfishes. Continued activity by our[1] and other groups, particularly by Stonik and Elyakov[2] and by Komori et al.,[3] has shown the large variety of these frequently highly oxygenated marine metabolites. Our investigation of the polar steroids mixture from the Pacific starfish *Myxoderma platyacanthum* H. L. Clark (family, Zoroasteridae; suborder, Asteriadina; order, Forcipulata) has led to the isolation and characterization of 11 steroid metabolites, eight of them novel, which are a further example of the structural variety of polyhydroxy steroids from starfish.[4]

One is the asterosaponin **8**, assigned as myxodermoside A, consisting of the common thornasterol 3-sulfate aglycone and a tetrasaccharide moiety attached at C-6. To the best of our knowledge this is the first reported isolation of a steroidal tetraglycoside from starfish; the previously described asterosaponins are penta- and hexaglycosides.[2] The remaining novel compounds **1–7** have the same nuclear 3β,5,6β,15α-hydroxylation pattern (**1** and **3** with sulfate conjugation in 15-hydroxy group) with different side chains. Five types of steroid side chains have been encountered (26-hydroxy; 26-carboxy; Δ^{22},27-nor-24-methyl-26-carboxy; 24-carboxymethyl; and 24-methyl-26-carboxy). In compound **3** the 26-hydroxyl group is glycosidated with a β-xylopyranosyl residue, while in **6** and **7** the carboxylic acid function is found as the amide derivative of taurine. To the best of our knowledge this is the first reported isolation from starfish of sterols with a methyl group oxidized to carboxyl. All compounds were isolated and purified by chromatography on Amberlite XAD-2 of the water extracts followed by chromatography on Sephadex LH-60, droplet counter current chromatography (DCCC), and HPLC. Since spectral data indicate that the steroids **1–7** possess virtually identical nuclei, but differed in the side chain, it was necessary to settle the nuclear substitution pattern of the steroids **2** and **1** (15-sulfated derivative of **2**).



Structure Elucidation of Steroids 1–3. The negative fast atom bombardment (FAB) mass spectrum of **1** exhibited a molecular anion peak at m/z 531, corresponding to a monosulfated derivative of a cholestane-pentol. On solvolysis in a dioxane–pyridine mixture, **1** afforded a desulfated derivative, FAB MS (negative ion mode) m/z 451, identical (^1H NMR, HPLC) with **2**. Examination of ^1H and ^{13}C NMR spectra of **1** and **2** (Tables I and II)

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(3) Dubois, M. A.; Higuchi, R.; Komori, T.; Takuma, S. *Liebigs Ann. Chem.* **1988**, 845 and references cited therein.
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Table I. Selected ^{1}H NMR Chemical Shifts (CD_3OD) of the Novel Steroids 1–7[a]

sterol	H-3α	H-6α	H-15β	H_3-18	H_3-19	H_3-21	$H_{2(3)}$-26	H_3-27	other signals
1	4.04 m	3.51 br s	4.51 dt (3, 9)	0.80 s	1.20 s	0.97 d (6.5)	3.44 dd (10.5, 5) 3.34[b]	0.93 d (7)	
2	4.04 m	3.50 br s	3.89 dt (3, 9)	0.76 s	1.20 s	0.97 d (6.5)	3.44 dd (10.5, 5) 3.34[b]	0.94 d (7)	
3	4.04 m	3.51 br s	4.51 dt (3, 9)	0.80 s	1.20 s	0.97 d (6.5)	3.70 dd (9.5, 5) 3.34[b]	0.96 d (7)	H-1′ 4.20 d (7), H-2′ 3.19 dd (7.5, 9), H-3′ 3.30,[b] H-4′ 3.50 m, H_2-5′ 3.87 dd (10.5, 5), 3.20 t (10.5)
4	4.04 m	3.50 br s	3.89 dt (3, 9)	0.78 s	1.20 s	1.04 d (6.5)	–	–	H-20[c] 1.97 m, H-22,23 5.31 m, H-24[c] 2.58 m, H_3-28, 1.045 d (7), H_2-25 2.22 dd (15, 6) to 2.11 dd (15, 7)
5	4.04 m	3.50 br s	3.89 dt (3, 9)	0.76 s	1.20 s	0.96 d (6.5)	0.93 d (7)[d]	0.87 d (7)[d]	H-20[c] 1.40 m, H-25[c] 1.78 m, H-28 2.32 dd (15, 5.5)
6	4.04 m	3.50 br s	3.89 dt (3, 9)	0.76 s	1.20 s	0.94 d (6.5)	–	1.12 d (7)	H-20[c] 1.40 m, H-25[c] 2.31 m, taurine residue 2.99 t (6), 3.62 t (6)
7	4.04 m	3.50 br s	3.89 dt (3, 9)	0.77 s	1.20 s	0.99 d (7)[e]	–	1.10 d (7)	H-3-28 1.01 d (6.5),[e] H-22,23 5.26 m, taurine residue 2.99 t (6.5), 3.62 m

[a] The chemical shift values are given in part per million (ppm) and were referred to CHD_2OD (3.34 ppm). The coupling constants are given in hertz and are enclosed in parentheses. [b] Under solvent signal. [c] Detected by decoupling. [d] The remaining H-28 signal is confused in the 2.00–1.5 ppm region. [e] Assignments can be reversed.

Table II. ^{13}C NMR Spectral Data[a]

C	1	2	3[b]	4	5	6[c]	7[c]	8[d]
1	31.7	31.7	31.7	31.7	31.7	31.7	31.7	36.0
2	33.6	33.6	33.6	33.6	33.6	33.6	33.6	29.4
3	68.4	68.3	68.4	68.4	68.3	68.4	68.4	77.9
4	41.6	41.7	41.6	41.5	41.7	41.7	41.6	30.7
5	76.6	76.4	76.6	76.6	76.5	76.7	76.6	49.4
6	76.6	76.4	76.5	76.7	76.5	76.6	76.5	80.4
7	34.9	36.1	35.1	35.5	35.3	35.4	35.4	41.7
8	31.3	31.3	31.4	31.3	31.4	31.4	31.3	35.4
9	46.6	46.6	46.6	46.8	46.7	46.7	46.7	145.6
10	39.4	39.4	39.4	39.4	39.4	39.4	39.4	38.4
11	22.1	22.2	22.1	22.2	22.2	22.2	22.1	116.7
12	41.6	41.7	41.6	41.7	41.7	41.7	41.6	42.5
13	44.1	45.1	44.1	45.1	45.1	45.1	44.9	41.5
14	61.2	63.6	61.2	64.1	63.8	63.7	63.7	54.9
15	81.8	74.3	81.8	74.5	74.4	74.4	74.2	23.3
16	38.8	41.5	38.7	41.7	41.5	41.6	42.0	25.2
17	55.3	55.0	55.3	54.1	55.1	55.1	54.9	59.7
18	13.8	13.8	13.8	14.1	13.8	13.8	14.0	13.6
19	17.2	17.4	17.4	17.2	17.3	17.3	17.3	19.4
20	34.8	36.6	34.8	40.4	36.8	36.4	40.7	73.7
21	19.1	19.2	19.1	21.2	19.2	19.2	21.2	27.1
22	37.4	37.4	37.3	136.4	34.7	37.1	137.7	54.9
23	24.6	24.5	24.3	133.8	28.7	25.0	132.6	211.8
24	34.8	34.9	36.6	34.9	42.8	35.8	41.1	54.1
25	36.9	36.9	34.8	44.2	30.9	42.4	48.2	24.4
26	68.5	68.4	76.2	176.5	18.7	178.2	178.2	22.7
27	17.3	17.2	17.5	20.8	20.1	18.2	18.2	22.5
28	–	–	–	–	37.8	–	15.2	–
29	–	–	–	–	176.5	–	–	–

