Minor and Trace Sterols in Marine Invertebrates. Part 20.[1] 3ξ-Hydroxymethyl-A-nor-patinosterol and 3ξ-Hydroxymethyl-A-nor-dinosterol. Two New Sterols with Modified Nucleus and Side-chain from the Sponge *Teichaxinella morchella*

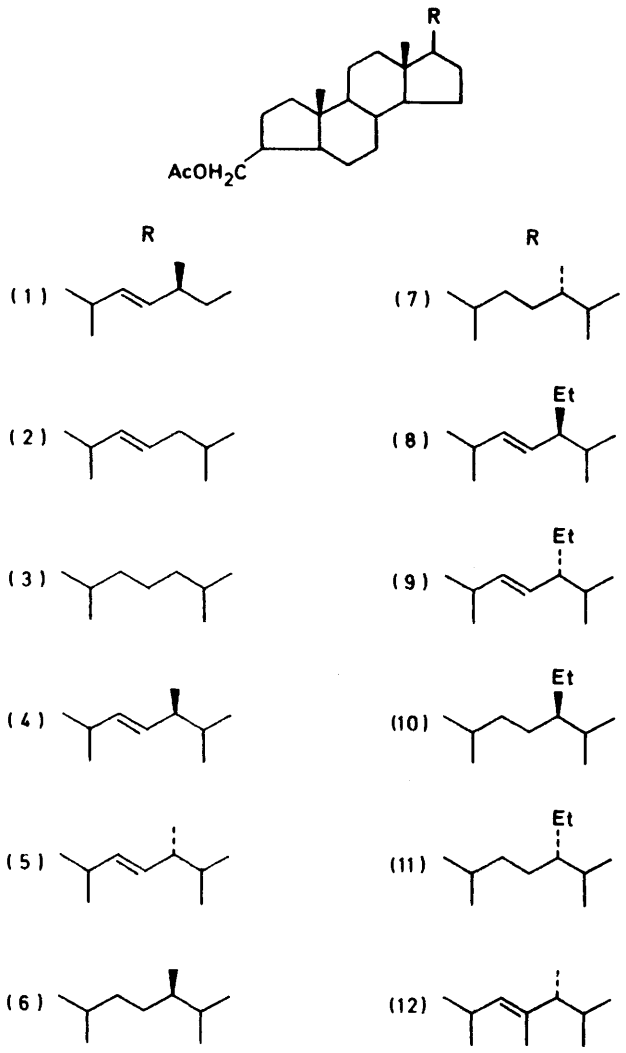
By **Lars Bohlin, Ulf Sjöstrand, and Carl Djerassi,*** Department of Chemistry, Stanford University, Stanford, California 94305, U.S.A.
Brian W. Sullivan, Institute of Marine Resources, Scripps Institution of Oceanography, La Jolla, California 92093, U.S.A.

The sponge *Teichaxinella morchella* from the Gulf of Mexico has been shown to contain solely 3ξ-hydroxymethyl-A-nor-sterols, of which two are the new 3ξ-hydroxymethyl-A-nor-patinosterol and 3ξ-hydroxymethyl-A-nor-dinosterol, isolated as acetates (1) and (12), respectively. The 24*R* and 24*S* epimers of both 24-methyl- [(4) and (5)] and 24-ethyl- [(8) and (9)] 3ξ-acetoxymethyl-A-nor-5α-cholest-22-ene were separated by reverse-phase HPLC while the corresponding saturated compounds were isolated as epimeric pairs. The 360-MHz n.m.r. spectra of the 3-acetoxymethyl-A-nor-sterols are discussed. The sole occurrence of A-nor-sterols and the absence of conventional sterols suggest that they are all bio-transformation products of dietary precursors. Attention is called to the potential chemo-taxonomic significance of this observation.

A-NOR-STEROLS have previously been found in five different sponges from four different locations, namely *Axinella verrucosa*[2] collected in the Bay of Naples, *Stylotella agminata*[3] and *Homaxinella trachy*[4] from outside Palau in the West Carolines, an *Axinella sp.*[3] found outside Tahiti, and *Phakellia aruensis* collected on the Great Barrier Reef. Such sterols with modified nuclei are very rare in nature and their occurrence in certain organisms can be considered *prima facie* evidence for the feasibility of modification by the sponge of dietary sterols with conventional nuclei (' normal sterols ').[6-8]

Three members of the class of C_{27} sterols with a modified cholestane-type side chain (27-nor-ergostane type) have previously been reported: amuresterol (13) found in an asteroid, *Asterias amurensis*,[9] occelasterol (14) isolated from an annelid, *Pseudopotamilla occelata*,[10] and patinosterol (15) from a scallop, *Patinopecten yessoensis*.[11] They are the first recorded examples of biochemical dealkylation in the sterol side-chain (recently also ficisterol)[12] and are presumably the biosynthetic precursors of the unique C_{26} sterols [*e.g.* (22*E*)-24-norcholesta-5,22-dien-3β-ol][13] of planktonic origin.

