Sterols in Marine Invertebrates. 32.[1] Isolation of 3β-(Hydroxymethyl)-*A*-nor-5α-cholest-15-ene, the First Naturally Occurring Sterol with a 15–16 Double Bond

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A new *A*-nor sterol, 3β-(hydroxymethyl)-*A*-nor-5α-cholest-15-ene, with an unusual unsaturation in the Δ^{15} position, has been found in the Pacific sponge *Homaxinella trachys* together with ten other *A*-nor sterols. The structure was elucidated by 360-MHz ^{1}H NMR, ^{13}C NMR, and mass spectral analysis as well as by chemical interconversion and comparison with synthetic 5α-cholest-15-en-3β-ol. ^{13}C NMR spectra of the title compound and of various 3β-(hydroxymethyl)-*A*-nor-5α-cholestanes were assigned as an aid in future structure elucidation of this class of sterols.

Sterols with normal skeletons have been isolated from several sponges of the family Axinellidae,[2-4] but this family is not homogeneous, as sterols with a contracted A-ring, the 3β-(hydroxymethyl)-*A*-norsteranes, have been found as the only type or as the major sterols of *Axinella verrucosa*,[5] *Hymeniacidon perlevis*,[6] *Stylotella agminata*,[7] *Teichaxinella morchella*,[8] *Axinella* sp.,[7] *Phakellia aruensis*,[2a] and *Acanthella aurantiaca*,[9] which belong to the same family. It has been shown[10] that *A*-nor sterols are formed in the sponge by conversion of dietary sterols; intermediates are Δ^4-cholesten-3-one and analogues with different side chains.

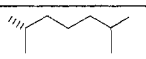
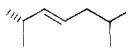
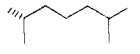
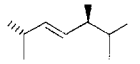
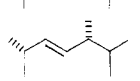
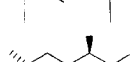
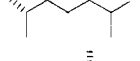
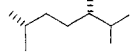
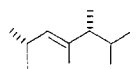
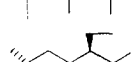
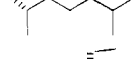
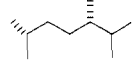
Our ongoing investigations of marine invertebrates have now uncovered another source of novel *A*-nor sterols, the Pacific sponge *Homaxinella trachys*.

Results and Discussion

An examination of the sterol mixture of the sponge *Homaxinella trachys* de Laubenfels from Palau in the Western Carolines led to the isolation of *A*-nor sterols, including the new sterol 3β-(hydroxymethyl)-*A*-nor-5α-cholest-15-ene, which is the first Δ^{15} sterol to be isolated from nature.[11,12] Cholesterol (**9**) was the only regular sterol in the mixture, but it was only a minor component. The abundances of the sterol components are given in Table I.

Sponges live in association with other organisms: microorganisms,[13] and especially bacteria, may account for a high percentage of the tissue volume of the sponge, but unicellular green and blue-green algae and dinoflagellates have also been reported in sponges. Because there is ample documentation[14] that microorganisms are capable of hydroxylating sterols in the 15- or 16-position, it is tempting to speculate that the new sterol **1** (Figure 1) was formed from dietary cholesterol (**9**) via ring contraction, hydroxylation in ring D, and dehydration. If this were correct, then one would also expect to find the Δ^{15} analogues of the other *A*-nor sterols (**2** and **4–8** in Table I) in the sterol mixture. However, such Δ^{15} sterols were absent. Therefore, we assume that **1** was formed by enzymatic ring contraction[10] from dietary cholesta-5,15-dien-3β-ol (**10**) which has not yet been found in any marine or terrestrial organism. This sterol (**10**) may be of planktonic origin and be derived from a 16-hydroxylated precursor since the ability to hydroxylate sterols in the 16-position is widespread in nature, as shown by the natural existence of steroidal sapogenins[15] and of steroidal alkaloids[16] hydroxylated in the 16-position.

Table I. Sterol Content of the Sponge *Homaxinella trachys*

nucleus[a]	side chain	abundance, %	GC RRT[b]
1, *A*-nor, Δ^{15}		7.7	0.93
2, *A*-nor, Δ^0		1.0	0.95
3, *A*-nor, Δ^0		32.7	0.98
4a, *A*-nor, Δ^0		5.6	1.12
4b, *A*-nor, Δ^0		7.2	1.12
5a, *A*-nor, Δ^0		10.1	1.28
5b, *A*-nor, Δ^0		15.0	1.28
6, *A*-nor, Δ^0		13.1	1.35
7a, *A*-nor, Δ^0		2.1	1.58
7b, *A*-nor, Δ^0		2.8	1.58
8, *A*-nor, Δ^0		0.8	2.41
9, conventional, Δ^5		2.1	1.00

[a] The symbol Δ^0 refers to the saturated 5αH nucleus. [b] Gas chromatographic conditions: 3% OV-17, 260 °C; standard cholesterol (**9**).