[a] At 62.9 MHz; values relative to CD_3OD = 49.00 ppm (central peak); assignments aided by DEPT technique (compounds 1, 4, 8). [b] Signals for the xylose unit at 105.4 (C-1), 75.0 (C-2), 77.8 (C-3), 71.3 (C-4), and 66.8 (C-5) ppm. [c] Signals for the taurine residue: at 36.5 ($CH_2SO_3^-$) and 5.16 (NCH_2) ppm. [d] Signals for the oligosaccharide chain in Table IV.

immediately indicated the presence of the 3β,5α,6β-trihydroxy moiety in both compounds, a common element in marine polyhydroxy sterols, also encountered in sterols from starfishes.[5] The ^{1}H NMR spectrum of 1 also contains a 1 H double triplet (J = 2.5, 10 Hz) at δ 4.51, shifted upfield to δ 3.89 in the desulfated 2, with the shape for a 15α-hydroxy[6,7] and two 1 H double doublets at δ 3.44 (J = 10, 5 Hz), and 3.32, this latter overlapping with CHD_2OD signal, characteristic for a 26-hydroxy group which is a very common feature among the polyhydroxy steroids from starfish.[1] ^{13}C NMR data (Table II) when compared with 15α-cholestanol,[8] amurensosides A and B (15α-sulfoxy steroidal glycosides from *A. amurensis*),[7] and the many 26-hydroxy steroids isolated from starfish,[9] give support for the 15α-sulfoxy-26-hydroxy structure in 1 and the 15α,26-dihydroxy structure in 2.

Compound 3, FABMS (negative ion mode), m/z 663, is the 26-*O*-β-xylopyranoside of the sulfated steroid 1. In addition to the signals for the aglycon already observed

(5) (a) Riccio, R.; Minale, L.; Pizza, C.; Zollo, F.; Pusset, J. *Tetrahedron Lett.* **1982**, 3307. (b) Minale, L.; Pizza, C.; Riccio, R.; Squillace Greco, O.; Zollo, F.; Pusset, J.; Menou, J. L. *J. Nat. Prod.* **1984**, *47*, 784.

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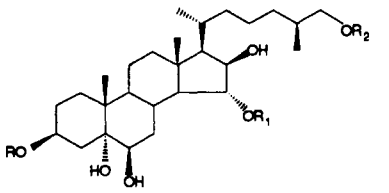
(9) See, for example: Riccio, R.; Minale, L.; Pagonis, S.; Pizza, C.; Zollo, F.; Pusset, J. *Tetrahedron* **1982**, *38*, 3615.

in the spectrum of **1**, the ^1H NMR spectrum contains the signals for a β-xylopyranosyl unit[7] (Table I). This was confirmed by acid methanolysis, which gave methyl-xylosides. Finally, the comparison of the ^{13}C NMR spectrum of **3** with that of its aglycon **1**, identified the site of glycosidation to be at C-26 (Table II); C-26 is deshielded by 7.7 ppm relative to **1** as expected upon glycosidation.[10]

The only feature needed to establish the complete structures of the steroids **1–3** was the stereochemistry at C-25. It has been shown[11–13] that (25*R*)- and (25*S*)-26-hydroxy steroids can be differentiated by the ^1H NMR of their (+)-(*R*)- and (−)-(*S*)-α-(trifluoromethyl)phenylacetic acid (MTPA, Mosher's reagent[14]) esters. In the spectra of the (+)-MTPA ester the 26-methylene protons of the 25*S* isomer appear as signals resonating much closer than in the corresponding 25*R* isomer, and the reverse in the (−)-MTPA esters, the resonance signals in the 25*R* isomer being closer than in 25*S* isomer. Thus we have treated **1** with (+)- and (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride affording the 3,26-di-(+)-MTPA and the 3,26-di-(−)-MTPA esters, respectively, and have recorded their ^1H NMR spectra. The 26-methylene proton signals of the (+)-MTPA ester appeared as dd at δ 4.14 and 4.24 (0.04 ppm between the inner lines of the two dd), while in the spectrum of the (−)-MTPA ester they appear slightly but clearly more separated (δ 4.14 and 4.26; 0.05 ppm between the inner lines of the two dd). On this basis we propose the 25*S* configuration for **1**, like the many 26-hydroxy steroids isolated from starfish.

Indeed we have always observed a larger difference between the chemical shifts of the 26-methylene protons in the spectra of the (25*R*) and (25*S*) 26-hydroxy steroids MTPA esters. In particular, in 26-hydroxy steroids unsubstituted at C-24, they were observed constantly as a 2 H doublet at δ 4.21 in (+)-MTPA ester in the 25*S* isomer (or (−)-MTPA ester of the 25*R* isomer) and as two 1 H double doublets at 4.08 and 4.24 ppm in (+)-MTPA ester of the 25*R* isomer (or (−)-MTPA ester of the 25*S* isomer).[11,12] We felt that the deviation from the "normal" values in the 3,26-di-(+)-MTPA ester of **1** could be mainly due to the presence of the bulky sulfoxy group at C-15α. To support this hypothesis, we prepared both (+)-MTPA and the (−)-MTPA esters of the known 15α-sulfoxy steroid **9**[15] also present in large amounts in the extractives of *M. platyacanthum*. The 26-methylene protons signals in the spectrum of 3,26-di-(+)-MTPA ester (**9a**) appear as two double doublets at δ 4.16 and 4.23 instead of the 2 H doublet at ca. δ 4.20, and in the spectrum of the 3,26-di-(−)-MTPA ester (**9b**) they are better separated signals at δ 4.13 and 4.26 (0.06 ppm between the inner lines of the two dd) than expected. Then, **9a** was solvolyzed into **9c**, and the removal of the sulfate group produced in the ^1H NMR spectrum the appearance of the expected doublet at δ 4.21 for the 26-methylene protons, in complete agreement with a 25*S* configuration. The 25*S* configuration was similarly confirmed for the known **10**; treatment of **10** with (+)- and (−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride gave the corresponding 26-MTPA esters showing signals for the 26-H_2 at δ 4.21 (2 H, doublet, J = 6.5 Hz) and at δ 4.12 (dd, J = 10.5, 6.5 Hz), 4.30 (dd, J = 10.5, 5.5 Hz) in the spectra of the (+)- and (−)-MTPA esters, respectively.



9, R = R_2 = H, R_1 = $SO_3^-Na^+$

10, R = R_1 = R_2 = H

9a, R = R_2 = (+)-MTPA, R_1 = $SO_3^-Na^+$

9b, R = R_2 = (−)-MTPA, R_1 = $SO_3^-Na^+$

9c, R = R_2 = (+)-MTPA, R_1 = H

Surprisingly, removal of the sulfate group in both **1a** and **1b**, giving **1c** and **1d**, respectively, did not produce significant changes in the ^1H NMR spectrum, the 26-methylene protons still appearing in both spectra as two double doublets, closer [δ 4.14–4.23 (0.03 between the inner lines of the two dd)] in **1c** [(+)-MTPA ester] and more separated [δ 4.13–4.26 (0.06 between the inner lines of the two dd system)] in **1d** [(−)-MTPA ester]. We suppose that the appearance of the 26-methylene protons in **1c** as two dd instead of the 2 H doublet could be due to the interaction of the hydroxyl group at C-15α with the MTPA grouping at C-26 producing some changes in the side chain conformation, which is prevented in compounds such **9–11**, because of the presence of the 16β-hydroxyl group projecting the side chain away from the nucleus. In conclusion, we believe that the method based on the ^1H NMR spectra of MTPA esters is reliable for the stereochemical assignments at C-26 of 26-hydroxy steroids. However, the chemical shift values of the 26-methylene protons in the MTPA esters also depend on the substituents on the ring D; but what is important is the comparison of the chemical shifts of the 26-methylene protons in the spectra of (+)- and (−)-MTPA esters.