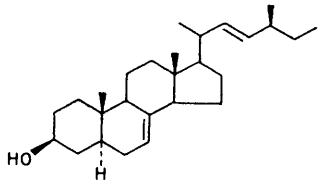
We now report the isolation and characterization of two new sterols, one (3ξ-hydroxymethyl-A-nor-patinosterol) with a *combination* of an A-nor-nucleus and a 27-nor-ergostane-type of side-chain (1) and the other (3ξ-hydroxymethyl-A-nor-dinosterol) with an A-nor-nucleus and a 23,24-dimethylated side-chain (12), from the sponge *Teichaxinella morchella*. In the earlier studies[2,3] of A-nor-sterols, the stereochemistry at the 24-position was not considered, and the separation of sterols epimeric in the 24-position has to our knowledge so far not been accomplished. Therefore we attempted to isolate all the epimers in the 24-position of the A-nor-sterols in the sterol mixture of the sponge by reverse-phase HPLC, and to characterize them by careful elucidation of their 360-MHz n.m.r. spectra.[14-17]



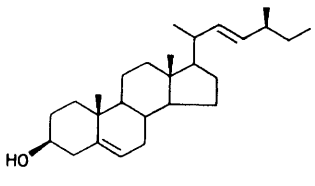
RESULTS AND DISCUSSION

A new source and location for A-nor-sterols has now been found in the sponge *Teichaxinella morchella*,[18] collected outside Yucatan in the Gulf of Mexico. All the A-nor-sterol-containing organisms have so far been shown to be sponges (Demospongiae) belonging to the family Axinellidae (order Axinellida, subclass Tetractinomorpha),[19] with the exception of *Stylotella agminata*. This species is classified in the family Hymeniacidonidae (order Halichondrida, subclass Ceractinomorpha).[5] However, it should be noted that this species has a strongly fibrous skeleton as reported for the order Axinellida.[5]

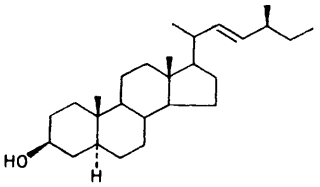
In spite of widely different collection localities, the sterol content is very consistent for all the six sponges, four containing *only* A-nor-sterols and two [*Stylotella agminata, Axinella sp.* (Tahiti)] also possessing minor amounts of conventional sterols. Bergquist *et al.*[5] also reported that several different samples of the species *Phakellia aruensis* collected at different times and locations (Great Barrier Reef) showed quantitatively and qualitatively very similar sterol contents. The order Axinellida has been reported to have a diverse sterol pattern.[5] However, five of the fifteen examined species from this order (five of twelve for the family Axinellidae) have been shown to contain A-nor-sterols with a very similar sterol pattern which, together with the diversity



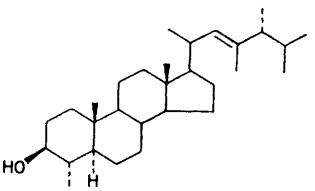
(13)



(14)



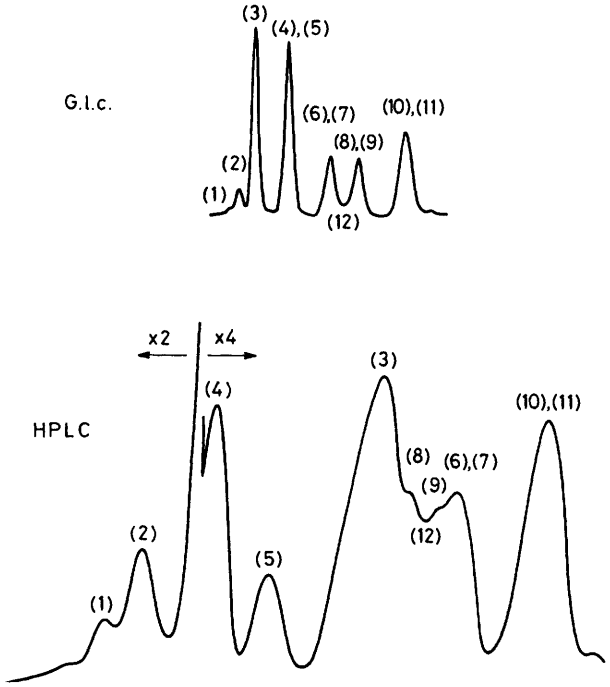
(15)



(16)

in location of the sponges, indicates a possible chemotaxonomic relationship within this family, which appears to contain a specific enzyme system capable of ring contraction of a variety of dietary sterol precursors.

