flow 5 mL/min, RT 30 min). 360-MHz NMR for **18** ($CDCl_3$) δ 0.683 (s, C18 H), 0.818 (s, C19 H), 0.858 (d, J = 6.7, C26 or C27 H), 0.861 (d, J = 6.5, C26 or C27 H), 0.891 (d, J = 6.2, C21 H), 3.60 (m, C3 H), 3.94 (ddd, $J_1 = J_2 = 4.6$, $J_3 = 3.2$, C15 H).

3β-Hydroxy-5α-cholestan-15-one 3-(Dimethyl-*tert*-butylsilyl) Ether (19). A 101.7-mg sample of the protected alcohol **17** in acetone (25 mL, distilled from $KMnO_4$) was cooled in ice, and 100 μL of a chromic acid solution was added (CrO_3, 26.73 g; concentrated H_2SO_4, 23 mL; water to 100 mL).[37] After a few minutes some ethanol was added, followed by $NaHCO_3$, and the mixture was partitioned between hexane and water. The residue of the hexane layer was purified over a Florisil column (5 g); the ketone **19** was eluted with hexane–benzene (3:2) and recrystallized from acetone–methanol: yield 66%; mp 211 °C; $[\alpha]_D$ +34° (c 6 × 10^{-3}, $CHCl_3$); 360-MHz NMR for **19** ($CDCl_3$) δ 0.042 (6 H, s, methyl groups at Si), 0.731 (s, C18 H), 0.798 (s, C19 H), 0.859 (d, J = 6.7, C26 or C27 H), 0.862 (d, J = 6.6, C26 or C27 H), 0.877 (9 H, s, t-Bu), 0.977 (d, J = 6.3, C21 H), 3.55 (m, C3 H).

5α-Cholestane-3β,15β-diol 3-(Dimethyl-*tert*-butylsilyl) Ether 15-(Ethyl carbonate) (21). The crude 15β-alcohol **20** was obtained in quantitative yield by $LiAlH_4$ reduction of **19** in ether; a 24-mg sample in anhydrous pyridine (4 mL) was cooled in ice, and ethyl chloroformate (0.3 mL, Aldrich) was added with stirring. The ice bath was removed, and stirring was continued until all solid material had disappeared. After 24 h some ice was added and most of the solvent was evaporated, the rest was removed by an aqueous workup (water, ether, 1 N HCl). TLC showed that there was only a small amount of starting material left. The main product had the R_f value of the Δ^{14} elimination product **16** (R_f 0.55 in hexane–benzene, 3:2; R_f 0.67 in hexane–ether, 2:1), whereas the minor product, the desired ethyl carbonate **21**, has lower R_f values: R_f 0.12 in hexane–benzene (3:2); R_f 0.61 in hexane–ether (2:1). The products were separated over Florisil (8 g): **16** (identified by NMR) was eluted with hexane–benzene (2:1), and **21** was eluted with benzene.

5α-Cholest-15-en-3β-ol (11). The crude 15β-carbonate **21** (6 mg) in Decalin (1 mL), vacuum sealed in a heavy-walled tube, was heated at 245 °C for 75 min (oil bath). The Decalin was evaporated (vacuum pump), whereupon TLC showed the reaction to be virtually complete. The product was isolated by column chromatography (Florisil, 5 g; eluent hexane–benzene, 4:1). The olefinic region of the NMR spectrum showed the product to be a 4:1 mixture of the Δ^{14} compound **16** and the isomeric Δ^{15} olefin **22**. Deprotection (vide supra) by using 2 mL of $LiBF_4$ solution (7 h, 45 °C) afforded a mixture of the corresponding free sterols which could be separated by reverse-phase HPLC: 5α-cholest-14-en-3β-ol (**15**) has RRT 0.85, while 5α-cholest-15-en-3β-ol (**11**) has RRT 0.91. Both sterols have the same RRT in GC (1.0). High-resolution MS (probe) of **11** (70 eV), m/z (assignment, relative intensity): 386.3550 ($C_{27}H_{46}O_1$, M^+, 100), 371.3287 ($C_{26}H_{43}O_1$, 22), 368.3431 ($C_{27}H_{44}$, 12), 353.3186 ($C_{26}H_{41}$, 9), 302.2580 ($C_{21}H_{34}O_1$, 12), 301.2569 ($C_{21}H_{33}O_1$, 12), 283.2392 ($C_{21}H_{31}$, 8), 273.2210 ($C_{19}H_{29}O_1$, 84), 260.2496 ($C_{19}H_{32}$, 18), 255.2129 ($C_{19}H_{27}$, 52), 234.1938 ($C_{16}H_{26}O_1$, 8), 233.1888 ($C_{16}H_{25}O_1$, 25), 220.2169 ($C_{16}H_{28}$, 3), 220.1828 ($C_{15}H_{24}O_1$, 31), 218.1664 ($C_{15}H_{22}O_1$, 14), 215.1795 ($C_{16}H_{23}$, 34), 206.2010 ($C_{15}H_{26}$, 66), 206.1616 ($C_{14}H_{22}O_1$, 4), 202.1676 ($C_{15}H_{22}$, 14).