Structure Elucidation of Steroid 4. Tables I and II contain ^1H and ^{13}C NMR spectral data of compounds **1–7** and they show conclusively that steroids **2** and **4–7** all have the 3β,5,6β,15α-tetrol structure but different side chains. In the 250-MHz ^1H NMR spectrum of **4** signals due to the terminal 26-hydroxy methylene group are absent, while two overlapping methyl doublets appear at δ 1.035 and 1.040 ppm, and a multiplet appears at δ 5.31, characteristic for the Δ^{22} double bond. The presence of a carboxyl group was indicated by the presence of IR absorption bands at 1705 (CO_2H), 1645, and 1558 (CO_2^-) cm^{-1}. The FAB mass spectrum (negative ion mode) of **4** exhibited an anion peak at m/z 463 ($[M - H]^-$). These data indicate a Δ^{22}, C_8 side chain with one methyl oxidized to carboxyl group. The 500-MHz ^1H NMR spectrum of **4** and double resonance experiments show the sequence $CH_3HCCH{=}CHCH(C\text{-}H_3)CH_2CO_2H$ [H-20, 1.97 m; H_3-21, 1.04 d; H-22, 5.28 dd (15, 8); H-23, 5.32 dd (15, 6); H-24, 2.58 m; H_2-28, 1.045 d; H_2-25, 2.23 dd (13.5, 6.5) and 2.12 dd (13.5, 8.0) ppm] and indicate the 24(ξ)-methyl-27-nor-3β,5α,6β,15α-tetra-

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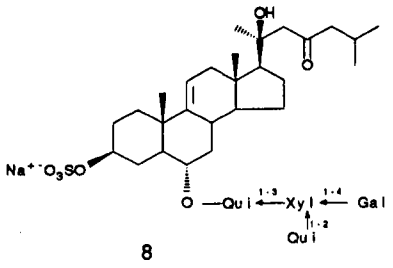
(11) D'Auria, M. V.; Minale, L.; Pizza, C.; Riccio, R.; Zollo, F. *Gazz. Chim. Ital.* **1984**, *114*, 469.
(12) Tachibana, K.; Sakaitani, M.; Nakanishi, K. *Tetrahedron* **1985**, *41*, 1027.
(13) D'Auria, M. V.; De Riccardis, F.; Minale, L.; Riccio, R. *J. Chem. Soc., Perkin Trans. 1*, in press.
(14) (a) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543. (b) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512. (c) Oh, S. S.; Butler, W. M.; Koreeda, M. *J. Org. Chem.* **1989**, *54*, 4499. (d) The term (+)- or (−)-MTPA ester refers to an ester obtained using the acid chloride prepared from (+)-(*R*)- and (−)-(*S*)-MTPA acid, respectively.
(15) Bruno, I.; Minale, L.; Riccio, R.; La Barre, S.; Laurent, D. *Gazz. Chim. Ital.* **1990**, *120*, 449.



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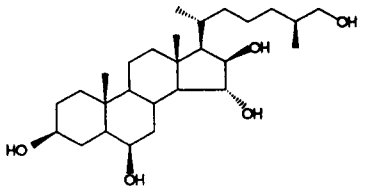
Qui=quinovose, Xyl=xylose, Gal=galactose; all sugars are in the pyranose form and linkages are β.



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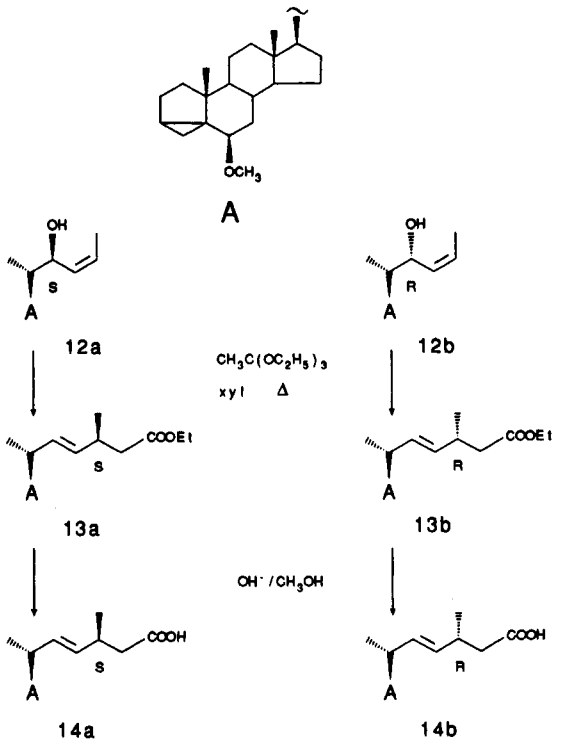
hydroxycholest-22(23)-en-26-oic acid structure for the new compound, which could be confirmed by direct comparison with (24*R*)- and (24*S*)-24-methyl-27-nor-Δ^{22E}-26-oic acid steroidal models, stereoselectively synthesized.

In designing the synthesis of the steroid **4** side chain, we have used the Claisen rearrangement, which Sucrow et al.[16] have specifically applied to steroidal side chain allylic alcohols. The method permits the construction of the stereogenic center at C-24 in a predictable way by exposing the suitable allylic C-22 alcohol to triethyl orthoacetate (Scheme I). The cis allylic alcohols **12a** (22*S*) and **12b** (22*R*)[13,17] were reacted with triethyl orthoacetate affording the olefinic esters **13a** (24*S*) and **13b** (24*R*), respectively, which were then converted into the corresponding acids **14a** and **14b**.

In the ^{1}H NMR spectra of the synthetic models, the side chain proton signals are virtually identical with those of the natural compound **4**. Indeed, one significant difference between the spectra of the natural steroid and those of the synthetic models was observed for the signals assigned to the 25-methylene protons, which in **14a** and **14b** appear as a sharp doublet at δ 2.25, while in **4** they were seen as two double doublets at 2.22 and 2.12. We attribute this deviation to the carboxyl function that in **4** is in the form of carboxylate. In confirmation, when we measured the ^{1}H NMR spectra of the synthetic models in CD_3OD/CD_3O^-, the 25-methylene protons appeared as two double doublets at δ 2.22 (dd, J = 13.5, 6.5 Hz) and 2.04 (dd, J = 13.5, 8 Hz) ppm. The ^{1}H and ^{13}C NMR spectra of the two synthetic steroids are too similar for a clear differentiation, and consequently for an unambigous stereochemical assignment at C-24 in **4**. However small differences are observed in the ^{1}H NMR spectra mainly dealing with the shifts of the olefinic protons (at 250 MHz in CD_3OD/CD_3O^-) double doublets centered at δ 5.32 with internal lines coincident in **14a** and two double doublets at δ 5.32 with the internal lines slightly separated by 1 Hz