Analysis of the acetylated (Ac_2O–pyridine, room temperature) sterol mixture showed a yellow spot with the same R_F-value as cholesterol acetate on t.l.c. when sprayed with cerium(IV) sulphate. This colour was the same as reported for free A-nor-sterols [2] but in the case of A-nor-sterol acetates no separation from cholesterol acetate could be seen in the t.l.c. systems used. The 220-MHz n.m.r. spectra of the sterol mixture showed two multiplets centred around δ 4.0, suggesting A-nor-sterols. No steroids with a conventional sterol skeleton could be detected. G.l.c.-analysis revealed eight peaks with five major ones (see Figure). Reverse-phase HPLC of the sterol mixture showed eleven peaks (see Figure) more or less separated, suggesting that some of the g.l.c. peaks contained more than one compound.



The g.l.c. and HPLC chromatograms of the sterol acetate mixture from the sponge *Teichaxinella morchella*

However, g.l.c.–m.s. analysis of the mixture gave just one molecular ion for each peak, suggesting the presence of isomers. Therefore, a careful fractionation of the sterol mixture by reverse-phase HPLC, with methanol as eluant, was performed. All the isolated sterol acetates show two double doublets at δ 4.102 and 3.936

TABLE 1

Yields and relative retention times of components of the sterol acetate mixture from the sponge *Teixachinella morchella*

Compound	Yield (%)	Relative retention times * G.l.c.	HPLC *
(1)	1.0	0.89	0.76
(2)	3.7	0.93	0.79
(3)	25.6	1.00	1.00
(4)	22.9	1.13	0.86
(5)	2.7	1.13	0.90
(6)	11.8	1.30	1.06
(7)		1.30	1.06
(8)	9.0	1.42	1.02
(9)	3.6	1.42	1.04
(10)	17.7	1.61	1.14
(11)		1.61	1.14
(12)	1.0	1.34	1.03

* Retention times are relative to A-nor-cholestanol (3).

with J 6.8, 9.0, and 10.6 Hz, and a multiplet at δ 2.332 which on irradiation caused the double doublets to collapse to two doublets with J 10.6 Hz. This ABX pattern is the same as earlier reported by Minale

and Sodano [2] for 3β-hydroxymethyl-A-nor-sterols. The acetate group causes the low field shift of the A-nor-signals.

Unsaturated A-Nor-sterol Acetates.—Sterols (1) and (2) were isolated cleanly in one run by reverse-phase HPLC. (2) revealed different patterns (see Table 2). The C-18 and C-19-proton signals are identical while the proton signals for C-21 show a slight difference. However, the most significant difference is that (2) has a dimethyl doublet at δ 0.858 for the C-26,27 proton signals while (1)

TABLE 2

N.m.r. data (360 MHz, $CDCl_3$) of some 3-acetoxymethyl-A-nor-sterols from the sponge *Teixachinella morchella* (δ from $SiMe_4$, *J* in Hz)