Acknowledgment. We thank Professor D. J. Faulkner and Dr. C. Ireland for the collection of the sponge, Mrs. Janice Thompson (Scripps Institution of Oceanography) for its identification, and Annemarie Wegmann and Dr. W. Uedelhoven for high-resolution mass spectra. The 360-MHz NMR spectra were recorded under the direction of Dr. Lois Durham in the Stanford Magnetic Resonance Laboratory (supported by NSF Grant No. GP-23633 and NIH Grant No. RR-00711). The work at Stanford University was made possible by a Grant (GM-06840) from the National Institutes of Health. M.L.E. is grateful to the DAAD, Bonn, West Germany, for a NATO research grant. The participation of Dr. Faulkner was made possible by an Office of Sea Grant (NOAA-04-9-M01-189), U.S. Department of Commerce. Operation and maintenance of the XL-100 spectrometer was supported by NSF Grant CHE 77-8810.

Registry No. **1**, 83681-79-2; **1Ac**, 83681-80-5; **2**, 55081-40-8; **3**, 3963-37-9; **3Ac**, 55081-43-1; **4a**, 83709-56-2; **4b**, 55081-41-9; **5a**, 83709-57-3; **5b**, 55088-75-0; **6**, 83681-81-6; **7a**, 55081-39-5; **7b**, 83709-58-4; **8**, 83681-82-7; **9**, 57-88-5; **11**, 83681-83-8; **12**, 80-97-7; **13**, 1225-43-0; **14**, 79632-13-6; **15**, 20780-35-2; **16**, 83681-84-9; **17**, 83681-85-0; **18**, 73389-49-8; **19**, 83681-86-1; **21**, 83681-87-2; DMTBSiCl, 18162-48-6; ethyl chloroformate, 541-41-3.

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Sterols in Marine Invertebrates. 33.[1] Structures of Five New 3β-(Hydroxymethyl)-*A*-nor Steranes: Indirect Evidence for Transformation of Dietary Precursors in Sponges

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Received May 25, 1982

Sixteen 3β-(hydroxymethyl)-*A*-nor steranes, of which five are new, have been found in the Red Sea sponge *Acanthella aurantiaca* (Family Axinellidae), which contains no sterols with conventional skeletons. The new structures were elucidated by 360-MHz ^1H NMR and mass spectral analysis. The 360-MHz ^1H NMR spectra of all *A*-nor sterols are summarized as an aid to the future rapid analysis of mixtures containing this class of marine sterols. The stereochemistry in the 3-position was proved by synthesis of 3α-(hydroxymethyl)-*A*-nor-5α-cholestane, which has different physical properties than the corresponding 3β compound.

In the search for new sterol structures, the finding of the unique class of nuclearly modified sterols,[1-7] the 3β-(hydroxymethyl)-*A*-nor steranes, is of considerable value for the understanding of the food chain, biosynthesis, and chemotaxonomy of certain sponges.

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(1) For part 32 in this series see: Eggersdorfer, M. L.; Kokke, W. C. M. C.; Crandell, C.; Hochlowski, J. E.; Djerassi, C. *J. Org. Chem.*, previous paper in this issue.

0022-3263/82/1947-5309$01.25/0

It has been shown[8-11] by tracer-incorporation studies that the *A*-nor sterols arise by modification of dietary sterols. This is so far the most convincing evidence for the ability of sponges to chemically modify sterols subsequent to dietary intake. Our rapidly increasing knowledge of the origin of marine sterols thus offers a means of examining the complicated food chain operating among these sponges. The majority of the sponges that contain *A*-nor sterols lack sterols with conventional nuclei. This probably means that these sponges have a very efficient enzyme system for converting absorbed sterols from the diet and transforming them into *A*-nor sterols. Therefore the existence in nature of some hitherto unknown sterols with conventional nuclei can be detected[1] through the isolation of the corresponding *A*-nor sterols.