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(1) For part 31 in this series see: Li, L. N.; Li, H. T.; Lang, R. W.; Itoh, T.; Sica, D.; Djerassi, C., *J. Am. Chem. Soc.*, in press.
(2) (a) Bergquist, P. R.; Hofheinz, W.; Oesterhelt, G. *Biochim. Syst. Ecol.* **1980**, *8*, 423–435. (b) Sjöstrand, U.; Kornprobst, J. M.; Djerassi, C. *Steroids* **1981**, *38*, 355–364.
(3) (a) Hofheinz, W.; Oesterhelt, G. *Helv. Chim. Acta* **1979**, *62*, 1307–1309. (b) Li, X.; Djerassi, C. *Tetrahedron Lett.*, in press.
(4) (a) Cafieri, F.; Fattorusso, E.; Frigerio, A.; Santacroce, C.; Sica, D. *Gazz. Chim. Ital.* **1975**, *105*, 595–602. (b) Itoh, T.; Sica, D.; Djerassi, C. *J. Chem. Soc.*, in press.
(5) Minale, L.; Sodano, G. *J. Chem. Soc., Perkin Trans 1* **1974**, 2380–2384.
(6) Kanazawa, A.; Teshima, S.; Hyodo, S. *Comp. Biochem. Physiol. B* **1979**, *62B*, 521–525.

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Table II. 360-MHz ^{1}H NMR Data ($CDCl_3$) for Naturally Occurring 3β-(Hydroxymethyl)-*A*-nor-5α-cholest-15-ene (1), Synthetic 5α-Cholest-15-en-3β-ol (11), and Their Saturated Analogues 3β-(Hydroxymethyl)-*A*-nor-5α-cholestane (3) and 5α-Cholestan-3β-ol (12)

	chemical shift,[a] δ					
compd	C18-H	C19-H	C21-H	C26,27-H	C15,16-H	C4-H or C3-H
1	0.734 (s)	0.755 (s)	0.901 (d, J = 6.6)	0.865 (d, J = 6.6), 0.869 (d, J = 6.6)	5.83 (1 H, m) 5.74 (1 H, m)	3.484 (1 H, dd, J_1 = J_2 = 10.0), 3.724 (1 H, dd, J_1 = 10.3, J_2 = 6.4)
3	0.647 (s)	0.737 (s)	0.899 (d, J = 6.5)	0.859 (d, J = 6.8), 0.862 (d, J = 6.6)		3.48 (1 H, dd, J_1 = J_2 = 10), 3.71 (1 H, dd, J_1 = 10, J_2 = 6)
11	0.731 (s)	0.822 (s)	0.896 (d, J = 6.5)	0.865 (d, J = 6.6), 0.869 (d, J = 6.7)	5.83 (1 H, m) 5.75 (1 H, m)	3.6 (m)
12	0.636 (s)	0.792 (s)	0.885 (d, J = 6.5)	0.848 (d, J = 6.5), 0.854 (d, J = 6.6)		3.6 (m)

[a] The number of hydrogens, the multiplicity, and/or the coupling constants (in hertz) are given in parentheses.

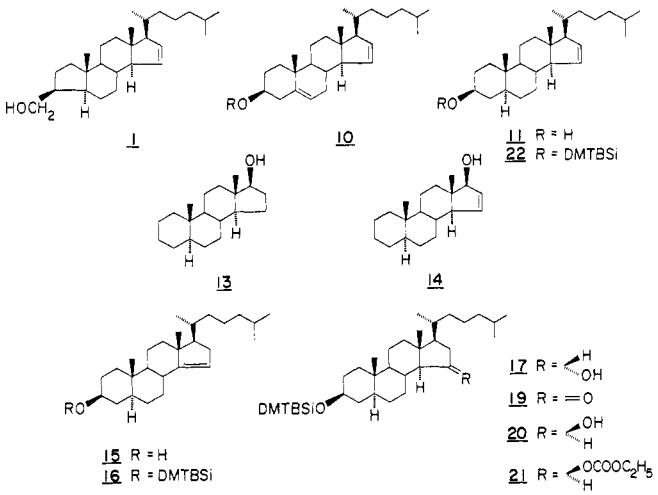

Figure 1. Structures of selected steroids.

Recently, the pharmaceutical industry[17] has shown interest in Δ^{15} steroids, but the compounds reported are derived from 18-homoandrosta-5,15-diene-3,17-dione and possess only short side chains. No synthetic Δ^{15} sterols with a "normal" (e.g., cholestane) side chain are known. To help us understand the NMR and mass spectra of the new sterol (1), it became necessary to synthesize a Δ^{15}-unsaturated member of the cholestane series.

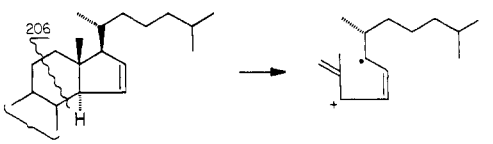

Figure 2. Characteristic fragmentation reaction of Δ^{15} sterols.