Compound [a]	18-H	19-H	21-H	22,23-H	26,27-H	28-H	29-H
(1)	0.661 (s)	0.752 (s)	0.987 (d, *J* 6.5)	5.166 (dd, *J* 7.5, 14.4) 5.108 (dd, *J* 7.2, 15.1)	0.829 (t, *J* 7.2)	0.923 (d, *J* 6.5)	
(2)	0.663 (s)	0.753 (s)	0.994 (d, *J* 6.8)	5.274 (dt, *J* 6.1, 14.8) 5.194 (dd, *J* 7.8, 15.1)	0.858 [d (6 H), *J* 6.8]		
(3)	0.648 (s)	0.751 (s)	0.899 (d, *J* 6.5)		0.863 (d, *J* 6.5) 0.859 (d, *J* 6.8)		
(4)	0.663 (s)	0.753 (s)	0.988 (d, *J* 6.5)	5.182 (dd, *J* 6.9, 15.8) 5.118 (dd, *J* 7.0, 15.1)	0.833 (d, *J* 6.5) 0.815 (d, *J* 6.5)	0.907 (d, *J* 6.8)	
(5)	0.663 (s)	0.753 (s)	0.997 (d, *J* 6.5)	5.201 (dd, *J* 6.9, 15.1) 5.147 (dd, *J* 7.4, 15.1)	0.832 (d, *J* 6.5) 0.814 (d, *J* 6.1)	0.908 (d, *J* 6.8)	
(6)	0.647 (s)	0.752 (s)	0.896 (d, *J* 6.5)		0.848 (d, *J* 6.8) 0.797 (d, *J* 6.8)	0.771 (d, *J* 6.8)	
(7) [b]	0.647 (s)	0.752 (s)	0.904 (d, *J* 6.5)		0.852 (d, *J* 6.8) 0.779 (d, *J* 6.8)	0.771 (d, *J* 6.8)	
(8)	0.668 (s)	0.754 (s)	1.006 (d, *J* 6.5)	5.143 (dd, *J* 8.6, 15.1) 5.003 (dd, *J* 8.6, 15.1)	0.843 (d, *J* 6.5) 0.792 (d, *J* 6.5)		0.802 (t, *J* 6.8)
(9)	0.667 (s)	0.754 (s)	1.009 (d, *J* 6.5)	5.151 (dd, *J* 8.6, 15.1) 5.009 (dd, *J* 8.6, 15.1)	0.840 (d, *J* 6.8) 0.788 (d, *J* 6.1)		0.808 (t, *J* 7.2)
(10) [b]	0.650 (s)	0.752 (s)	0.908 (d, *J* 6.1)		0.828 (d, *J* 6.8) 0.809 (d, *J* 6.8)		0.842 (t, *J* 8.6)
(11) [b]	0.650 (s)	0.752 (s)	0.911 (d, *J* 6.1)		0.828 (d, *J* 6.8) 0.809 (d, *J* 6.8)		0.851 (t, *J* 7.2)
(12) [c]	0.682 (s)	0.756 (s)	0.922 (d, *J* 6.5)	4.871 (d, *J* 9.6)	0.778 (d, *J* 6.5) 0.837 (d, *J* 6.5)	0.929 (d, *J* 6.8)	
(15) Patinosterol	0.660 (s)	0.802 (s)	0.985 (d, *J* 6.8)	5.167 (dd, *J* 7.5, 15.1) 5.108 (dd, *J* 7.3, 15.1)	0.828 (t, *J* 7.5)	0.923 (d, *J* 6.8)	

[a] The sterols (1)—(12) show an acetate signal ($MeCO_2CH_2$) at δ 2.03 as a singlet, and the signals typical for A-nor-sterols (–CH_2-OCOMe) at δ 4.102 (1 H, dd, *J* 6.8 and 10.6 Hz) and δ 3.936 (1 H, dd, *J* 9.0 and 10.6 Hz). [b] The epimeric pairs (6), (7) and (10), (11) were not isolated. Their signals were assigned from the mixture by comparison with the 360-MHz n.m.r data of the corresponding authentic 'normal' sterols, and from literature data.[14] [c] Compound (12) also shows 23-Me at δ 1.495 (d, *J* 1.2).

G.l.c.–m.s. showed the same molecular ion and fragmentation pattern in spite of different relative retention times (r.r.t.) on HPLC and g.l.c. (see Figure and Table 1). High-resolution m.s. showed characteristic peaks for a C_{27} sterol acetate with a saturated nucleus, and a C_8H_{15} side chain containing one double bond in the 22-position.[20]

Comparison of the 360-MHz n.m.r. spectra of (1) and has a methyl triplet at δ 0.829 and a methyl doublet at δ 0.923, indicating that (1) and (2) are structural isomers. Furthermore, the olefinic C-22,23-proton signals show a more complex pattern in (2) than in (1) (see Table 2) indicating the presence of a methyl group in the 24-position. Comparison of spectral data of authentic patinosterol (see Table 2) shows (1) to be (22*E*,24*S*)-

3ξ-acetoxymethyl-24-methyl-27-nor-A-nor-5α-cholest-22-ene. The 24*S* stereochemistry in patinosterol (15) has been demonstrated by Kobayashi and Mitsuhashi by comparison with partially synthesized occelasterol (14). In the same way (2) was identified as 3ξ-acetoxymethyl-A-nor-5α-cholest-22-ene.

Sterols (4) and (5) showed a baseline separation on HPLC but only one peak could be seen on g.l.c. (see Figure). G.l.c.–m.s. shows the same molecular ion and fragmentation pattern for both (4) and (5) indicating that they are epimers. The mass spectrum shows the typical peaks for a C_{28} sterol acetate analogous to (1) and (2). The 360-MHz n.m.r. spectra of (4) and (5) display a very similar pattern with differences for the C-21- and C-22,23-proton signals (see Table 2) indicating that they are stereoisomers. Comparison with the 360-MHz n.m.r. spectra of authentic 24*S*- and 24*R*-methyl-22,23-dehydrocholesterol and with the n.m.r. data of 24-methyl isomers described by Rubenstein *et al.*[14] identified (4) as (22*E*,24*S*)-3ξ-acetoxymethyl-24-methyl-A-nor-5α-cholest-22-ene, and (5) as the corresponding 24*R* isomer. The identical chemical shift for the C-21-proton signals for (22*E*,24*S*)-3ξ-acetoxymethyl-24-methyl-A-nor-5α-cholest-22-ene (4) (δ 0.988) and A-nor-patinosterol (1) (δ 0.987) independently confirms the 24*S* configuration for (1).