What is the biological function of such sterols? Does the sponge change the structure because its membranes need a sterol which fits a special phospholipid? Earlier[12] many sterols with complex side chains, but with a conventional nucleus, have been found in marine invertebrates, which has been attributed[13,14] in several instances to a specialized membrane function and possible unusual fatty acids. However, if we find sterols with alterations in both the nucleus and the side chain compared to cholesterol, does this imply the existence of a corresponding phospholipid which is changed in both the hydrophilic and hydrophobic part?

Results and Discussion

Earlier, five sponges,[5] belonging to the family Axinellidae, have been shown to contain exclusively or overwhelmingly *A*-nor sterols, while two belonging to the family Hymeniacidonidae contain in addition substantial amounts of sterols with a conventional nucleus (Δ^5 or 5α-H).

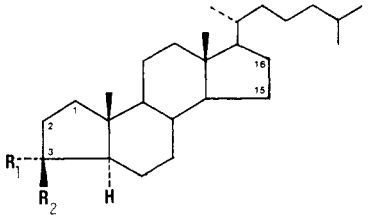
We have now found another sponge, *Acanthella aurantiaca*, that solely contains *A*-nor sterols from another different geographical location (Red Sea). This sponge also belongs to the family Axinellidae, which points to a chemotaxonomic relationship of the species in this family.

TLC-analysis of the sterol mixture showed the typical characteristics for *A*-nor sterols as reported in an earlier publication,[5] without the presence of sterols with conventional skeletons. GLC analysis revealed six major peaks which according to mass spectral analysis were shown to be the known *A*-nor sterols found by Minale and Sodano[2] and subsequently by others.[3,5,6] However, some minor sterols with longer and shorter RRTs (relative retention times) were also present in the GC trace, thus prompting the present study.

The sterol mixture was subjected repeatedly to HPLC separation (see Experimental Section). The resulting fractions were subjected to GC and GC/MS which together with the RRT (GC and HPLC) served for tentative structure assignment of several of the sterols. However, to determine strereochemical differences in the side chain and to search for new structures, we performed 360-MHz ^{1}H NMR measurements on fractions that contained a single pure component.

The stereochemistry of the hydroxymethyl group in the 3-position of *A*-nor sterols has been assigned the β configuration by Minale and Sodano[2] by conversion of the natural *A*-nor sterols to methyl *A*-nor-5α-sterane-3β-carboxylates and comparison to synthetic methyl *A*-nor-5α-cholestane-3β-carboxylate. However, a direct comparison between 3β- and 3α-(hydroxymethyl)-*A*-nor sterols is necessary to exclude the possibility that they show the same physical properties. Therefore, 3α-(hydroxymethyl)-*A*-nor-5α-cholestane (**16**) was synthesized[15] and compared with the corresponding natural compound.

A clear distinction could be made between 3β-(hydroxymethyl)- (**6**) and 3α-(hydroxymethyl)- (**16**) *A*-nor-5α-cholestane by TLC (R_f 0.42 and 0.51, respectively), GC (RRT = 1.00 and 0.82, respectively), reverse-phase HPLC (RRT = 1.00 and 0.75 respectively), and NMR (vide infra). Even the mass spectra show interesting differences: $M^+ - CH_3$ (m/z 373) is significantly stronger in **16** compared to that in **6** (41% vs. 13%). Furthermore, the m/z 357 fragment (loss of CH_3O) has about double the intensity compared to m/z 355 (loss of CH_5O) in **6** while in the 3α compound (**16**) the opposite intensities occur. The fragmentation of **6** is discussed in our earlier publication.[3]



6 R_1 = H; R_2 = CH_2OH

16 R_1 = CH_2OH; R_2 = H

Known 3β-(Hydroxymethyl)-*A*-nor Steranes. The structures of compounds **3–6**, **7a,b**, **10a,b**, **11a,b**, and **12a,b** (Table I) were shown to be identical with the compounds reported in our earlier publications[1,5] by comparison of GC and HPLC relative retention times together with mass spectral and 360-MHz ^{1}H NMR data. In one publication[5] the *A*-nor sterols were isolated and characterized as acetates. To determine if the separation was the same with the free sterols and also to obtain their 360-MHz ^{1}H NMR data, we examined the free sterol mixture. However, the separation is more effective with the sterol acetates as shown by the C-24 isomers **11a** and **11b** which can be separated as acetates[5] but not as free sterols (see Table I). Nevertheless, the chemical shifts for both isomers (**11a** and **11b**) could clearly be seen in the 360-MHz ^{1}H NMR spectrum, and by comparison with the spectra of the isolated compounds,[5] the two compounds could be identified in the mixture. The epimeric pairs **10a,b** and **12a,b** were also identified in this manner. Compound **14**, containing the gorgosterol side chain typical of coelenterates, has

