Sterols in Marine Invertebrates. 22.[1] Isolation and Structure Elucidation of Conicasterol and Theonellasterol, Two New 4-Methylene Sterols from the Red Sea Sponges *Theonella conica* and *Theonella swinhoei*

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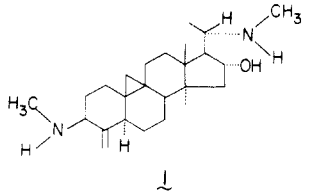
Two new and unusual sterols with unsaturation in the $\Delta^{8(14)}$ position and a heretofore unprecedented 4-methylene nucleus, conicasterol (**12c**, 4-methylene-24(*R*)-methylcholest-8(14)-en-3β-ol) and theonellasterol (**12d**, 4-methylene-24(*S*)-ethylcholest-8(14)-en-3β-ol), were isolated as the principal sterol constituents from the Red Sea sponges *Theonella conica* (Kieschnick) and *Theonella swinhoei* (Gray), respectively. The structures were determined by chemical and spectral analysis and comparison to the spectral data of the newly synthesized 4-methylenecholestan-3β-ol (**11a**).

Although an ever increasing number of 4α-methyl sterols from marine sources has been reported by this group[2] and others,[3] the biosynthetically unusual 4-methylene sterols have never been reported from either marine or even terrestrial sources. These sterols could be viewed as a shunt in the oxidative demethylation of the 4α-methyl series through the dehydration of the primary alcohol formed in the first oxidation of the 4α-methyl sterols.[4] However, the closely related steroidal alkaloid cyclobuxine-B (1) and other related alkaloids from the terrestrial plants of the *buxus* species have been reported to contain the 4-methylene functionality.[5] Two other notable cases that contain the exocyclic methylene moiety in a similar structural environment in the diterpene series,[6] agbaninol (2) and two related compounds agbanindiol A and B, and in the triterpene series,[7] cymbopogonol (3), have also been reported. This unusual feature combined with the relatively rare $\Delta^{8(14)}$ double bond[2] created some difficulties with direct correlations to known spectral parameters. We therefore synthesized 4-methylenecholestan-3β-ol (**11a**) for spectral comparisons.



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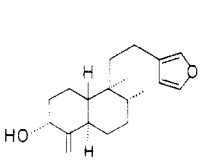

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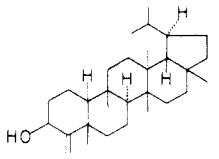

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Results and Discussion

Synthesis of 4-Methylenecholestan-3β-ol (11a). Reaction of the known epoxide (**15a**)[8] with lithium diisopropylamide in ether at room temperature by the method of Miller and Behare gave, among other products, the desired 3β-alcohol **11a**.[9] The β-epoxide was synthesized from 4β-methylcholestan-3β-ol (**13a**)[10] by standard methods outlined in Scheme I. The 4-methylene alcohol **11a** was synthesized to gain insight into the ^{1}H NMR additivity effects of the 4-methylene group on the C-18 and C-19 angular methyls in accordance with the methods of Zürcher,[11] since such standard reference values were not available.

Scheme I

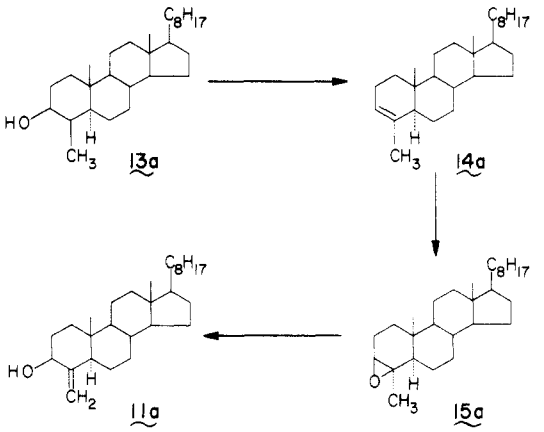


Table I. ^{1}H Chemical Shift Increments (in ppm)