Structure Elucidation of the New Sterol (1). ^{1}H NMR was the method of choice to prove that the sterols 1–8 in the mixture were of the rare *A*-nor type, because the protons at C-4 (the hydroxyl-bearing carbon atom) give rise to two one-proton muliplets at about δ 3.4 and 3.7 ($CDCl_3$). In this manner one can also differentiate[9] between 3β-(hydroxymethyl)-*A*-norsterols and their 3α epimers, which have not yet been detected in nature. It has been reported[5] that mass spectrometry can also be used to detect *A*-nor sterols through a diagnostic ($M^+ - 31$) peak associated with the loss of the hydroxymethyl substituent, but there are exceptions to this rule. In the case of *A*-nor sterols with a double bond or a cyclopropyl group in the side chain, e.g., *A*-nordinosterol (6) and *A*-norgorgostanol (8),[7] side-chain fragmentation presumably dominates the mass spectrum, because the ($M^+ - 31$) peak is not detectable.

The acetate of the new sterol of molecular weight 386 was the sole constituent of a fraction with a low R_f value on argentic silica gel TLC plates. Although this R_f value was initially taken to indicate the presence of a methylene group in the side chain, this assumption was not consistent with the NMR spectrum, which showed the presence of a normal cholesterol side chain, nor with the mass spectrum, which included a peak[18] at m/z 273 (loss of side chain without hydrogen transfer), thus supporting a structure with a saturated side chain and one degree of unsaturation in the ring system. The presence of a regular *A*-nor sterol skeleton was established unambiguously by catalytic hydrogenation with $Rd(P(C_6H_5)_3)Cl$ to give the known[5] 3β-(hydroxymethyl)-*A*-nor-5α-cholestane (3).

(7) Bohlin, L.; Gehrken, H. P.; Scheuer, P. J.; Djerassi, C. *Steroids* **1980**, *35*, 295–304.
(8) Bohlin, L.; Sjöstrand, U.; Sullivan, B. W.; Djerassi, C. *J. Chem. Soc. Perkin Trans. 1* **1981**, 1023–1028.
(9) Bohlin, L.; Sjöstrand, U.; Sodano, G.; Djerassi, C., following paper in this issue.
(10) (a) Bibolini, L.; Minale, L.; Sodano, G. *J. Chem. Soc., Chem. Commun.* **1978**, 524–525. (b) De Rosa, M.; Minale, L.; Sodano, G. *Experientia* **1975**, *31*, 408–410. (c) De Rosa, M.; Minale, L.; Sodano, G. *Ibid.* **1976**, *32*, 1112–1113. (d) De Stefano, A.; Sodano, G. *Ibid.* **1980**, *36*, 630–632.
(11) The isolation of another Δ^{15} sterol, 24(*Z*)-5α-cholesta-8(14),15,24(28)-stigmastatrien-3β-ol, was claimed (Fioriti, J. A.; Kolor, M. G.; McNaught, R. P. *Tetrahedron Lett.* **1970**, 2971–2974), but this structure was later shown to be incorrect (Frost, D. J.; Ward, J. P. *Recl. Trav. Chim. Pays-Bas* **1970**, *89*, 1054–1056). Stěpán, J.; Tkáč, A.; Hanzlíček, L. *Med. Exp.* **1961**, *4*, 19–29. These authors mentioned a possible Δ^{15} sterol in their investigation of the lipopeptidic complex from the cerebrospinal fluid of patients with a schizophrenic syndrome, but the tentative structure assignment was made solely by reference to IR absorption bands of the complex mixture.
(12) The same sterol has recently also been isolated from a Red Sea sponge.[9]
(13) (a) Bergquist, P. R. "Sponges"; University of California Press: Berkeley and Los Angeles, 1978. (b) Vacelet, J. *J. Microsc. (Paris)* **1971**, *12*, 363–381. (c) Braekman, J. C.; Kaisin, M.; Tursch, B. "Abstracts of Papers from the 3rd International Symposium on Marine Natural Products"; Brussels 1980; p P15.
(14) (a) Charney, W.; Herzog, H. L. "Microbial Transformations of Steroids"; Academic Press: New York, 1967. (b) Capec, A.; Hanč, O.; Tandra, M. "Microbial Transformations of Sterols"; W. Junk: The Hague and Academia: Prague, 1966. (c) Iizuka, H.; Naito, A. "Microbial Transformations of Steroids and Alkaloids"; University of Tokio Press: Tokyo and University Park Press: State Park, CA, 1967. (d) Fonken, G. S.; Johnson, R. A. "Chemical Oxidations with Microorganisms"; Marcel Dekker: New York, 1972.
(15) It has been shown that 3β,16β-dihydroxycholestane is a precursor of sapogenins. See: Tschesche, R.; Leinert, J. *Phytochemistry* **1973**, *12*, 1619–1620.
(16) Kaneko, K.; Nakaoka, U.; Tanaka, M.; Yoshida, N.; Mitsuhashi, H. *Phytochemistry* **1981**, *20*, 157–160.
(17) (a) Wiechert, R. "Main Plenary Lectures, 1st International Conference on Biotechnology and Biologically Active Products"; Bulgarian Academy of Sciences: Sofia, Bulgaria, 1981, Vol. 1, pp 29–67. (b) Hofmeister, H.; Wiechert, R.; Annen, K.; Laurent, H.; Steinbeck, H. U.S. Patent 4 081 537, 1978.
(18) Wyllie, G. S.; Djerassi, C. *J. Org. Chem.* **1968**, *33*, 305–312.