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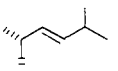
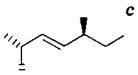
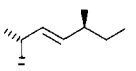
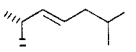
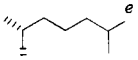
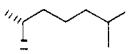
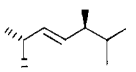
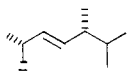
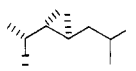
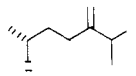
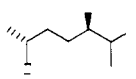
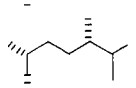
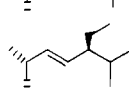
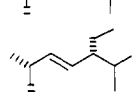
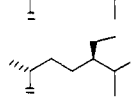
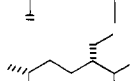
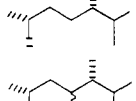
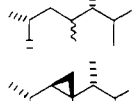
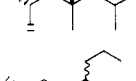
Table I. Molecular Ions, Percent Yield, and GC and HPLC RRT[a] Values of the 3β-(Hydroxymethyl)-*A*-nor Steranes from the Red Sea Sponge *Acanthella aurantiaca*

compd	structure of side chain[b]	*m/e* for M⁺	% yield[d]	RRT GC	RRT HPLC
1		372	1	0.65	0.61
2	 [c]	384	T	0.86	0.66
3		386	3	0.92	0.71
4		386	5	1.00	0.76
5	 [e]	386	6	1.00	0.80
6		388	20	1.00	1.00
7a		400	22	1.10	0.83
7b		400	6	1.10	0.88
8		400	3	1.42	1.22
9		400	T	1.30	0.80
10a		402	5	1.31	1.07
10b		402	4	1.31	1.07
11a		414	2	1.42	1.03
11b		414	3	1.42	1.03
12a		416	9	1.61	1.14
12b		416	7	1.61	1.14
13		416	T	1.63	1.29
14		428	1	2.30	1.48
15		430	T	1.91	1.24

[a] Retention time relative to that of 3β-(hydroxymethyl)-*A*-nor-5α-cholestane (**6**). [b] Unless indicated otherwise the nucleus is the conventional 3β-(hydroxymethyl)-*A*-nor-5α-androstane. [c] Δ^7. [d] T = trace amount. [e] $\Delta^{15(16)}$.

earlier been isolated in this laboratory by Bohlin et al.,[3] and comparison of **14** with this compound showed them to be identical.

The 24-methylene sterol **9** was not isolated because of its very small amount but was seen as an impurity in the 360-MHz ^{1}H NMR spectrum of **5**. Comparison of the GC and HPLC relative retention times with those of an authentic sample of 3β-(hydroxymethyl)-24-methylene-*A*-nor-5α-cholestane[7] tentatively shows that our sponge also contains this sterol.

New 3β-(Hydroxymethyl)-*A*-nor Steranes. The minor sterol **1** was eluted very early from the HPLC (RRT = 0.61) and GC (RRT = 0.65) columns, thus suggesting that **1** probably has a shorter side chain than cholesterol. The mass spectrum shows **1** to be a C_{26} homologue of (22*E*)-3β-(hydroxymethyl)-*A*-nor-5α-cholest-22-ene (**4**) which was confirmed by 360-MHz ^{1}H NMR (three doublets and two singlets in the methyl region and two olefinic protons; cf. Table II). By comparison to the spectral data of an authentic sample[16] of 24-nor-22-dehydrocholestanol, **1** was shown to be (22*E*)-3β-(hydroxymethyl)-24-nor-*A*-

(16) Carlson, R. M. K. Ph.D. Thesis, Stanford University, 1977.