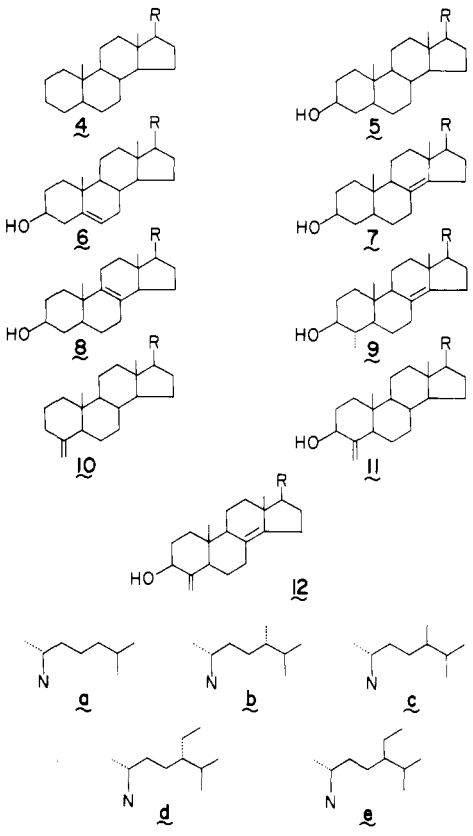
compd	C_{18}	C_{19}
4a	0.65	0.78
10a[10]	0.651	0.687
$\Delta\delta$	+0.001	−0.093
5a	0.65	0.81
11a	0.653	0.686
$\Delta\delta$	+0.003	−0.124
$\Delta\delta_{av}$	+0.002	−0.108

*To whom correspondence should be addressed at Stanford University.

(1) For the preceding paper see: Blanc, P.-A.; Djerassi, C. *J. Am. Chem. Soc.*, in press.
(2) (a) Kokke, W. C. M. C.; Fenical, W.; Djerassi, C. *Phytochemistry*, in press. (b) Bohlin, L.; Kokke, W. C. M. C.; Fenical, W.; Djerassi, C., submitted for publication in *Phytochemistry*. (c) Djerassi, C. *Pure Appl. Chem.*, in press.
(3) Schmitz, F. J. In "Marine Natural Products"; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. 1, pp 241-297.
(4) Nes, W. R.; McKean, M. L. "Biochemistry of Steroids and Other Isopentenoids"; University Park Press: Baltimore, 1973; pp 325-410.
(5) For review of *Buxus* alkaloids see: Manske, R. H. F. "The Alkaloids"; Academic Press: New York, 1973; Vol. XIV, pp 1-82.
(6) Ekong, D. E. U.; Okogun, J. I. *J. Chem. Soc.* C **1969**, 2153-2156.
(7) Yokoyama, Y.; Tsuyuki, T.; Nakamura, N.; Takahashi, T.; Hanson, S. W.; Matsushita, K. *Tetrahedron Lett* **1980**, 3701-3702.
(8) Nelson, J. A.; Kahn, S.; Spencer, T. A.; Sharpless, K. B.; Clayton, R. B. *Bioorg. Chem.* **1975**, *4*, 363-376.
(9) Miller, R. B.; Behare, E. S. *J. Am. Chem. Soc.* **1974**, *96*, 8102-8106.
(10) Czarny, M. R.; Maheshwari, K. K.; Nelson, J. A.; Spencer, T. A. *J. Org. Chem.* **1975**, *40* 2079-2085.
(11) Zürcher, R. F. *Helv. Chem. Acta* **1963**, *46*, 2054-2088.

0022-3263/81/1946-1836$01.25/0

Chart I



Comparison of the known shifts of 5α-cholestane (**4a**)[12] and 5α-cholestan-3β-ol (**5a**)[12] to 4-methylenecholestane (**10a**) and 4-methylenecholestan-3β-ol (**11a**, Table I) indicated the average increment values for C-18 and C-19 to be 0.002 and −0.108 ppm, respectively. These values were useful in predicting the chemical shifts of C-18 and C-19 in the natural products.

Structural Elucidation of Conicasterol (12c) and Theonellasterol (12d). The major component isolated from the sterol fraction of the sponge *T. conica* was conicasterol (**12c**). TLC analysis showed the relative R_f value of this compound (R_f 0.43) to be above that of cholesterol (R_f 0.28), 3β-hydroxymethyl-*A*-norcholestane (R_f 0.33), or 4α-methylcholestanol (R_f 0.39). Upon charring with ceric sulfate and sulfuric acid solution, the different sterol series displayed different colors. Cholesterol immediately turned bright red, changed to a brown color after 10 min, and remained that color after 1 h; *A*-norcholestanol charred yellow, went to purple, and remained purple; 4α-methylcholestanol charred orange and then went to purple and then blue; conicasterol charred brown and remained brown. The mass spectrum displayed a parent ion at m/z 412 ($C_{29}H_{48}O$, 100%), indicating two more carbons and one more site of unsaturation than possessed by cholesterol. Loss of C_9H_{19} at m/z 285 ($C_{20}H_{29}O$, 6.1%) suggested that one of the additional carbons was located on the side chain. This was confirmed by careful analysis of the high-field region of the 360-MHz ^1H NMR spectrum. Close inspection of the ^1H NMR spectrum (Table II) of conicasterol (**12c**; see Chart I) indicated almost identical chemical shifts of the methyl protons C-21, C-26, C-27, and C-28 to those in the reference compound 4α,24(*R*)-di-