The ^{1}H NMR spectrum showed the presence of a 1,2-disubstituted ethylene moiety, which appeared as two one-proton multiplets, thus leaving four possible positions (Δ^1, Δ^6, Δ^{11}, Δ^{15}) for the double bond. However, the complexity of the multiplets was consistent with neither an ABx system nor an ABxy system, thus suggesting Δ^{15} as the most likely structural alternative.[19] A detailed analysis of the ^{13}C NMR spectra of **1** and of some other *A*-nor sterols also supported a structure with a Δ^{15} double bond (vide infra).

^{1}H NMR (360 MHz) data for the naturally occurring Δ^{15}-*A*-nor sterol **1**, synthetic 5α-cholest-15-en-3β-ol (**11**) and their analogues with a saturated ring system (**3**, **12**) are given in Table II. In order to determine which methyl singlet corresponded to C-18 and which to C-19 we compared the NMR spectra of two sets of compounds: the *A*-nor sterols **1** and **3** and the normal sterols **11** and **12**. The angular methyl groups of the Δ^{15} sterols have been assigned in such a manner that the Zürcher additive values[20] for a Δ^{15} double bond of both sets are consistent with each other. From the data in Table II one calculates that these values for the *A*-nor sterols are 0.087 and 0.018, and for the normal sterols they are 0.095 and 0.030 for C-18 and C-19, respectively.

Mass Spectra of Δ^{15} Sterols. The mass spectra of **1** and **11** both show a strong molecular ion peak (100%) and also pronounced peaks for loss of the side chain[18] (*m/z* 273) and for loss of the side chain and water (*m/z* 255). What is unusual about these mass spectra is a strong (37% and 66%, respectively) peak associated with an ion of composition $C_{15}H_{16}$ (*m/z* 206). This fragment appears to be unique for Δ^{15} sterols and is most likely formed by C-ring cleavage to give a stable ionized conjugated triene (Figure 2).[21]

^{13}C NMR Spectra. We have already summarized[9] the ^{1}H NMR data of *A*-nor sterols and their utility in structure elucidation. We now report a study of their ^{13}C NMR spectra which offers additional information for structure assignment.

The shifts for 3β-(hydroxymethyl)-*A*-nor-5α-cholestane (**3**) are assigned on the basis of its SFORD (single frequency off-resonance decoupled) spectrum, similarity to 5α-cholestan-3β-ol,[22] predictions from a ^{13}C predicting program,[23] and a shift study. This information is summarized in Table III. The signals for carbons 12–18 and 20–27 were immediately assigned on the basis of their similarity to corresponding carbons in 5α-cholestan-3β-ol. The remaining angular methyl group (C-19) was then ascribed to the as yet unassigned quartet at 14.61 ppm. The downfield triplet at 66.80 ppm was attributed to the hydroxyl-bearing C-4, and the remaining quaternary carbon (C-10) to the singlet at 44.00 ppm. Breaking up the remaining carbons into multiplicity groups and then considering predicted resonances (and their confidence values) and Eu(fod) shift values (and approximage distances from the carbons to the shift reagent as calculated from a Dreiding model) led to the assignment of the remaining carbons. We note that the results of the shift study are consistent only with a 3β-orientation of the hydroxymethyl group. Minale and Sodano,[5] who were the first to isolate *A*-nor sterols, used chemical methods to establish the configuration of the hydroxymethyl group as 3β.

Table III. Data for Assignment of the ^{13}C NMR Spectrum of 3β-(Hydroxymethyl)-*A*-nor-5α-cholestane (**3**)[24]

C	mult	δ_{pred} (cert)	δ_{obsd}	$\Delta\delta_{max}$	dist
1	t	42.9 (2)	39.06	1.34	5.4
2	t	26.6 (1)	22.79	1.86	4.3
3	d	39.1 (1)	42.66	0.61	3.3
4	t	66.7 (2)	66.80	14.89	2.0
5	d	50.0 (1)	52.50	1.41	4.5
6	t	24.9 (2)	27.28	2.24	4.3
7	t	31.9 (3)	32.74	0.73	6.0
8	d	35.0 (3)	35.57	0.65	6.4
9	d	53.3 (2)	55.40	0.65	6.4
10	s	39.5 (1)	44.00	1.46	5.1
11	t	20.8 (3)	23.30	0.49	
12	t	39.8 (4)	39.88	0.33	
13	s	42.6 (4)	42.95	1.09	
14	d	56.4 (2)	56.21	0.36[a]	
15	t	24.2 (4)	24.46	0.19	
16	t	28.2 (4)	28.21	0.15	
17	d	56.3 (4)	56.21	0.17[a]	
18	q	12.1 (4)	12.24	0.24	
19	q	14.4 (2)	14.61	1.15	4.6
20	d	35.8 (4)	35.76	0.12	
21	q	18.7 (4)	18.76	0.14	
22	t	36.2 (4)	36.20	0.03	
23	t	23.9 (4)	23.88	0.08	
24	t	39.5 (4)	39.50	0.06	
25	d	28.0 (4)	27.99	0.04	
26	q	22.7 (4)	22.57	0.03	
27	q	22.7 (4)	22.79	0.06	

[a] Interchangeable values.