Scheme I. Synthesis of the Epimeric Steroidic Δ^{22E}, 27-Nor-24-methyl-26-oic Acid Side Chains



in **14b**). In the spectrum of **4** the olefinic pattern is superimposable with that observed in **14a**. On this basis we tentatively suggest the 24*S* stereochemistry for **4**, as in amuresterol [(22*E*,24*S*)-24-methyl-27-nor-5α-cholesta-7,22-diene], which has been isolated from a different species of starfish.[18]

Structure Elucidation of Steroid 5. Negative-ion FABMS of the steroid **5** exhibits a pseudo-molecular ion at m/z 493 ([M − H]$^-$), and the FT IR spectrum shows a carboxyl band at 1705 cm^{-1}. In addition to the signals ascribable to the tetracyclic nucleus already observed in **2** and **4**, the ^{1}H NMR spectrum shows three methyl doublets at δ 0.88 (J = 6.5 Hz), 0.94 (J = 6.5 Hz), and 0.97 (J = 6.2 Hz) ppm. Irradiation at δ 1.78 (H-25) ppm simultaneously transformed the doublets at 0.88 (H_3-26 or -27) and 0.94 (H_3-27 or -26) into singlets, while irradiation at δ 1.40 (H-20) collapsed the doublet at δ 0.97 (H_3-21) into a singlet. These experiments, along with the FABMS and IR data, suggested a stigmastan-29-oic acid side chain. ^{13}C NMR data (Table II) gave support to this conclusion. (24*S*)- and (24*R*)-3β-acetoxy-5α-stigmast-7-en-29-oic acid ethyl ester were synthesized and significant differences were observed for the resonances of the isopropyl methyl protons and isopropyl methyl carbons as well.[19] In the ^{1}H NMR spectrum of the 24*S* isomer the isopropyl methyl protons appear as overlapping doublets (δ 0.85 d and 0.86 d) separated by 0.01 ppm, while in that of the 24*R* isomer they resonate as well-separated (Δδ 0.06 ppm) signals (δ 0.82 d and 0.88 d). The same tendency was observed in the ^{13}C NMR spectra; in the spectrum of the 24*S* isomer the C-26 and C-27 resonances are seen at 18.9 and 19.3 ppm (Δδ = 0.4 ppm), while in that of the 24*R* isomer they are split by 1.4 ppm (18.3–19.7 ppm). Our values, δ_H 0.88 and 0.94 (Δδ = 0.06 ppm), and δ_C 18.7 and 20.1 ppm (Δδ

(16) (a) Sucrow, W.; Girgensohn, B. *Chem. Ber.* **1970**, *103*, 750. (b) Sucrow, W.; Shubert, B.; Rihter, W.; Slopianka, M. *Ibid.* **1971**, *104*, 3689. (c) Sucrow, W.; Caldeira, P. P.; Slopianka, M. *Ibid.* **1973**, *106*, 2236. (d) Sucrow, W.; Slopianka, M.; Caldeira, P. P. *Ibid.* **1975**, *108*, 1101. (e) Sucrow, W.; Slopianka, M. *Ibid.* **1975**, *108*, 3721.

(17) Hirano, Y.; Djerassi, C. *J. Org. Chem.* **1982**, *47*, 2420.

(18) (a) Kobayashi, M.; Mitsuhashi, H. *Tetrahedron* **1974**, *30*, 2147. (b) Kobayashi, M.; Mitsuhashi, H. *Steroids* **1974**, *24*, 399.

(19) Anastasia, M.; Allevi, P.; Giuffreda, P.; Riccio, R. *Tetrahedron* **1986**, *42*, 4843.

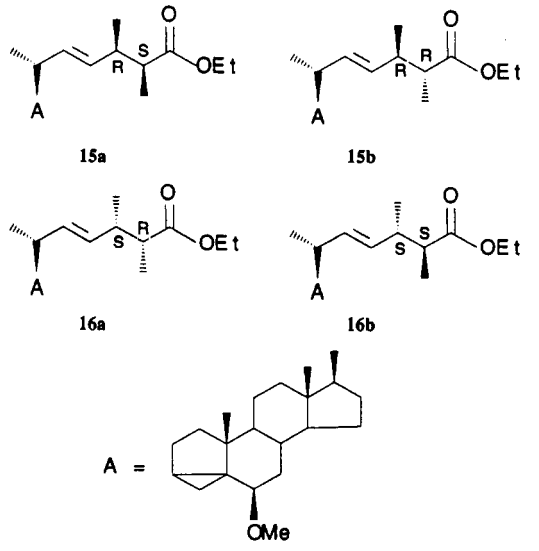
= 1.4 ppm), are in better agreement with those of the 24*R* isomer.

Thus the structure of the novel steroid **5** is determined as (24*R*)-3β,5,6β,15α-tetrahydroxy-5α-stigmastan-29-oic acid.

Structure Elucidation of Steroid 6. The negative-ion FAB mass spectrum exhibits a pseudo-molecular ion at *m/z* 572 ([M]⁻), while the positive-ion FAB MS gave molecular ion species at *m/z* 618 (major, 100%) and 634 (10%), which were interpreted as due to [M + Na]⁺ and [M + K]⁺, where M corresponds to a molecular weight of 595 Da. Thus **6**, which has a polarity corresponding to that of the sulfated **1**, **3**, and **8**, is the sodium salt of an anion of molecular weight of 572 Da. An examination of ^{1}H and ^{13}C NMR spectra indicates that **6** possesses the same 3β,5,6β,15α-tetrahydroxy steroidal nucleus as the previous **2**, **4**, and **5**. Present in the ^{1}H NMR spectrum of **6** are only two doublets at δ 0.94 (H_3-21, coupled with a multiplet at δ 1.40, H-20) and 1.12 (H_3-27, coupled with a multiplet downfield shifted to δ 2.31, H-25), corresponding to a cholesterol side chain in which one of the methyl groups has been oxidized. Also present are two extra methylene triplets at δ 2.99 and 3.62, coupled to each other by 6 Hz. Intense carbonyl absorptions in the FT IR spectrum of **6** at 1655 "Amide I band" and 1558 "Amide II band" cm^{-1} indicate the presence of an amide function, while two strong bands at 1216 and 1048 cm^{-1} are suggestive for a sulfonic acid salt.[20] All these data led to the structure of the sodium salt of 2′-*N*-ethanesulfonic acid 3β,5,6β,15α-tetrahydroxy-5α-cholestan-27-amide (**6**). In confirmation, acid hydrolysis (6 N HCl, 110 °C, 2 h) of **6** gave taurine. ^{13}C NMR data (Table II) gave conclusive support to the structure **6**. The stereochemistry at C-25 remains to be determined; we prefer the 25*S* configuration by analogy with the many (25*S*)-26-hydroxy steroids isolated from starfish.