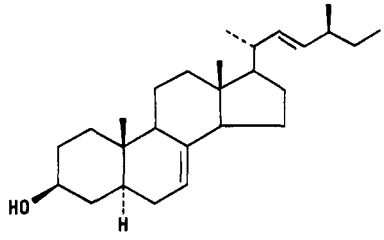
Table II. 360-MHz ^{1}H NMR Data[a] of the 3β-(Hydroxymethyl)-*A*-nor Steranes from the Red Sea Sponge *Acanthella aurantiaca* and of Two Partially Synthetic Reference Compounds (16 and 16Ac)

	chemical shift, δ							
sterol	C18-H	C19-H	C21-H	C22,23-H	C26,27-H	C28-H	C29-H	other signals
1	0.660 [s]	0.740 [s]	0.986 [d, J = 6.1]	5.207 [m]	0.938 [d, 6 H, J = 6.5]			
2	0.537 [s]	0.747 [s]	1.013 [d, J = 6.5]	5.162 [m, 2 H]	0.838 [t, J = 7.4]	0.930 [d, J = 6.5]		
3	0.661 [s]	0.739 [s]	0.989 [d, J = 6.5]	5.140 [m]	0.829 [t, J = 7.6]	0.923 [d, J = 6.1]		
4	0.663 [s]	0.739 [s]	0.995 [d, J = 6.5]	5.230 [m]	0.859 [d, J = 6.8], 0.855 [d, J = 6.5]			
5	0.737 [s]	0.758 [s]	0.904 [d, J = 6.5]		0.871 [d, J = 6.5], 0.867 [d, J = 6.5]			C15,15-H: 5.830, m, 5.739, m
6	0.647 [s]	0.737 [s]	0.899 [d, J = 6.5]		0.862 [d, J = 6.8], 0.859 [d, J = 6.8]			
7a	0.663 [s]	0.739 [s]	0.989 [d, J = 6.5]	5.153 [m]	0.834 [d, J = 6.1], 0.816 [d, J = 6.8]	0.908 [d, J = 6.8]		
7b	0.663 [s]	0.740 [s]	0.998 [d, J = 6.5]	5.172 [m]	0.833 [d, J = 6.1], 0.815 [d, J = 6.5]	0.908 [d, J = 6.8]		
8	0.594 (0.628) [s]	0.736 (0.680) [s]	0.982 (1.093) [d, J = 6.5 (6.8)]		0.912 (0.985) [d, J = 6.8], 0.889 (0.959) [d, J = 6.5]			cyclopropyl: 0.183, 2H, m, 0.371, 2H, m, (0.196, 1H, m), (0.286, 1H, m), (0.395, 2H, m)
10a	0.650 [s]	0.739 [s]	0.897 [s]		0.848 [d, J = 6.8], 0.799 [d, J = 6.8]	0.769 [d, J = 6.5]		
10b	0.650 [s]	0.739 [s]	0.905 [d, J = 6.5]		0.851 [d, J = 6.8], 0.780 [d, J = 6.8]	0.771 [d, J = 6.5]		
11a	0.667 [s]	0.740 [s]	1.010 [d, J = 6.5]	5.075 [m]	0.841 [d, J = 6.8], 0.793 [d, J = 6.5]		0.802 [t, J = 6.5]	
11b	0.667 [s]	0.740 [s]	1.010 [d, J = 6.5]	5.082 [m]	0.839 [d, J = 6.8], 0.788 [d, J = 6.5]		0.808 [t, J = 7.2]	
12a	0.650 [s]	0.738 [s]	0.908 [d, J = 6.5]		0.830 [d, J = 6.5], 0.809 [d, J = 6.5]		0.842 [t, J = 7.6]	
12b	0.650 [s]	0.738 [s]	0.908 [d, J = 6.5]		0.830 [d, J = 6.5], 0.809 [d, J = 6.5]		0.850 [t, J = 7.9]	
13	0.667 [s]	0.740 [s]	0.881 [d, J = 6.8][b]		0.726 [d, J = 7.2],[b] 0.703 [d, J = 6.8][b]	0.816 [d, J = 6.8][b]	0.881 [d, J = 6.8][b]	
14	0.629 (0.675) [s]	0.738 (0.696) [s]	0.994 [br s], (1.140, d, J = 6.5)	0.894 (0.921) [s]	0.949 (1.030) [d, J = 6.5 (7.2)], 0.935 (1.018) [d, J = 6.8]	0.854 (0.899) [d, J = 6.5]		cyclopropyl region: −0.137 [1 H, dd], 0.203 [2 H, m], 0.448 [1 H, m], (−0.108, 1 H, dd), (0.216, 2 H, m), (0.490, 1 H, m), C30-H: 0.877 (0.940), [d, J = 7.2 (6.8)]
15	0.649 (0.670) [s]	0.739 (0.682) [s]	0.905 (1.042) [d, J = 6.5]		0.824 (0.924) [d, J = 6.5], 0.808 (0.905) [d, J = 6.8]			
16[c]	0.654 [s]	0.725 [s]	0.900 [d, J = 6.5]		0.861 [d, 6 H, J = 6.5]			
16Ac[c]	0.655 [s]	0.720 [s]	0.901 [d, J = 6.5]		0.861 [d, J = 6.5], 0.865 [d, J = 6.8]			