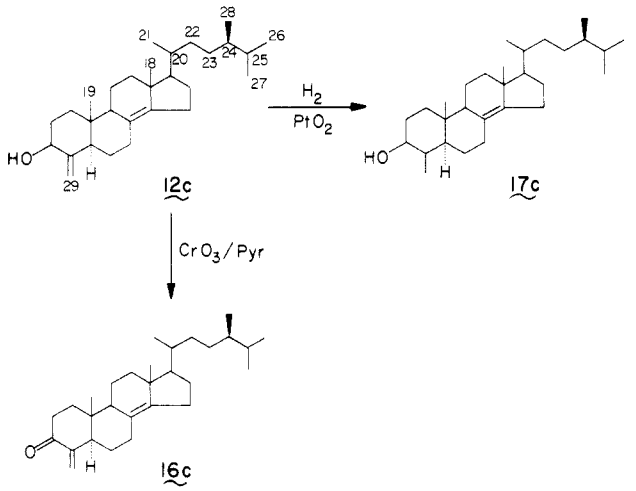
Table II. Selected ^1H NMR (360 MHz, $CDCl_3$) Chemical Shifts[a]

compd	C-18	C-19	C-21	C-26	C-27	C-28	C-29	methylene	4α-Me	C-3α H
10a	0.651 (s)	0.687 (s)	0.902 (d, J = 6.5)	0.863 (d, J = 6,7)	0.867 (d, J = 6.7)			4.441 (s), 4.72 (s)		
11a	0.653 (s)	0.686 (s)	0.907 (d, J = 6.5)	0.863 (d, J = 6.8)	0.866 (d, J = 6.8)			4.628 (d, J = 1.2), 5.042 (s)		3.985 (dd, J = 5.8, 11.6)
12c	0.836 (s)	0.588 (s)	0.927 (d, J = 6.6)	0.777 (d, J = 6.7)	0.802 (d, J = 6.7)	0.851 (d, J = 7.0)		4.631 (s), 5.073 (s)		4.022 (dd, J = 5.2, 10.8)
12d	0.836 (s)	0.588 (s)	0.941 (d, J = 6.6)	0.811 (d, J = 6.6)	0.828 (d, J = 6.6)		0.856 (t, J = 7.4)	4.629 (s), 5.069 (s)		4.027 (dd, J = 5.2, 10.8)
9c	0.836 (s)	0.713 (s)	0.925 (d, J = 6.6)	0.781 (d, J = 6.1)	0.801 (d, J = 6.2)	0.849 (d, J = 9.3)			0.988 (d, J = 6.3)	3.095 (ddd, J = 15.0, 11.5, 5)
9d	0.837 (s)	0.714 (s)	0.939 (d, J = 6.5)	0.811 (d, J = 6.6)	0.832 (d, J = 7.0)		0.857 (t, J = 7.4)		0.989 (d, J = 6.3)	3.093 (ddd, J = 15.0, 11.5, 5)
17c	0.834 (s)	0.740 (s)	0.921 (d, J = 6.0)	0.773 (d, J = 6.6)	0.799 (d, J = 6.9)	0.846 (d, J = 7.0)			0.903 (d, J = 7.1)	3.756 (m)

[a] Given as δ values. J values are in hertz.

(12) Iida, T.; Tamura, T.; Wainai, T.; Mashimo, K.; Matsumoto, T. *Chem. Physics Lipids* **1977**, *19*, 169–178.
(13) Tsuda, M.; Parish, E. J.; Schroepfer, G., Jr. *J. Org. Chem.* **1979**, *44*, 1282–1289.