24-Methyl-3β-(hydroxymethyl)-*A*-nor-5α-cholestane (**5a,b**) and 24-ethyl-3β-(hydroxymethyl)-*A*-nor-5α-cholestane (**7a,b**) were isolated as mixtures of epimers at C-24. The assignment of the nuclear carbons (Table IV) is based on their similarity to 3β-(hydroxymethyl)-*A*-nor-5α-cholestane (**3**), while side-chain carbon assignments are based on the work of Wright et al.[22]

The assignments for 3β-(acetoxymethyl)-*A*-nor-5α-cholestane (**3Ac**, Table IV) were made on the basis of its similarity to the 3β-hydroxymethyl parent **3**, on the multiplicities, and on acetylation shifts. Carbons 1 and 6–27 were assigned on the basis of the appearance of corresponding resonances in the spectrum of **3**. Acetylation shifts (α + 2.8, β − 4.3, γ small),[26] chemical shift theory, and multiplicities permitted assignment of the remaining carbons.

The Δ^{15} location of the double bond in 3β-(hydroxymethyl)-*A*-nor-5α-cholest-15-ene (**1**) was confirmed by

(19) We note that acetates of **1** and of Δ^{14} sterols (Kokke, W. C. M. C.; Fenical, W.; Djerassi, C. *Phytochemistry* **1981**, *20*, 127–134) behave like sterically unhindered methylene compounds such as 24-methylenecholesterol acetate in argentic silica gel TLC. This property facilitates isolation of Δ^{14} and Δ^{15} sterols from mixtures.

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(24) Shifts are in parts per million from Me_4Si. Multiplicities were obtained from a SFORD spectrum. The predicted resonances were generated by a ^{13}C NMR predicting program[23] from a structure constructed via the CONGEN program. The certainty level (cert) is a measure of confidence in a predicted resonance, with 4 being the maximum and 0 the minimum. "Dist" is the distance from the shift reagent to the carbon of interest as obtained from a Dreiding model. δ_{obsd} is the assigned observed resonance. $\Delta\delta_{max}$ is the magnitude of downfield motion by the corresponding resonance upon addition of the maximum amount (i.e., molar ratio of EuFOD/sterol of 1:1) of shift reagent.

(25) Shifts are in parts per million from Me_4Si. The letters in parentheses indicate the following: a and b, resonances may be interchanged within a column, and those indicated are the preferred assignments; c, assignment of carbons 20 and 22–27 are in question due to lack of an SFORD spectrum and significant deviations from typical side-chain resonances; d, these minor isomer (see Table I) peaks were buried in other peaks, and these values were calculated from comparison of the *S*-isomer (**5b**) resonance with that given by Wright et al.[22] and then applying any correction to the *R*-isomer resonances.

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Table IV. ^{13}C NMR Spectral Data of 5α-Cholestan-3β-ol (12), the Androstan-17β-ols 13 and 14, and *A*-Nor Sterols 5a,b, 7a,b, 3, 3Ac, and 1Ac[25]

	chemical shift									
C	12	3	5b	5a	7b	7a	3Ac	13	14	1Ac (c)
1	37.0	39.1	39.1	39.1	39.1	39.1	39.0	38.8	38.6	38.8
2	31.5	22.8	22.8	22.8	22.8	22.8	22.6	22.2	22.2	22.6
3	71.4	42.7	42.6	42.6	42.7	42.7	38.5	26.8	26.8	38.5
4	38.2	66.8	66.9	66.9	66.9	66.9	68.3	29.1	28.9 (a)	68.3
5	44.9	52.5	52.5	52.5	52.5	52.5	52.4	47.2	47.3	52.6
6	28.8	27.3	27.3	27.3	27.3	27.3	27.2	29.1	29.0 (a)	27.3
7	32.1	32.7	32.8	32.8	32.7	32.7	32.7	31.8	31.9	32.7
8	35.5	35.6	35.6	35.6	35.6	35.6	35.5	35.8	33.2	32.2
9	54.4	55.4	55.4	55.4	55.4	55.4	55.4	55.0	55.4	55.9
10	35.5	44.0	44.0	44.0	44.0	44.0	44.1	36.4	36.5	44.1
11	21.3	23.0	23.3	23.3	23.3	23.3	23.3	20.5	20.3	24.3
12	40.1	39.9	39.9	39.9	39.9	39.9	39.9	36.9	34.8	37.7
13	42.6	43.0	43.0	43.0	43.0	43.0	43.0	43.1	53.0	49.9
14	56.5	56.2	56.2	56.2	56.2	56.2	56.2	51.3	57.7	62.4 (a)
15	24.3	24.5	24.5	24.5	24.4	24.4	24.4	34.4	132.0	131.1
16	28.3	28.2	28.2	28.2	28.2	28.2	28.2	30.6	134.2	133.5
17	56.3	56.2	56.0	56.1	56.1	56.1	56.2	82.1	85.7	61.5 (a)
18	12.1	12.2	12.2	12.2	12.2	12.2	12.2	11.2	12.3	12.9
19	12.3	14.6	14.6	14.6	14.6	14.6	14.5	12.3	12.3	14.5
20	35.8	35.8	36.2	35.9	36.2	36.1	35.8			29.7
21	18.7	18.8	18.9	18.7	18.9	18.9	18.7			18.7
22	36.2	36.2	33.7	33.7	33.9	33.9	36.2			36.5
23	23.9	23.9	30.6	30.3	26.4	26.1	23.9			29.7 (b)
24	39.5	39.5	39.1	38.8	46.1	45.8	39.5			39.5
25	28.0	28.0	31.5	32.4	29.0	29.2	28.0			29.3 (b)
26	22.6	22.6	17.6	20.2 (d)	19.0	19.8	22.6			22.8
27	22.8	22.8	20.5	18.3 (d)	19.6	19.0	22.8			22.9
28			15.5	15.4 (d)	23.0	23.0				
29					12.2	12.2				
30							21.0			21.1
31							170.8			170.9