Separation of sterols (8) and (9) was more difficult and required repeated HPLC. Both had the same mass spectrum showing characteristic peaks for C_{29} sterol acetates analogous to (4) and (5). The 360-MHz n.m.r. of pure (8) and (9) showed signals typical for an ethyl group in the 24-position,[14] with principal differences for the C-29 and C-22,23 proton signals (see Table 2). Therefore we can assign (8) as 24*S*- and (9) as (24*R*)-3ξ-acetoxymethyl-(22*E*)-24-ethyl-A-nor-5α-cholest-22-ene in the same way as for the corresponding 24-methyl analogues (4) and (5).

The sterol (12) was isolated as a minor component by repeated HPLC. The mass spectrum showed the same fragmentation pattern as (8) and (9) suggesting that (12) is a structural isomer. This was confirmed by 360-MHz n.m.r. spectral analysis which showed the typical pattern of a dinosterol side chain [21] (see Table 2) which together with the A-nor-signals define (12) as A-nor-dinosterol. Theoretically, the precursor for this sterol could be dinosterol (16) [22] which is a typical dinoflagellate sterol. However, this would presuppose biosynthetic demethylation at C-4; a more likely precursor is the corresponding Δ^5-3β-ol, isolated from various marine organisms,[23,24] because this route has been shown to occur in the biosynthesis of A-nor-cholestanol.[6-8]

Saturated A-*Nor-sterols.*—The major sterol (3) (25.6%) was isolated in one run by reverse-phase HPLC. The mass spectrum demonstrated the presence of a fully saturated C_{27} sterol acetate with a different fragmentation pattern from the unsaturated A-nor-sterols. The 360-MHz n.m.r. spectrum showed a C-18-proton signal at δ 0.648 instead of δ 0.66 for the unsaturated ones, showing that the double bond in the 22-position has some influence. The structure of (3) as A-nor-cholestanol is confirmed by comparison to authentic A-nor-cholestanol earlier isolated in this laboratory.[3]

The sterols (6) and (7) could not even be separated on repeated reverse-phase HPLC. The mass spectrum showed typical peaks for a C_{28} sterol acetate analogous to (3). Three methyl signals were doubled in the 360-MHz n.m.r. spectrum (see Table 2) indicating the presence of epimers, which is confirmed by comparison of the n.m.r. spectra of corresponding Δ^5-sterols and literature data.[14] Sterol (7) represents 70% of this mixture. On the basis of the n.m.r. shifts [14] (7) is assigned the 24*S* and (6) the 24*R* stereochemistry of 3ξ-acetoxymethyl-24-methyl-A-nor-5α-cholestane. The main difference between (6) and (7) is the chemical shifts for the C-26,27 methyl groups.

The sterols (10) and (11) could not even be separated by repeated reverse-phase HPLC. The mass spectrum showed (10) and (11) to be the C_{29} analogues of (6) and (7), while the 360-MHz n.m.r. spectrum revealed the presence of epimers in the ratio of 1 : 1. However, in this case the main difference is the chemical shift for the C-29-methyl group (see Table 2). The assignment of (10) as 24*R* and (11) as the 24*S* epimer of 3ξ-acetoxymethyl-24-ethyl-A-nor-5α-cholestane was accomplished as for (6) and (7).

Utility of Reverse-phase HPLC for Isomer Separation.—Reverse-phase HPLC has been used for the separation of sterols by several authors [25,26] as reviewed by Heftmann and Hunter.[27] Separation of isomeric pairs of sterols by HPLC has been reported for 25*R*- and 25*S*-26-hydroxycholesterol [28] and for epimers of hydroperoxy- and epoxy-cholesterols.[29]

The reverse-phase HPLC of the sterol mixture of the sponge *Teichaxinella morchella* (see Figure and Table 1) shows that the unsaturated series of sterols [(1), (2); (4), (5); (8), (9)] have a shorter retention time than the corresponding saturated analogues [(3); (6), (7); (10), (11)]. Furthermore, the different isomeric pairs in both series are well separated from each other, indicating that one of the separation factors is the number of carbons in the side-chain, which is also emphasized by the appearances of the structural isomer (12) together with (8) and (9). Both the separation of the structural isomers (1) and (2) and the epimers (4), (5), and (8), (9) was achieved, which indicates that even the stereochemistry of the side-chain has an influence on the retention time. However, in the case of the saturated epimeric pairs (6), (7) and (10), (11) separation by reverse-phase HPLC could not be accomplished even after re-running several times, showing that the C-22-double bond has a strong effect on the separation of the different epimers.