Structure Elucidation of Steroid 7. An examination of ^{1}H and ^{13}C NMR spectra immediately indicated the presence of the 3β,5,6β,15α-tetrahydroxy steroidal nucleus and the presence of the same taurine residue as in **6**. The FT IR spectrum exhibits the amide bands at 1653 and 1558 cm^{-1} and the sulfonic acid salt bands at 1277 and 1048 cm^{-1} already observed in the spectrum of **6**. The ^{13}C NMR signal at 178.2 ppm supports the presence of the amide function. The negative-ion FABMS exhibits an anion peak at *m/z* 584 ([M]⁻) and the positive-ion FABMS give molecular ion species at *m/z* 630 ([M + Na]⁺, 100%) and 646 ([M + K]⁺, 10%), all 12 mass units shifted relative to **6** [(negative ion) *m/z* 572; (positive ion) *m/z* 618 and 634]. The ^{1}H NMR spectrum of **7** contains three methyl doublets at δ 0.99, 1.01, and 1.10 ppm and a 2 H multiplet at δ 5.26 characteristic for a Δ^{22} double bond. All these data accommodate into the structure **7** with a Δ^{22E}, 26-amide ergostane side chain. For studies of the stereochemistry at C-24 and C-25 of 24-methyl-26-hydroxy steroids isolated from echinoderms we synthesized[13] stereospecifically all the possible configurations of the 24-methyl-26-hydroxy steroidal side chain from the corresponding 24-methyl-26-carboxy side chains, these latter obtained via a Claisen rearrangement reaction with the ethyl orthopropionate on the cis allylic C-22 alcohols **12a** and **12b**. Every allylic alcohol was converted into a mixture of two Δ^{22E} esters epimeric at C-25 (**15a,b** from **12a** and **16a,b** from **12b**) which were separated by HPLC, the erythro isomer (**15b** and **16b**) being the major compound of both mixtures.[13] The erythro isomers (**15b** and **16b**) could be easily differentiated from the threo isomers (**15a** and **16a**) by comparing their ^{1}H NMR data (Table III). The major differences deal with the ^{1}H NMR shifts of the olefinic protons, which in the erythro isomers are seen as two well separated double doublets at δ 5.16 and 5.33–5.31 ppm while in the threo isomers are observed as almost coincident signals at δ 5.27 (5.28) ppm. Our pattern, a 2 H m at δ 5.26, is in close agreement with those of the threo isomers. Small differences dealing with the shifts of the side chain methyl protons were also observed in the spectra of the synthetic models, and more significant differences are seen between the spectra of the two erythro isomers **15b** (three well-separated doublets at δ 0.99, 1.05, and 1.09) and **16b** (one 3 H doublet at 1.00 d and a 6 H apparent triplet at 1.07 because of the coincident overlap of the low-field arm of one doublet at δ 1.06 with the high-field arm of the other at δ 1.08) than between those of the threo isomers. Thus, we equilibrated the natural steroidal amide **7** with 10% KOH in aqueous ethanol at reflux for 36 h affording a mixture of the erythro isomer (**7a**, major compound) and of the starting material, which were separated by HPLC. The methyl doublets pattern in the ^{1}H NMR spectrum of the newly formed erythro isomer (three well-separated doublets at δ 0.98, 1.04, and 1.09 ppm) compared better with **15b** (24*R*,15*R*) than with **16b** (24*S*,25*S*). On this basis we propose the threo 24*R*,25*S* stereochemistry for the natural steroid **7**.

Table III. Side Chain ^{1}H NMR Data (CD_3OD) of the Model Steroids 15a–16b, the Natural 7, and Its Epimer at C-25 7a



		olefinic H's	methyl doublets
15a (24*R*,25*S*)	threo	5.27 m	1.00 d, 1.03 d, 1.10 d
16a (24*S*,25*R*)	threo	5.28	1.01 d, 1.03 d, 1.11 d
15b (24*R*,25*R*)	erythro	5.16 dd, 5.31 dd	0.99 d, 1.05 d, 1.09 d
16b (24*S*,25*S*)	erythro	5.16 dd, 5.33 dd	1.00 d, 1.06 d, 1.08 d
7 (24*R*,25*S*)	threo	5.26 m	0.99 d, 1.01 d, 1.10 d
7a (24*R*,25*R*)	erythro	5.15 dd, 5.30 dd	0.98 d, 1.04 d, 1.09 d

Structure Elucidation of Myxodermoside A. The FAB (negative ion mode) mass spectrum gives a molecular anion peak at *m/z* 1097 and a major fragment at *m/z* 997 ([M − 100]⁻), corresponding to the C-20–C-22 cleavage of the steroid side chain with 1 H transfer (retro-aldol cleavage). On acid methanolysis it gave methyl xyloside, methyl quinovoside, and methyl galactoside (GLC) in the proportions 1:2:1.

The ^{1}H NMR spectrum of the intact saponin reveals signals due to the aglycon protons identical with those observed in the spectra of the many asterosaponins containing 3-*O*-sulfothornasterol A aglycon.[7,21] The thorna-

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Table IV. ^{13}C NMR Shifts (CD_3OD) of Sugar Carbon Atoms of 8

sugar carbon atoms	Qui I	Xyl	Qui II	Gal
1	105.0	104.2	105.1	104.2
2	74.2	82.6	75.3	72.0
3	90.2	75.6	76.8	74.8
4	74.4	77.9	76.2	70.1
5	72.2	64.5	73.8	77.1
6	17.9	-	18.5	62.3

sterol A structure for the aglycon of the glycoside 8 was also supported by ^{13}C NMR spectroscopy (Table II), which also confirmed that the oligosaccharide is attached at C-6 and the sulfate at C-3. A detailed comparison of the ^{13}C NMR data (Table IV) with those of thornasteroside A, i.e. (20*S*)-6α-*O*-{β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}-20-hydroxy-23-oxo-5α-cholest-9(11)-en-3β-yl sulfate,[21,22] and other asterosaponins containing the same sequence Qui→$^{1-2}$(X$^{1-4}$-)Xyl$^{1-3}$→Qui→aglycon[7,23] showed that the shifts of the signal due to the trisaccharide sequence Qui-Xyl-Qui are virtually superimposable in all spectra. After substracting from the ^{13}C NMR signals those due to the sequence Qui-Xyl-Qui-aglycon, the remaining signals were consistent with a terminal galactose unit. Thus the structure of the novel myxodermoside A can be defined as sodium (20*S*)-6α-*O*-{β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}-20-hydroxy-23-oxo-5α-cholest-9(11)-en-3β-yl sulfate (**8**).

Myxodermoside A has been examined for the effects on the development of fertilized sea urchin eggs and showed a modest activity (15% inhibition of cell division at 10^{-5} M concentration) in comparison with thornasteroside A and other pentaglycosides which in turn were more active than the hexaglycosides.[24]

Compounds **1** and **4–7** were inactive at the concentration (10^{-5} M) tested.

Known Compounds 9–11. The structures of the known compounds **9–11** have been established by direct comparison (FAB MS, ^1H NMR, HPLC) with authentic samples, whose physical data are described in the refs 15, 5b, and 25, respectively. The 25*S* configuration in the samples isolated from *M. platyacanthum* (**9** and **10**) has been established as described above. The 25*S* configuration in **11** has been assumed by analogy.

Experimental Section

General Methods. ^1H and ^{13}C NMR spectra were recorded on a Bruker WM-250 spectrometer. The DEPT experiments were made by using polarization transfer pulse of 90° and 135°, respectively, obtaining in the first case only the CH group signals and in the other case positive signals for CH and CH_3 and negative ones for CH_3 groups. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. FAB mass spectra were obtained by dissolving the samples in a glycerol matrix and placing them on a copper probe tip prior to bombardement with Xe atoms of energy 2.6 kV. Reverse-phase HPLC was performed by using a RI detector and a Waters μ-Bondapak C_{18} column (7.8 mm i.d., 30 cm). Droplet counter current chromatography (DCCC) was performed on an apparatus manufactured by Tokyo Rikakikai Co., equipped with 300 tubes. GLC analyses were performed on a SE-30 capillary column. The purity of all title compounds was judged to be >90% by HPLC and ^1H NMR. All new natural compounds were also characterized by ^{13}C NMR spectroscopy.