Table V. Disubstituted Olefin ^{13}C Resonances[27,28]

steroid	shift[a]	steroid	shift[a]
cholest-1-ene	124.5, 135.5	cholest-1-en-3β-ol	128.8, 137.9
cholest-2-ene	125.6, 125.7	cholest-2-en-3β-ol	128.0, 130.4
cholest-3-ene	125.1, 131.2	cholest-6-en-3β-ol	129.3, 130.6
cholest-6-ene	121.7, 128.6	cholest-11-en-3β-ol	125.3, 138.8
cholest-11-ene	125.6, 138.6	androst-15-en-17β-ol	132.0, 134.2
		3β-(hydroxymethyl)-*A*-nor-5α-cholest-15-ene	131.1, 133.5

[a] The chemical shifts are in parts per million from Me_4Si.

comparing the olefinic resonances with those of other structures having the disubstituted double bond at various locations (Table V). Assignments were made (Table IV) on the basis of similarity with **3Ac** and a model study (vide infra) on the effects of the introduction of a 15–16 double bond on the C and D ring carbon resonances. Carbons 1–7, 10, 19, and 21 were assigned on the basis of similarities with **3Ac** which had been previously assigned (Table IV). Carbons 14–17 were then assigned on the basis of their distinctive movement downfield relative to **3Ac**. Assignment of carbons 8, 9, 11, 13, and 18 was made by comparing androstan-17β-ol (**13**)[28] with androst-15-en-17β-ol (**14**)[27] and extrapolating the effects of the introduction of the 15–16 double bond to the **3Ac**–**1Ac** pair. This left carbons 20 and 22–27 of the new sterol 1, which should have been found resonating at "typical" values (as in **3**). Since this was not the case, and the requisite model systems were not available, the assignment of those carbons is tentative.

Synthesis of 5α-Cholest-15-en-3β-ol (11). The most obvious route to Δ^{15}-unsaturated sterols is dehydration of sterols with a hydroxyl group in the 15- or 16-position, and we selected the former alternative. The readily available 5α-cholest-14-en-3β-ol (**15**)[29] was protected[30] as the dimethyl-*tert*-butylsilyl (DMTBSi) ether (**16**) which was hydroborated[31] to give the 15α-alcohol **17** and then converted into the 15β isomer **20**[32] by Jones oxidation followed by $LiAlH_4$ reduction.

We intended to introduce the Δ^{15} double bond by ester pyrolysis, as mechanistic studies have shown that ester pyrolysis is a syn-elimination reaction. Thus, pyrolysis of an ester of a 15β- or 16β-alcohol such as an *S*-methylxanthate[33] or an ethyl carbonate should afford exclusively the Δ^{15} olefin. The *S*-methyl xanthate of the 15β-alcohol **20** could not be isolated: apparently it was unstable under the reaction conditions, and the only isolated product was shown by NMR to be the pure Δ^{14} olefin **16**. Elimination

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was also a problem when the 15β-alcohol **20** was treated with ethyl chloroformate in pyridine. The main product was again the Δ^{14} olefin **16**, but the desired 15β-ethyl carbonate **21** was also obtained, albeit in low (25%) yield. Pyrolysis of **21** (solution in decalin/245 °C/75 min) afforded a 1:4 mixture of the Δ^{15} and Δ^{14} isomers (**16** and **22**) from which, after deprotection,[34] 5α-cholest-15-en-3β-ol (**11**) was isolated by reverse-phase HPLC. As shown in Table II, the ^{1}H NMR signals of the olefinic protons of the synthetic Δ^{15} sterol **11** coincided with those of the Δ^{15}-*A*-nor sterol **1**.