Using g.l.c. (OV-17 3%) the unsaturated compounds also have a shorter retention time than the saturated ones, and a good separation between the different methyl analogues in both series can be achieved. However, with the exception of an incomplete separation between the structural isomers (1) and (2), the g.l.c. method could not separate the epimeric pairs.

This leads to the conclusion that in many instances

reverse-phase HPLC is the preferred method for the separation of epimeric pairs of sterols. More detailed study of the scope and limitation of reverse-phase HPLC for sterol epimer separation is indicated.

EXPERIMENTAL

General Methods.—Analytical t.l.c. was performed on precoated (Analtech, Uniplate) silica gel GF (250 μ) glass plates (2.5 × 10 cm) developed in hexane–diethyl ether (9 : 1 for acetates and 1 : 1 for free sterols) and visualized with cerium(IV) sulphate in sulphuric acid.

G.l.c. was performed using a Hewlett–Packard 402A chromatograph equipped with a flame-ionization detector [1.80 m × 4 mm i.d. U-shaped glass column containing 3% OV-17 on gas Chrom Q (Applied Sci. Inc.); temperature 260 °C; carrier gas He (100 ml min^{-1})]. A Carlo–Erba Fractovap 4160 gas chromatograph was also used [a coiled SE-52 (95-5 methyl phenyl silicone) 15 m × 0.32 mm i.d. column at 260 °C, flame-ionization detector, hydrogen as carrier gas].

For isolation a Water Associates HPLC system [M 6000 pump, U6 K injector, R 401 differential refractometer and a Whatman Partisil M9 10/50 ODS-2 column (8 mm × 50 cm)] was used at a flow rate of 1.5 ml min^{-1} (450 lb in^{-2}) with absolute methanol as mobile phase. A solution of 15 mg sterol mixture in 5 ml methanol was injected each time.

Low-resolution g.l.c.–m.s. analysis was performed on a Varian MAT-44 spectrometer system with an electron ionization energy of 70 eV using a coiled g.l.c. column (1.80 m × 2 mm i.d.) containing 3% OV-17 on GasChrom Q (Applied Sci. Inc.) at 260 °C.

High-resolution mass spectra were recorded on a Varian MAT 711 double-focusing spectrometer equipped with a PDP-11/45 computer for data acquisition and reduction. 360-MHz N.m.r. spectra ($CDCl_3$ or C_6D_6 with $SiMe_4$ as internal standard) were recorded on a Bruker HXS 360 spectrometer at the Stanford Magnetic Resonance Laboratory.

Extraction and Isolation of the Sterol Mixture.—The sponge was collected off Puerto Morellos (Yucatan, Mexico) at a depth of 50 ft in January 1979, and identified by Janice Thompson at Scripps Institution of Oceanography as *Teichaxinella morchella.*[18] After storage in ethanol the sponge was homogenized in ethanol and the mixture filtered. The filtrate was taken to dryness and the residue dissolved in ethyl acetate. The material on the filter was extracted at room temperature with ethyl acetate and filtered. Both ethyl acetate solutions were combined, washed with water, and evaporated to give the extract (0.39 g), which was then acetylated (pyridine–Ac_2O at room temperature). The sterol esters were then isolated from the acetylated extract by column chromatography (Sephadex LH 20) using ethanol as eluant, yielding crude sterol mixture (0.19 g), which was filtered on a silica gel column and eluted with hexane containing increasing amounts of diethyl ether. The sterol acetate mixture was then run on $AgNO_3$–t.l.c. giving one major band (R_F 0.5) which was scraped off and eluted with diethyl ether, giving a clean sterol mixture (*ca.* 40 mg).

Physical Data.—For g.l.c. and HPLC relative retention times see Figure and Table 1. For 360-MHz n.m.r. see Table 2.