[a] Values in parentheses are data from C_6D_6 spectra. Coupling constants are given in hertz. All other data are from $CDCl_3$ spectra. [b] Assignment of the five methyl doublets is tentative. [c] Note that this is not a 3β- but a 3α-(hydroxymethyl)-*A*-nor sterane.

nor-5α-cholest-22-ene. Since 24-nor sterols appear to be of planktonic origin, the isolation of the corresponding *A*-nor analogue **1** indicates that the sponge's diet includes such plankton which produce 24-nor sterols.

Except for the recently described[1] Δ^{15}-*A*-nor sterol **5**, only *A*-nor sterols with a saturated nucleus have been found earlier.[2-7] We now report another *A*-nor sterol with a nuclear (Δ^7) double bond. The structure for this compound (**2**) was elucidated by virtue of its earlier RRT in HPLC and GC (compared to the nuclear saturated analogue **3**) and by the mass spectrum which showed the typical *m/z* 246 peak[17] for Δ^7 sterols. The 360-MHz ^{1}H NMR spectrum of **2** displays the same pattern in the methyl region as compound **3** but with some changed chemical shifts. In particular the chemical shift for the C18 H signal is considerably shifted to higher field and by following Zürcher's rules[18] it shows compound **2** to have a Δ^7 double bond. This was also confirmed by the integral for the olefinic protons (three protons in **2**, two protons in **3**). The methyl region of the 360-MHz ^{1}H NMR spectrum of authentic[19] amuresterol (**17**) was found to be



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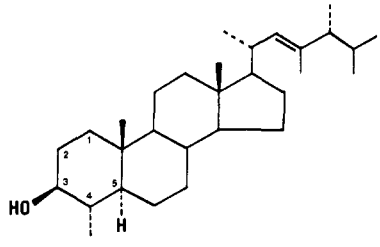
identical with that of **2** with the exception of the C19 H signal which is influenced by the *A*-nor ring. Therefore, the new sterol is 3β-(hydroxymethyl)-*A*-noramuresterol (**2**).

22(*R*),23(*R*)-Methylenecholesterol has been isolated in this laboratory from a variety of marine organisms by Blanc and Djerassi,[20] and we have now detected (through the characteristic NMR spectrum; cf. Table II) the corresponding *A*-nor sterol **8** [3β-(hydroxymethyl)-22(*R*),23-(*R*)-methylene-*A*-nor-5α-cholestane).

Dinosterol (**18**), which has a 4α-methyl group, can be



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hydrogenated stereoselectively[21,22] to give a saturated sterol with two methyl substituents in the side chain (23ξ,24*R*). The structure of the new *A*-nor sterol **13** was established by NMR comparison with this hydrogenation product of dinosterol (**18**). The respective 360-MHz ^{1}H NMR spectra were very similar if the doublet for the 4-methyl group of dinosterol (**18**) is neglected. The only difference is that the hydrogenated dinosterol has two doublets at 0.880 and 0.873 ppm[22] while **13** has one doublet at 0.881 ppm with double intensity. One possibility for this difference could be a stereochemical difference at C23, because the stereochemistry at this position is not known. Since the mass spectrum shows **13** to be a C_{29} homologue of 3β-(hydroxymethyl)-*A*-nor-5α-cholestane (**6**), the 3β-(hydroxymethyl)-23ξ,24(*R*)-dimethyl-*A*-nor-5α-cholestane structure assignment for **13** seems secure.