Experimental Section

General Methods. Analytical GC was carried out with a Hewlett-Packard Model 402 gas chromatograph with a flame-ionization detector (3% OV-17 column, 2 or 4 mm i.d. × 1.80 m, 260 °C). Reverse-phase HPLC was performed by using Waters equipment (M6000A pump, U6K injector, R401 refractometer) and a Whatman Partisil M9 10/50 ODS-2 column (9 mm i.d. × 50 cm). The eluent was absolute methanol, unless indicated otherwise. The beginning of the solvent peak (and not the point of injection) was used to calculate relative and absolute retention times (RRT and RT) in HPLC. Cholesterol was used as a standard, both in GC and HPLC. Low-resolution GC/MS was performed on a Varian MAT-44 (3% OV-17 column, 260 °C). 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. The 360-MHz ^{1}H NMR spectra were recorded on a Brucker HXS-360 spectrometer, and ^{13}C NMR spectra were recorded with a Varian XL-100 Fourier transform spectrometer under the following conditions: frequency 25.1 MHz, spectral width 3000 and 5000 Hz, flip angle ca. 30°, internal pulse lock to D in $CDCl_3$ (solvent), ambient temperature, concentration 20–60 mg/mL. The coupling constants are given in hertz. Shift studies were run by using incremental additions of an Eu(fod)/$CDCl_3$ solution. IR spectra were run on a Nicolet MX-1 FT-IR instrument by using KBr pellets. Melting points were measured on a Thomas-Hoover Unimelt capillary melting point apparatus, and they were not corrected. Angles of rotation were determined by using a Rudolph Research Autopol III polarimeter. Silica gel 60 (E. Merck, 70–230 mesh), Grace grade 62 silica gel (70–200 mesh), and Florisil (100–200 mesh) were used for column chromatography; analytical TLC was performed on precoated silica gel sheets (E. Merck No. 5539). Argentic silica gel plates were prepared in the manner of Idler.[35]

Collection of the Sponge and Isolation of the Sterols. The sponge was collected in Palau at the south-west end of a small bridge connecting Malakal and Koror at a depth of 6 ft. It is bright orange and grows in fast-flowing water in tidal channels. The freshly collected sponge was stored in dry ice and shipped to California, where it was left in a freezer prior to the workup. A freeze-dried sponge (209 g) was homogenized in a Waring blender in methylene chloride–methanol (1:1); the homogenate was filtered, and the material on the filter was homogenized four more times in the same solvent mixture. The combined filtrates were evaporated; the residue was partitioned between $CHCl_3$ and water, and the $CHCl_3$ layer was evaporated to give 32.5 of extract. Sterols were isolated from this extract by using a silica gel column (650 g) and hexane–toluene (3:2) and hexane–ether mixtures (2:1 and 1:1). The crude sterols were further purified over a Florisil column (150 g) to yield 2.7 g of free sterols. Sterol acetates, prepared by acetylation with pyridine–acetic anhydride at room temperature, were separated into four fractions by argentic silica gel TLC:[36] 0, R_f 0.06–0.16; 1, R_f 0.31–0.33; 2, R_f 0.33–0.38; 3, R_f 0.38–0.44. Fraction 0 contained only 3β-(hydroxymethyl)-*A*-norcholest-15-en-3β-ol (**1**) which was obtained in essentially quantitative yield (7.7% of the sterol mixture; see Table I). All components of fractions 1–3, including epimers at C-24 with a Δ^{22} double bond,[8] were separated by reverse-phase HPLC.

3β-(Hydroxymethyl)-*A*-nor-5α-cholest-15-ene (1): mp 151–153 °C; $[\alpha]_D$ −37° (*c* 6 × 10^{-4}, $CHCl_3$), IR (KBr) 1635 cm^{-1} (C=C stretch); high-resolution GC/MS (70 eV), *m/z* (assignment, relative intensity) 386.3541 (M^+, $C_{27}H_{46}O_1$, 100), 371.3283 ($C_{26}H_{43}O_1$, 28), 368.3384 ($C_{27}H_{44}$, 15), 355.3381 ($C_{26}H_{43}$, 23), 353.3228 ($C_{26}H_{41}$, 7), 302.2631 ($C_{21}H_{34}O_1$, 4), 301.2586 ($C_{21}H_{33}O_1$, 7), 273.2247 ($C_{19}H_{29}O_1$, 66), 260.2483 ($C_{19}H_{32}$, 3), 255.2112 ($C_{19}H_{27}$, 28), 233.1894 ($C_{16}H_{25}O_1$, 22), 220.1822 ($C_{15}H_{24}O_1$, 7), 219.2095 ($C_{16}H_{27}$, 3), 219.1725 ($C_{15}H_{33}O_1$, 6), 218.1682 ($C_{15}H_{22}O_1$, 7), 215.1785 ($C_{16}H_{23}$, 9), 213.1640 ($C_{16}H_{21}$, 6), 206.2008 ($C_{15}H_{26}$, 37), 202.1736 ($C_{15}H_{22}$, 7).

3β-(Hydroxymethyl)-23,24(*R*)-dimethyl-*A*-nor-5α-cholest-22(*E*)-ene (6; *A*-nordinosterol): mp 168–172 °C; high-resolution GC/MS (70 eV), *m/z* (assignment, relative intensity) 414.3856 (M^+, $C_{29}H_{50}O_1$, 12), 396.3775 ($C_{29}H_{48}$, 2), 371.3289 ($C_{26}H_{43}O_1$, 7), 353.3195 ($C_{26}H_{41}$, 3), 343.2990 ($C_{24}H_{39}O_1$, 3), 325.2888 ($C_{24}H_{37}$, 2), 302.2583 ($C_{21}H_{34}O_1$, 21), 287.2403 ($C_{20}H_{31}O_1$, 10), 284.2513 ($C_{21}H_{32}$, 5), 273.2215 ($C_{19}H_{29}O_1$, 46), 269.2244 ($C_{20}H_{29}$, 3), 260.2095 ($C_{18}H_{28}O_1$, 2), 257.2255 ($C_{19}H_{29}$, 22), 255.2093 ($C_{19}H_{27}$, 5), 243.2124 ($C_{18}H_{27}$, 2), 233.1864 ($C_{16}H_{25}O_1$, 1), 230.1992 ($C_{17}H_{26}$, 2), 217.1937 ($C_{16}H_{25}$, 2), 215.1795 ($C_{16}H_{23}$, 2), 203.1806 ($C_{15}H_{23}$, 2), 202.1707 ($C_{15}H_{22}$, 2), 201.1650 ($C_{15}H_{21}$, 2), 69.0704 (C_5H_9, 100).