(22E,24S)-3ξ-*Acetoxymethyl*-24-*methyl*-27-*nor*-A-*nor*-5α-*cholest*-22-*ene* (A-*nor*-*patinosterol*) (1). High-resolution m.s.: *m/z* 428.359 92 ($C_{29}H_{48}O_2$; 74%; M^+), 368.344 36 ($C_{27}H_{44}$; 13), 353.324 51 ($C_{26}H_{41}$; 8), 345.273 84 ($C_{23}H_{37}O_2$; 16), 344.269 96 ($C_{23}H_{36}O_2$; 66), 329.253 61 ($C_{22}H_{33}O_2$; 14), 316.236 47 ($C_{21}H_{32}O_2$; 26), 315.230 01 ($C_{21}H_{31}O_2$; 34), 285.255 17 ($C_{21}H_{33}$; 9), 284.249 11 ($C_{21}H_{32}$; 8), 269.221 64 ($C_{20}H_{29}$; 6), 258.225 35 ($C_{19}H_{30}$; 19), 257.223 17 ($C_{19}H_{29}$; 100), 255.208 21 ($C_{19}H_{27}$; 23), and 215.180 40 ($C_{16}H_{23}$; 11).

(22E)-3ξ-*Acetoxymethyl*-A-*nor*-5α-*cholest*-22-*ene* (2). G.l.c.–m.s.: *m/z* 428 (10%, M^+), 368 (2), 353 (2), 345 (5), 344 (22), 343 (13), 329 (5), 316 (6), 315 (12), 314 (7), 285 (1), 284 (5), 269 (5), 258 (7), 257 (36), 256 (11), 255 (11), 215 (5), and 201 (5).

3ξ-*Acetoxymethyl*-A-*nor*-5α-*cholestane* (3). G.l.c.–m.s.: *m/z* 430 (4%, M^+), 371 (31), 370 (100), 369 (13), 356 (14), 355 (45), 354 (6), 257 (17), 231 (8), 230 (22), 217 (39), 216 (66), 215 (100), and 201 (18).

(22E,24S)-3ξ-*Acetoxymethyl*-24-*methyl*-A-*nor*-5α-*cholest*-22-*ene* (4). G.l.c.–m.s.: *m/z* 442 (10%, M^+), 344 (11), 343 (8), 339 (3), 329 (2), 316 (7), 315 (13), 314 (7), 285 (2), 284 (2), 269 (3), 258 (5), 257 (28), 256 (7), 255 (12), 215 (4), and 201 (4).

(22E,24R)-3ξ-*Acetoxymethyl*-24-*methyl*-A-*nor*-5α-*cholest*-22-*ene* (5). G.l.c.–m.s.: *m/z* 442 (10%, M^+), 344 (10) 343 (8), 339 (4), 329 (2), 316 (6), 315 (12), 285 (3), 284 (3), 269 (3), 258 (5), 257 (28), 256 (8), 255 (11), 215 (5), and 201 (4).

(24S)-3ξ-*Acetoxymethyl*-24-*methyl*-A-*nor*-5α-*cholestane* (6) *and* (24R)-3ξ-*acetoxymethyl*-24-*methyl*-A-*nor*-5α-*cholestane* (7). G.l.c.–m.s.: *m/z* 444 (6%, M^+), 385 (30), 384 (98), 383 (16), 371 (2), 370 (12), 369 (40), 257 (18), 231 (12), 230 (24), 217 (40), 216 (67), 215 (100), and 201 (21).

(22E,24S)-3ξ-*Acetoxymethyl*-24-*ethyl*-A-*nor*-5α-*cholest*-22-*ene* (8). G.l.c.–m.s.: *m/z* 456 (9%, M^+), 353 (5), 344 (6), 343 (6), 329 (1), 316 (6), 315 (10), 314 (7), 285 (3), 284 (2), 269 (2), 258 (4), 257 (21), 256 (6), 255 (9), 215 (4), and 201 (3).

(22E,24R)-3ξ-*Acetoxymethyl*-24-*ethyl*-A-*nor*-5α-*cholest*-22-*ene* (9). G.l.c.–m.s.: *m/z* 456 (10%, M^+), 353 (4), 344 (6), 343 (7), 329 (1), 316 (5), 315 (9), 314 (7), 285 (3), 284 (3), 269 (3), 258 (4), 257 (22), 256 (5), 255 (10), 215 (4), and 201 (3).

(24R)-3ξ-*Acetoxymethyl*-24-*ethyl*-A-*nor*-5α-*cholestane* (10) *and* (24S)-3ξ-*acetoxymethyl*-24-*ethyl*-A-*nor*-5α-*cholestane* (11). G.l.c.–m.s.: *m/z* 458 (5%, M^+), 399 (33), 398 (98), 397 (14), 384 (11), 383 (33), 382 (7), 257 (22), 231 (10), 230 (22), 217 (40), 216 (67), 215 (100), and 201 (20).

(22E,24R)-3ξ-*Acetoxymethyl*-23,24-*dimethyl*-A-*nor*-5α-*cholest*-22-*ene* (A-*nor*-*dinosterol*) (12). G.l.c.–m.s.: *m/z* 456 (1%, M^+), 353 (1), 345 (1), 344 (3), 329 (1), 316 (2), 315 (6), 314 (1), 284 (1), 269 (1), 258 (1), 257 (4), 256 (1), 255 (3), 215 (1), and 201 (1).