Table V. Steroidal Constituents Isolated from the Starfish *Myxoderma platyacanthum*

compd	amount, mg	rotations[a] $[\alpha]_D$, deg	HPLC[b] mobility, min	FABMS (negative ion)
1	34	+2.2	11.6[c]	531 $[M]^-$
2	4.4	+13.0	19.6[e]	451 $[M - H]^-$
3	3.7	+8.5	19.6[d]	663 $[M - H]^-$
4	7.4	+18.5	16.0[e]	463 $[M - H]^-$
5	8.9	+6.7	30.4[e]	493 $[M - H]^-$
6	4.8	+26.9	9.6[d]	572 $[M]^-$
7	9.8	+10.9	15.6[d]	584 $[M]^-$
8	21.8	+3.1	16.2[d]	1097 $[M]^-$
9	33	+12.8	14.4[d]	547 $[M]^-$
10	20	+12.1	9.80[e]	467 $[M - H]^-$
11	1.4	0	8.40[e]	451 $[M - H]^-$

[a] Rotations taken for solutions in methanol (*c* ranging from 1 to 0.1). [b] C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.). [c] In MeOH–H_2O (1:1). [d] In MeOH–H_2O (45:55). [e] In MeOH–H_2O (7:3).

Extraction and Isolation. The animals, *M. platyacanthum* H. L. Clark (4 kg, wet weight), were collected off the Gulf of California in June 1987. The animals were chopped and soaked in water (3 × 5 L; 3 h each time). The aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (1 kg). This column was washed with water (1 bed volume, 1 L) and then with methanol (2 L). The methanol eluate was taken to dryness to give glassy material (5 g), which was chromatographed on a column of LH-60 (4 × 80 cm) using MeOH–H_2O (2:1) as eluent. Fractions of 5 mL were collected and checked by thin-layer chromatography on SiO_2 in 1-butanol–acetic acid–H_2O (12:3:5) and $CHCl_3$–methanol–H_2O (40:9:1). Fractions 63–90 (1.5 g) contained the more polar compounds, the sulfated derivatives (**1–3**, **8**, and **9**) and the sulfonic salts (**6** and **7**), while fractions 91–129 (1 g) contained the less polar steroids **2**, **4**, **5**, **10**, and **11**.

The first eluting fractions (fr. 63–90) were submitted to droplet counter current chromatography (DCCC, 1-butanol–acetone–H_2O (3:1:5), ascending mode, the lower phase was used as stationary phase, flow rate 24 mL/h; 2-mL fractions were collected and monitored by TLC). Four main fractions were combined, i.e. fr. 186–256 (**1** + **9**), fr. 288–330 (**7**), fr. 331–351 (**6** + **8**), and fr. 352–384 (**3**), which were then submitted to HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.) with MeOH–H_2O (45:55 or 50:50 in the case of the fr. 186–256) at a flow of 5 mL min^{-1} to give pure compounds.

The last-eluted fractions (fr. 91–129, 1 g) from the column of Sephadex LH-60 were submitted to DCCC with $CHCl_3$–methanol–H_2O (7:13:8) in the ascending mode (the lower phase was used as stationary phase, flow rate 20 mL/min, 4.5-mL fractions were collected) to give six main fractions, i.e. 36–48 (**10**), 49–60 (cholesteryl sulfate), 85–96 (**2**), 97–115 (**4** + **11**), 116–160 (**4** + **5**), and 161–180 (**5**), which were then purified by HPLC on a C_{18} μ-Bondapak (30 cm × 7.8 mm i.d.) with MeOH–H_2O (7:3). The results are summarized in Table V, which also contains physical data (HPLC, [α]) and FAB MS data for each compound (all amorphous solids).

The ^1H and ^{13}C NMR spectral data of the new compounds are given in Tables I, II, and IV, FT IR spectral data are in the text.

Solvolysis of 1 Giving 2. A solution of **1** (5 mg) in dioxane (0.1 mL) and pyridine (0.1 mL) was heated at 140 °C for 2 h in a stoppered reaction vial. After the solution had cooled, H_2O was added, and the solution was extracted three times with 1-butanol. Removal of solvent left the pentol **2** as a glassy material. After purification by reverse-phase HPLC, the ^1H NMR and FAB MS spectral data were superimposable with those of native **2**.

Methoxy-α-(trifluoromethyl)phenylacetate Esters 1a–d. Compound **1** (4 mg) was treated with (+)-methoxy(trifluoromethyl)phenylacetyl chloride (10 μL) in dry pyridine (0.3 mL)

(21) Kitagawa, I.; Kobayashi, M. *Chem. Pharm. Bull.* **1978**, *26*, 1864.
(22) Minale, L.; Riccio, R.; Squillace Greco O.; Pusset, J.; Menou, J. L. *Comp. Biochem. Physiol.* **1985**, *80B*, 113.
(23) See, for example: (a) Bruno, I.; Minale, L.; Pizza, C.; Zollo, F.; Riccio, R.; Mellon, F. A. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1875. (b) Riccio, R.; Squillace Greco, O.; Minale, L. *Ibid.* **1985**, 655.
(24) Fusetani, N.; Kato, Y.; Hashimoto, K.; Komori, T.; Itakura, Y.; Kawasaki, T. *J. Nat. Prod.* **1984**, *47*, 997.
(25) Minale, L.; Pizza, C.; Zollo, F.; Riccio, R. *Tetrahedron Lett.* **1982**, *23*, 1841.

for 1 h at room temperature. After removal of the solvent, the residue was purified by HPLC [C_{18} μ-Bondapak 30 cm × 3.8 mm i.d.; MeOH–H_2O (7:3)] to give the 3,26-di-(+)-MTPA ester **1a** (glassy material): FABMS (negative ion mode) m/z 963 ([M – H]⁻); ^{1}H NMR (CD_3OD) δ 0.80 (3 H, s, 18-H_3), 0.97 (6 H, d, J = 7 Hz, 21-, 27-H_3), 3.50 (1 H, br s, 6α-H), 4.14 (1 H, dd, J = 10, 5.5 Hz) to 4.24 (1 H, dd, J = 10, 5.5 Hz, 26-H_2), 4.50 (1 H, dd, J = 3, 9 Hz, 15β-H), 5.50 (1 H, m, 3α-H) ppm.

The 3,26-di-(–)-MTPA ester **1b** was prepared using (–)-methoxy(trifluoromethyl)phenylacetyl chloride: FAB MS (negative ion mode) m/z 963 ([M – H]⁻); ^{1}H NMR identical with that of the above **1a** except for the signals for 26-H_3, which appeared slightly split at δ 4.14 (1 H, dd, J = 10, 5.5 Hz) and 4.26 (1 H, dd, J = 10, 5.5 Hz).

A solution of **1a** (3 mg) in dioxane (0.1 mL) and pyridine (0.1 mL) was heated at 140 °C for 2 h in a stoppered reaction vial. After the solution had cooled, H_2O (1 mL) was added, and the solution was extracted with 1-butanol. Removal of solvent left the glassy material of the *desulfated* **1c**. Purification by reverse-phase HPLC using 20% aqueous methanol afforded 1.8 mg of the *desulfated* **1c**: FABMS (negative ion mode) m/z 883 [M – H]⁻; ^{1}H NMR (CD_3OD) almost identical with that of **1a** except for the signals of 15β-H moved upfield to δ 3.90 (1 H, dt, J = 3, 9 Hz) and 26-H_2 slightly closer at δ 4.15 (dd, J = 10, 5.5 Hz) to 4.24 (dd, J = 10, 5.5 Hz) ppm.