Deprotection of DMTBSi ethers[34] was always carried out with a solution of the following composition: CH_2Cl_2, 20 mL (dried by filtration through Al_2O_3 activity I); acetonitrile, 10 mL (freshly distilled from CaH_2); $LiBF_4$, 0.33 g. The usual workup when the reaction is complete was as follows: evaporate the solvents; purify the residue over a short column. It is not necessary to partition the residue between water and hexane or ether in order to remove the inorganics.

5α-Cholest-14-en-3β-ol Dimethyl-*tert*-butylsilyl Ether (16).[30] 5α-Cholest-14-en-3β-ol (**15**,[29] 1.0 g), DMTSiCl (Fluka; 0.973 g, 2.5 equiv), imidazole (Fluka, "puriss. P.A."; 0.880 g, 5.0 equiv), DMF 50 mL (distilled from CaO), and CH_2Cl_2 (50 mL, dried by filtration through activity I Al_2O_3) were placed in a flask and heated in an oil bath at 35 °C. The reaction was followed by TLC and was complete after 100 min. After addition of water the reaction mixture was extracted with hexane. The hexane layer was washed with water and evaporated, and the residue was purified over a silica gel column (40 g; eluent hexane–toluene, 3:2) to provide **16** in essentially quantitative yield. For removal of minor impurities, if any, a small sample of **16** was purified by argentic silica gel TLC[36] [developer hexane–benzene (3:1), one development] and recrystallized from acetone. Pure **16** has the following: mp 143.0–143.5 °C; $[\alpha]_D$ +24.4° (*c* 0.01, $CHCl_3$); 360-MHz NMR ($CDCl_3$) δ 0.049 (6 H, s, methyl groups at Si), 0.809 (s, C18-H), 0.867 (6 H, d, *J* = 6.7, C26,27 H), 0.882 (9 H, s, *t*-Bu); 0.893 (s, C19 H), 0.908 (d, *J* = 6.4, C21 H), 3.55 (m, C3 H), 5.13 (s, C15 H); 360-MHz NMR (C_6D_6) δ 0.133 (6 H, s, methyl groups at Si), 0.707 (s, C19 H), 0.921 (6 H, d, *J* = 6.6, C26,27 H), 0.970 (s, C18 H), 1.012 (d, *J* = 5.8, C21 H), 1.042 (9 H, s, *t*-Bu), 3.6 (m, C3 H), 5.27 (s, C15 H).

5α-Cholestane-3β,15α-diol 3-(Dimethyl-*tert*-butylsilyl) Ether (17). 5α-Cholest-14-en-3β-ol dimethyl-*tert*-butylsilyl ether (**16**; 1.24 g in a 100-mL three-necked flask equipped with a condensor, dropping funnel, thermometer, and stirrer bar) under argon was placed in an ice bath. BH_3–THF (Aldrich, 1 M, 15 mL) was added dropwise, followed by anhydrous ether (10 mL). After 30 min, the ice bath was removed, the mixture was allowed to reach room temperature, and water was added carefully, followed by 10% NaOH (20 mL) and 30% H_2O_2 (15 mL). After the usual workup, the alcohol **17** was isolated in 50% yield by silica gel column chromatography (40 g; eluent hexane–ether, 9:1). The purity of **17** was satisfactory (NMR, HPLC), but an analytical sample for the determination of physical constants was purified over an ODS-2 column (MeOH; RRT 0.64, standard cholesterol). After recrystallization from acetone **17** had the following: mp 84–88 °C; $[\alpha]_D$ +35.2° (*c* 7 × 10^{-3}, $CHCl_3$); 360-MHz NMR for **17** ($CDCl_3$) δ 0.048 (6 H, s, methyl groups at Si), 0.677 (s, C18 H); 0.809 (s, C19 H); 0.858 (s, *J* = 6.5, C26 or C27 H), 0.861 (s, *J* = 6.6, C26 or C27 H), 0.881 (9 H, s, *t*-Bu), 0.890 (d, *J* = 6.2, C21 H), 3.55 (m, C3 H), 3.93 (ddd, $J_1 = J_2 = 4.6$, $J_3 = 3.3$, C15 H).

The protected diol **17** (16.5 mg) was left overnight in the $LiBF_4$ solution[34] (3 mL, vide supra). Reaction was complete the next morning. Crude 5α-cholestane-3β,15α-diol (**18**), a known compound,[31] was purified over an ODS-2 column (MeOH–water, 9:1;

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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).

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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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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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