We thank Dr. Peter Djura, Roger Walker, and Robert Armstrong of the Scripps Institution of Oceanography for collection of the sponge. High-resolution m.s. combined with a computer was programmed and operated by Annemarie Wegmann. The n.m.r. spectra were recorded under the supervision of Dr. Lois J. Durham. We thank the National Institute of Health, the National Science Foundation, the Swedish Academy of Pharmaceutical Sciences (to L. B.), and the Swedish Natural Science Research Council (to U. S.) for financial support.

[0/948 *Received*, 20*th June*, 1980]

REFERENCES

[1] Part 19, L. N. Li, U. Sjöstrand, and C. Djerassi, *J. Am. Chem. Soc.*, in the press

[2] L. Minale and G. Sodano, *J. Chem. Soc., Perkin Trans. 1*, 1974, 3480.

[3] L. Bohlin, H. P. Gehrken, P. J. Scheuer, and C. Djerassi, *Steroids*, 1980, **35**, 295.
[4] M. Eggersdorfer and C. Djerassi, unpublished observation.
[5] P. R. Bergquist, W. Hofheinz, and G. Oesterhelt, *Biochem. Syst. Ecol.*, in the press.
[6] M. De Rosa, L. Minale, and G. Sodano, *Experientia*, 1975, **31**, 408.
[7] M. De Rosa, L. Minale, and G. Sodano, *Experientia*, 1976, **32**, 1112.
[8] L. Bibolino, L. Minale, and G. Sodano, *J. Chem. Soc., Chem. Commun.*, 1978, 524.
[9] M. Kobayashi and H. Mitsuhashi, *Tetrahedron*, 1974, **30**, 2147.
[10] M. Kobayashi and H. Mitsuhashi, *Steroids*, 1974, **24**, 399.
[11] M. Kobayashi and H. Mitsuhashi, *Steroids*, 1975, **26**, 605.
[12] M. W. Khalil, L. J. Durham, C. Djerassi, and D. Sica, *J. Am. Chem. Soc.*, 1980, **102**, 2133; M. Karpf and C. Djerassi, *Tetrahedron Lett.*, 1980, **21**, 1603.
[13] D. R. Idler, P. M. Wiseman, L. M. Safe, *Steroids*, 1970, **16**, 451. For subsequent references, see F. J. Schmitz in ' Marine Natural Products ', ed. P. J. Scheur, Academic Press, New York, 1978, vol. I, pp. 241—297.
[14] I. Rubenstein, L. J. Goad, A. D. H. Clague, and L. J. Mulheirn, *Phytochemistry*, 1976, **15**, 195.
[15] N. Theobald and C. Djerassi, *Tetrahedron Lett.*, 1978, 4369.
[16] W. C. M. C. Kokke, W. H. Fenical, C. S. Pak, and C. Djerassi, *Tetrahedron Lett.*, 1978, 4373.
[17] T. Iida, T. Tamura, and T. Matsumoto, *J. Lipid Res.*, 1980, **21**, 326.
[18] F. Wiedenmayer, *Experientia Supplementum*, 1977, **28**, 154.
[19] P. R. Bergquist, ' Sponges ', University of California Press, Berkeley and Los Angeles, 1978.
[20] C. Djerassi, *Pure Appl. Chem.*, 1978, **50**, 171.
[21] W. C. M. C. Kokke, W. Fenical, and C. Djerassi, *Phytochemistry*, in the press.
[22] Y. Shimizu, M. Alam, and A. Kobayashi, *J. Am. Chem. Soc.*, 1976, **98**, 1059.
[23] A. Kanazawa, S. I. Teshima, and T. Ando, *Comp. Biochem. Physiol. B*, 1977, **57**, 317.
[24] W. C. M. C. Kokke, W. Fenical, L. Bohlin, and C. Djerassi, *Comp. Biochem. Physio.*, in the press.
[25] H. Colin, G. Guiochon, and A. Siouffi, *Anal. Chem.*, 1979, **51**, 1661.
[26] J. R. Thowsen and G. J. Schroepfer, *J. Lipid Res.*, 1979, **20**, 681.
[27] E. Heftmann and I. R. Hunter, *J. Chromatogr.*, 1979, **165**, 283.
[28] J. Redel and J. Capillon, *J. Chromatogr.*, 1978, **151**, 418.
[29] G. A. S. Ansari and L. L. Smith, *J. Chromatogr.*, 1979, **175**, 307.