Similarly **1b** (3 mg) was desulfated affording **1d**, FAB MS (negative ion mode) m/z 883 [M – H]⁻, ^{1}H NMR very similar with that of **1b** except for signals of 15β moved upfield to δ 3.90 and 26-H_2 slightly more separated at δ 4.13 (dd, J = 10, 5.5 Hz) and 4.26 (dd, J = 10, 5.5 Hz) ppm.

MTPA Esters 9a–c. Compound **9** (2.8 mg) was esterified with (+)-methoxy(trifluoromethyl)phenylacetyl chloride as above to give the 3,26-di-(+)-MTPA ester **9a** (2.5 mg), purified by reverse-phase HPLC using 30% aqueous methanol, glassy: FAB MS (negative ion mode) m/z 979 [M – H]⁻; ^{1}H NMR (CD_3OD) δ 0.96 (6 H, d, J = 7 Hz, 21-, 27-H_3), 1.00 (3 H, s, 18-H_3), 1.20 (3 H, s, 19-H_3), 4.16 (1 H, dd, J = 10, 5.5 Hz) to 4.23 (1 H, dd, J = 10, 5.5 Hz, 26-H_2), 4.32 (2 H, complex, 15β- 16α-H), 5.50 (1 H, m, 3α-H) ppm.

The 3,26-di-(–)-MTPA ester **9b** (glassy material) was prepared using (–)-methoxy(trifluoromethyl)phenylacetyl chloride: FABMS (negative ion mode) m/z 979 [M – H]⁻; ^{1}H NMR (CD_3OD) identical with that of **9a** except for signals of 26-H_2 split at 4.13 (1 H, dd, J = 10, 5.5 Hz) and 4.26 (1 H, dd, J = 10, 5.5 Hz) and 21- and 27-H_3 appearing as two separated doublets at δ 0.93 and 0.96 ppm.

Compound **9a** (2.4 mg) was solvolyzed as described above to give desulfated **9c** as a glassy material: FAB MS (negative ion mode) m/z 899 [M – H]⁻; ^{1}H NMR (CD_3OD) δ 0.93 (3 H, s, 18-H_3), 0.96 (6 H, d, J = 7 Hz, 21-, 27-H_3), 1.20 (3 H, s, 19-H_3), 3.76 (1 H, dd, J = 9, 2.5 Hz, 15β-H), 4.00 (1 H, dd, J = 7.5, 2.5 Hz, 16α-H), 4.21 (2 H, d, J = 5.5 Hz, 26-H_2), 5.50 (1 H, m, 3α-H) ppm.

(22*E*,24*S*)-6β-Methoxy-3α,5-cyclo-27-nor-5α-ergost-22-en-26-oic Acid (14a). The (22*S*,23*Z*)-allylic alcohol **12a**[13,17] (1 g) was heated under reflux in dry xylene (50 mL) with triethylorthoacetate (10 mL) and acetic acid (0.2 mL); 7 mL of solvent were removed by distillation after 1.5 h, and reflux was continued for additional 1.5 h. Usual workup gave the ester **13a** (1.1 g): HRMS calcd for $C_{30}H_{48}O_3$ (M^+) m/z 456.3603, found 456.3591; $[\alpha]_D$ +33°; ^{1}H NMR ($CDCl_3$) δ 0.45 (1 H, m, 4-H), 0.65 (1 H, m, 4-H), 0.9 (1 H, m, 3-H), 0.73 (3 H, s, 18-H_3), 1.03 (6 H, d, J = 6.5 Hz, 21- and 28-H_3), 1.04 (3 H, s, 19-H_3), 1.24 (3 H, t, J = 7 Hz, OCH_2CH_3), 2.25 (2 H, d, J = 6.5 Hz), 3.33 (3 H, s, OCH_3), 4.11 (2 H, q, J = 7 Hz, OCH_2CH_3), 5.25 (2 H, m, 22- and 23-H) ppm, which was in part (37 mg) converted to the corresponding acid **14a** by refluxing in 10% KOH ethanolic solution for 20 min. Usual workup gave 20 mg of the acid **14a**: HRMS calcd for $C_{28}H_{44}O_3$ (M^+) m/z 428.3290, found 428.3277; $[\alpha]_D$ +31°; ^{1}H NMR (CD_3OD), nuclear signals almost identical with the ester **13a**, δ (side chain) 1.04–1.05 (overlapping doublets, 21- and 28-H_3), 1.90 (m, 20-H), 2.25 (2 H, d, J = 6.5 Hz, 25-H_2), 2.58 (1 H, m, 24-H), 5.31 (2 H, m, 22- and 23-H); ^{1}H NMR (CD_3OD/CD_3O^-) δ (side chain) 1.04–1.05 (overlapping doublets, 21- and 28-H_3), 2.04 (1 H, dd, J = 14, 8.5 Hz, 25-H), 2.22 (1 H, dd, J = 14, 6.5 Hz, 25-H), 2.58 (1 H, m, 24-H), 5.32 (2 H, m, 22-, 23-H); ^{13}C NMR (CD_3OD) C-1 34.4, C-2 25.8, C-3 22.8, C-4 13.9, C-5 36.4, C-6 84.0, C-7 36.1, C-8 31.7, C-9 50.0, C-10 44.5, C-11 23.8, C-12 41.5, C-13 43.9, C-14 57.4, C-15 25.2, C-16 29.5, C-17 56.8, C-18 12.9, C-19 19.8, C-20 41.2, C-21 21.3, C-22 137.1, C-23 133.0, C-24 34.8, C-25 43.0, C-26 176.4, C-28 21.0, OCH_3 57.8 ppm.

(22*E*,24*R*)-6β-Methoxy-3α,5-cyclo-27-nor-5α-ergost-22-en-26-oic Acid (14b). The (22*R*,23*Z*)-allylic alcohol **12b**[13,17] (0.5 g) was heated under reflux in xylene (20 mL) with triethyl orthoacetate (5 mL) as above to give the ester **13b** (0.6 g): HRMS calcd for $C_{30}H_{48}O_3$ (M^+) m/z 456.3605, found 456.3603; $[\alpha]_D$ +22°; ^{1}H NMR ($CDCl_3$) virtually identical with that of **13a**. Treatment of **13a** with 10% KOH in ethanol as above gave the corresponding acid **14b**: HRMS calcd for $C_{28}H_{44}O_3$ (M^+) m/z 428.3290, found 428.3282; $[\alpha]_D$ +17°; ^{1}H NMR (CD_3OD) identical with **14a** except the two overlapping methyl doublets more split at δ 1.03 and 1.05 ppm; ^{1}H NMR (CD_3OD/CD_3O^-) identical with **14a** except the methyl doublets at δ 1.03–1.04 (21- and 28-H_3) and the olefinic signals further split (δ 5.32, the inner lines of the two dd splitting by ca. 1 Hz); ^{13}C NMR (CD_3OD) identical within ±0.1 ppm with that of **14a**.