Sterol **15** was eluted very late from the HPLC column (RRT = 1.29), indicating two or three more methyl groups than cholesterol in the side chain. This was further established by the mass spectrum (M^+, *m/e* 430) which displayed the fragmentation of a C_{30} homologue of 3β-(hydroxymethyl)-*A*-nor-5α-cholestane (**6**). The 360-MHz ^{1}H NMR spectrum of authentic 24ξ-*n*-propylcholest-5-en-3β-ol[23] shows very similar chemical shifts to those of **15** with the exception of the Δ^5 double bond influence on the C18 H, C19 H, and C21 H signals. From these facts we conclude that **15** is 3β-(hydroxymethyl)-24ξ-*n*-propyl-*A*-nor-5α-cholestane. This type of side chain has earlier been found in a cultured alga by Rohmer et al.[23]

General Comments on NMR Spectra of *A*-Nor Sterols. High-resolution NMR is a very useful tool to determine the type of nucleus and side chain of isolated sterols. A conventional saturated nucleus (5α-androstane) shows a multiplet at about 3.6 ppm for the 3α-H while for saturated *A*-nor sterols two doublets of doublets appear at about 3.6 ppm instead. These signals are the AB quartet of an ABx system associated with the hydroxymethyl group in the A ring.

Analysis of the methyl region of the *A*-nor sterols reveals the structure and stereochemistry of the side chain by comparison to authentic sterols with conventional nuclei since change of the structure in the A ring does not affect the chemical shifts of the methyl groups in the side chain.

Comparison of the *A*-nor sterols as free sterols with corresponding acetates shows a downfield shift (about 0.010 ppm) of the C19 H signal of the acetates[5] (see Table II) and a downfield shift of the hydroxymethyl signals (3.6–4.02 ppm).

A Δ^{22} double bond (**1**, **3**, **4**, **7a,b**, **11a,b**) moves the C18 H signal about 0.010 ppm downfield compared to that in the corresponding saturated compounds. Additional alkylation in the 24-position does not seem to interfere with the C18 H signal even if the 24-ethyl compounds (**11a,b**) have slightly higher values (see Table II).

Alkylation in both 23- and 24-positions (**13**) shows a considerable downfield shift of the C18 H signal (0.020 ppm). An even stronger difference (0.053 ppm, high field) in chemical shift of the C18 H signal is caused by a cyclopropane group in the 22,23-position (**8**; see Table I).

Unsaturation in the nucleus of the 3β-(hydroxymethyl)-*A*-nor steranes affects the chemical shifts of the hydroxymethyl protons but not the coupling constants. A Δ^7 double bond has the strongest effect while the $\Delta^{15(16)}$ double bond just slightly shifts the protons (see Table III). The Δ^7 double bond affects both C18 H and C19 H signals while $\Delta^{15(16)}$ only affects the C18 H signal (see Table II).

The stereochemistry of the hydroxymethyl group can also be established by high-resolution NMR. Both the chemical shifts and the coupling constants for the AB protons are different for 3β-(hydroxymethyl)-*A*-nor-5α-cholestane (**6**) compared to those for 3α-(hydroxymethyl)-*A*-nor-5α-cholestane (**16**; see Table III). Both **6** and **16** show the same geminal coupling constant (10.1 Hz)

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(22) Zielinski, J., unpublished experiments from this laboratory.
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Table III. Comparison of ^{1}H NMR Data for 3-Hydroxymethyl and 3-Acetoxymethyl Protons of *A*-Nor Sterols

side chain	nucleus	R	chemical shift,[a] δ CH_2OR	
6	5α-H	H	3.715 (3.539) [dd, J = 6.5, 10.1]	3.478 (3.297) [dd, J = 9.0, 9.7]
6	5α-H	Ac	4.102 (4.226) [dd, J = 6.8, 10.5]	3.936 (4.018) [dd, J = 9.0, 10.6]
16	5α-H	H	3.695 [dd, J = 4.0, 10.1]	3.489 [dd, J = 6.1, 10.1]
16	5α-H	Ac	4.069 [dd, J = 5.6, 10.8]	3.953 [dd, J = 7.0, 10.8]
2	Δ^7	H	3.769 [dd, J = 6.6, 10.4]	3.545 [dd, J = 9.0, 10.1]
5	$\Delta^{15(16)}$	H	3.724 [dd, J = 6.5, 10.2]	3.486 [dd, J = 9.5, 9.6]

[a] Values in parentheses are data from C_6D_6 spectra. Coupling Constants are given in hertz.

but different vicinal coupling constants (6.5, 9.0 Hz and 4.0, 6.1 Hz, respectively), confirming that they have different stereochemistry in the 3-position. Furthermore, the chemical shift for the X-proton is 2.1 ppm for the 3β compound (**6**) while it is shifted considerably to 1.86 ppm in the corresponding 3α compound (**16**). Irradiation of the X proton for both **6** and **16** collapses the AB multiplet to a quartet with a geminal coupling of 10.1 Hz. Irradiation of one of the quartets (A) collapses the other quartet (B) to a doublet with its respective vicinal coupling (see Table III) and also simplifies the X-proton multiplet. Furthermore, the angular methyl group at C19 is shifted upfield (0.737 compared to 0.725 ppm) in the 3α compound (**16**). This difference is considerably increased for the corresponding acetates (0.751 compared to 0.720 ppm). The methyl signals for C18 H and the side chain are almost the same (see Table II).