Ethyl (22*E*,24*R*,25*S*)- and (22*E*,24*R*,25*R*)-6β-Methoxy-3α,5-cyclo-5α-ergost-22-en-26-oate (15a and 15b). The (22*S*,23*Z*)-allylic alcohol **12a**[13,17] (1 g) was heated under reflux in dry xylene (300 mL) with triethyl orthopropionate (10 mL) and propionic acid (0.2 mL); 7 mL of solvent were removed by distillation after 1.5 h, and reflux was continued for additional 1.5 h. Usual workup gave the epimeric mixture **15a** and **15b**, which in part (16 mg) was fractionated by HPLC [Whatman Partisil M9 10/25 column; hexane–ethyl acetate (99:1), flow rate 10 mL/min] to give pure erythro **15b** (10 mg; t_R 18 min): HRMS calcd for $C_{31}H_{50}O_3$ (M^+) m/z 470.3760, found 470.3747; ^{1}H NMR (CD_3OD) δ 0.44 (1 H, m, 4-H), 0.66 (1 H, m, 4-H), 0.9 (1 H, m, 3-H), 0.73 (3 H, s, 18-H_3), 1.03 (3 H, s, 19-H_3), 1.26 (3 H, t, J = 7 Hz, OCH_2CH_3), 2.77 (1 H, bt, J = 3 Hz, 6-H), 3.33 (3 H, s, OCH_3), 4.13 (2 H, q, J = 7 Hz, OCH_2CH_3), signals for side chain protons in Table III, and the threo **15a** (3 mg) still in admixture with the erythro **15b** (side chain ^{1}H NMR data in Table III).

Ethyl (22*E*,24*S*,25*R*)- and (22*E*,24*S*,25*S*)-6β-Methoxy-3α,5-cyclo-5α-ergost-22-en-26-oate (16a and 16b). The (22*R*,23*Z*)-allylic alcohol **12b**[13,17] (500 mg) was reacted with triethyl orthopropionate as above to give the epimeric mixture **16a** and **16b**, which in part (20 mg) was separated by HPLC in the same conditions as above to give pure erythro isomer **16b** (13 mg; t_R 25 min) [HRMS calcd for $C_{31}H_{50}O_3$ (M^+) m/z 470.3760, found 470.3747; side chain ^{1}H NMR (CD_3OD) data in Table III] and almost pure threo isomer **16a** (4 mg; t_R 27 min), side chain ^{1}H NMR (CD_3OD) data in Table III.

Acid Hydrolysis of the Compound 6. A solution of **6** (1 mg) in aqueous 1 N HCl (0.3 mL) was heated at 110 °C in a stoppered reaction vial for 2 h. After having cooled, the reaction vial was analyzed by chromatography [TLC on SiO_2 in 1-butanol–acetic acid–H_2O (60:15:25) and phenol saturated by H_2O]. Taurine was identified by chromatography (spray reagent: 1% ninhydrin in acetone).

Equilibration of the Amide 7. The amide **7** (5.4 mg) was refluxed with 10% KOH in aqueous ethanol (1:9, 3 mL) for 36 h. The product was isolated in the usual manner and chromatographed in HPLC (C_{18} μ-Bondapak, 30 cm × 7.8 mm i.d., MeOH–H_2O, 45:55) to give the erythro epimer **7a** (2.8 mg, t_R 13.2 min): FAB MS (negative ion mode) m/z 584 ([M]⁻); ^{1}H NMR (CD_3OD) identical with that of **7** except the side chain protons which are in Table III, and smaller amounts (0.8 mg, t_R 15.8 min) of the starting material **7**.

Methanolysis of Myxodermoside A (8). Sugar Analysis. A solution of myxodermoside (**8**, 1.5 mg) in anhydrous 2 N HCl in MeOH (0.5 mL) was heated at 80 °C in a stoppered reaction vial for 8 h. After having cooled, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2. The residue was trimethylsilylated with Trisil Z (Pierce Chemical Co.) for 15 min at room temperature. GLC analysis (135 °C) using a 25-m column of SE-30 (hydrogen carrier flow 10 mL/min) gave peaks which coeluted with those of silylated methyl xylosides, methyl quinovosides, and methyl galactosides in the ratio 1:2:1.

Bioassay. Compounds **1**, **4–7**, and **8** were tested for inhibition of cell division of fertilized sea urchin eggs according to Ruggeri and Nigrelli.[26] Only the asterosaponin **8** showed moderate activity

(15% inhibition) at 10^{-5} M concentration; under the same conditions the known asterosaponins thornasteroside A[21] and marthasteroside B[23a] and C[23a] were active (ca. 50% inhibition) at 10^{-7} M.

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Registry No. 1, 130799-35-8; 2, 130799-36-9; 3, 130829-31-1; 4, 130799-37-0; 5, 130799-38-1; 6, 130829-32-2; 7, 130829-33-3; 8, 130829-34-4; 9, 129725-35-5; 10, 130856-16-5; 11, 82485-96-9; 12a, 81477-23-8; 12b, 81477-24-9; 13a, 81477-27-2; 13b, 81502-57-2; 14a, 130829-35-5; 14b, 130930-22-2.

Supplementary Material Available: NMR spectra for compounds 1-8 (20 pages). Ordering information is given on any current masthead page.

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1,4-Dioxaspiro[2.2]pentanes. Synthesis, Spectroscopic Properties, and Reactions with Nucleophiles

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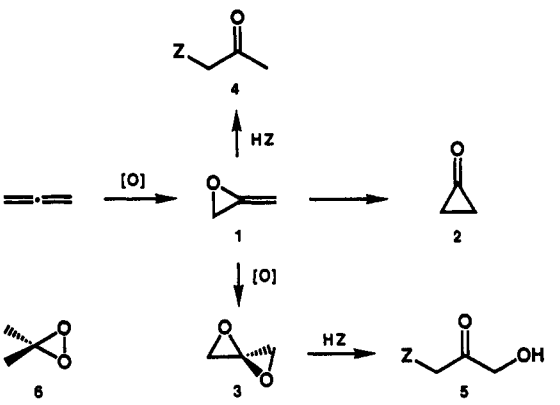
A number of simple allenes have been converted cleanly to the corresponding 1,4-dioxaspiro[2.2]pentanes in good yields by oxidation with dimethyldioxirane. Mono- and trisubstituted allenes give the anti diastereomers with good stereoselectivity, whereas 1,3-disubstituted allenes show only a slight preference for the anti,anti isomers, unless steric effects are extreme. Stereochemical assignments are based on steric considerations and a consistent set of NMR characteristics that permit structural attributions. The addition of a range of nucleophiles (water, alcohols, amines, thiophenol, acetate, chloride, and fluoride) to these intermediates proceeded smoothly under buffered conditions to give highly functionalized products of general type **5**. These reactions were shown to take place with the appropriate selectivities for S_N2 substitutions in instances where these features were observable, namely, regioselective attack at the less-substituted epoxide carbon and inversion of configuration at the site of substitution. Under acidic conditions dioxaspiropentanes gave mixtures of other types of products, which appear to arise from carbocationic processes.

Earlier studies on the epoxidation of allenes have revealed a rich chemistry that has been established to involve reactive intermediates (Scheme I) such as allene oxides (**1**), cyclopropanones (**2**), and 1,4-dioxaspiro[2.2]pentanes (spirodioxides **3**).[1-3] Although these species have been isolated and characterized on occasion, they are usually transformed further under the reaction conditions to a variety of stable products including nucleophilic adducts **4** and **5**.[4,5] The overall transformation of allenes, via the corresponding spirodioxides **3**, to products of type **5** constitutes a potentially efficient protocol for the introduction of different functionality at each of the three allenic carbons. Such a conversion should prove to be of considerable utility in the synthesis of densely functionalized molecules of the type that abound in nature. However, prior to the present study only three relatively hindered examples of spirodioxides had been described adequately in the literature.[2,6] Furthermore, the nucleophilic adducts **5** were not normally major products of the oxidation processes, especially when peracids were used as the oxidizing agents. In this case, the carboxylic acids formed as byproducts in the epoxidations promote acid-catalyzed transformation to other products. In addition, these acids are necessarily among the nucleophilic species that add to any spirodioxides that might be generated, thus limiting the nature of adducts **5**.

Scheme I



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