Experimental Section

General Methods. TLC was performed on precoated (Analtech, Uniplate) silica gel GF (250 μm) glass plates (2.5 × 10 cm) with hexane/diethyl ether (1:1) as the eluant and ceric sulfate in sulfuric acid as the spray reagent.

GLC was performed by using a Hewlett-Packard 402A chromatograph equipped with a flame-ionization detector: 1.80 m × 4 mm i.d. U-shaped column containing 3% OV-17 on Gas Chrom Q (Applied Science Inc.); temperature 260 °C; carrier gas He (100 mL/min). A Waters Associates HPLC system (M6000 pump, U6K injector, R401 differential refractometer) and a Whatman Partisil M9 10/50 ODS-2 column (50 cm × 9.40 mm i.d.) were used for isolation at a flow rate of 3 mL/min (1000 psi) with absolute methanol as the solvent. The sterol mixture (136 mg) was injected in portions of 15 mg dissolved in 5 mL of methanol.

Low-resolution of GC/MS analyses were performed on a Varian MAT-44 spectrometer at 70 eV and a coiled GC column (1.80 m × 2 mm i.d.) with 3% OV-17 on Gas Chrom Q (Applied Science Inc.) at 260 °C.

The 360-MHz ^{1}H NMR spectra ($CDCl_3$ and C_6D_6) were measured on a Bruker HXS-360 spectrometer at the Stanford Magnetic Resonance Laboratory.

High-resolution mass spectra were recorded on a MAT 711 double-focusing spectrometer equipped with a PDP-11/45 computer for data acquisition and reduction.

Extraction and Isolation of the Sterol Mixture. The sponge (454 g, dry weight after extraction) was collected during a trip on the Red Sea, preserved in acetone in 7 days, and then extracted three times with acetone at room temperature for 3 days. After concentration of the mixture, the aqueous residue was extracted with ether (two times), and the combined ethereal extracts were taken to dryness. The residue (11.2 g) was chromatographed on a silica gel column (320 g, Merck) to give on elution with petroleum ether and increasing amounts of diethyl ether a crude sterol fraction (0.37 g) which was further purified from some contaminants by crystallization from methanol to give the sterol mixture (0.30 g).

The individual sterols were isolated by repeated reversed-phase HPLC runs under the above-described conditions. The relative retention times of all sterols, both in GC and HPLC, are given in Table I.

It is not possible to give melting points and angles of rotation of the new sterols (**1**, **2**, **8**, **13**, **15**) because only very small amounts were isolated: they were all minor or trace sterols (see Table I).

3α-(Hydroxymethyl)-*A*-nor-5α-cholestane (**16**) was synthesized by following the method described by Whitham and Wickramasinghe.[15]

Acknowledgment. Grants from the National Institutes of Health (No. GM-06840 and GM-28352) are gratefully acknowledged as well as grants from the National Science Foundation (No. GP-23633) and the National Institutes of Health (No. RR-00711) for use of a 360-MHz NMR spectrometer. A high-resolution mass spectrometer combined with a computer was programmed and operated by Annemarie Wegmann, and the NMR spectra were taken under the supervision of Dr. Lois Durham. L.B. is grateful to the Swedish Academy of Pharmaceutical Sciences for financial support and U.S. to the Swedish National Science Research Council. We thank Professor M. Sara (University of Genova) for identifying the sponge.

Registry No. **1**, 83704-10-3; **2**, 83704-11-4; **3**, 83704-12-5; **4**, 55081-40-8; **5**, 83681-79-2; **6**, 3963-37-9; **6Ac**, 55081-43-1; **7a**, 83709-56-2; **7b**, 55081-41-9; **8**, 83704-13-6; **9**, 76907-63-6; **10a**, 83709-57-3; **10b**, 55088-75-0; **11a**, 55081-42-0; **11b**, 83730-42-1; **12a**, 55081-39-5; **12b**, 83709-58-4; **13**, 83704-14-7; **14**, 83730-43-2; **15**, 83704-15-8; **16**, 3963-34-6; **16Ac**, 83730-44-3.