Scheme II



methylcholest-8(14)-en-3β-ol (**9c**),[2b] thus confirming not only the gross structure of the side chain but also the configuration of C-24 as *R*.[2]

Assignments of the side-chain carbons C-20 → C-28 in the ^{13}C NMR spectrum were based upon analogy to the known values for 24(*R*)-methyl-5α-cholestan-3β-ol (**5c**)[14] and 24(*R*)-methylcholest-5-en-3β-ol (**6c**).[14] These were in good agreement and supported the proposed side-chain structure. With the confirmation of the side chain, the other carbon and sites of unsaturation must be within the nucleus. The appearance of two proton signals at 5.073 and 4.631 ppm indicated that there was an exocyclic methylene, while the absence of any other olefinic protons indicated a fully substituted double bond which could only be placed in the $\Delta^{8(9)}$ or $\Delta^{8(14)}$ positions.

Comparison of the methylene protons and the 3α-proton signals of synthetic 4-methylenecholestan-3β-ol (**11a**) and conicasterol (**12c**) showed (Table II) them to be identical, strongly suggesting a similar A ring structure. Use of the relaxation reagent Gd(fod)$_3$ indicated that the *Z* proton was located at 5.073 ppm by its line broadening.[15]

Careful oxidation[16] (Scheme II) of conicasterol (**12c**) gave the enone (**16c**) with a UV maximum at 236 nm (cyclohexane), thus confirming the presence of the exocyclic methylene group at C-4. Hydrogenation of **12c** from the α side also supported this notion by the downfield shift of the C-19 methyl proton ($\Delta\delta$ = 0.152 ppm) caused by the formation of the 4β-methyl group in the reduction product 4β,24(*R*)-dimethyl-5α-cholest-8(14)-en-3β-ol (**17c**).

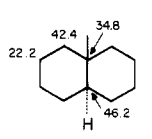
Placement of the tetrasubstituted double bond was based upon comparisons to the known spectral properties of other $\Delta^{8(14)}$ sterols. The mass spectrum displayed loss of the side chain and two carbons (*m/z* 258) which is indicative[2] of an 8(14) double bond. This information, however, was not very conclusive due to the very weak intensity (3.0%) of this peak. Carbon-13 NMR data were more convincing (Table III). The quaternary sp^2 carbons and angular methyl chemical shifts fit very well with the known shifts of 5α-cholest-8(14)-en-3β-ol (**7a**)[13] as opposed to the other possibility, cholest-8(9)-en-3β-ol (**8a**).[13] These data along with the calculated angular methyl proton chemical shifts for **12c** (C-18, δ 0.851; C-19, δ 0.603) confirmed the structure for conicasterol (**12c**) as 4-methylene-24(*R*)-methylcholest-6(14)-en-3β-ol.

Table III. ^{13}C NMR Assignments[a] of Selected Compounds

carbon	8a[13]	7a[13]	6c[14]	5c[14]	12c
1	35.1	36.5	37.34	37.07	34.55
2	31.5	31.5	31.76	31.58	33.16
3	70.9	71.0	71.87	71.40	73.37
4	38.2	38.2	42.40	38.28	153.11
5	40.7	44.2	140.75	44.92	49.49+
6	25.4	28.9	121.72	28.79	27.05
7	27.1	29.6	31.99	32.14	29.37
8	128.0	126.1	31.99	31.57	125.69
9	134.8	49.2	50.24	54.43	49.28+
10	35.6	36.7	36.58	35.57	40.00
11	22.7	19.9	21.16	21.30	20.45*
12	36.9	37.2	39.86	40.10	37.38
13	42.0	42.6	42.40	42.64	42.76
14	51.8	142.4	56.85	56.56	142.93
15	23.9	25.7	24.36	24.25	24.68
16	28.7	27.0	28.27	28.27	25.80
17	54.8	56.8	56.21	56.31	56.90
18	11.2	18.2	11.90	12.10	18.22
19	12.9	12.8	19.44	12.35	13.20
20	36.1	34.4	35.96	35.93	36.77
21	18.8	19.0	18.77	18.68	19.10
22	36.1	35.9	33.80	33.74	33.55
23	23.7	23.7	30.37	30.35	30.21
24	39.4	39.5	38.92	38.88	38.95
25	27.9	27.9	32.49	32.45	32.39
26	22.4	22.5	20.26	20.22	20.21*
27	22.7	22.7	18.32	18.78	18.22
28			15.44	15.41	15.39
29					102.82

[a] Assignments marked with an asterisk or a plus can also be reversed.

Complete assignment of the remaining carbons was achieved by analogy to compound **7a** with the exception of the remaining A ring carbons. These carbons were assigned on the basis of correlations to the pertinent bicyclic models 9-methyldecalin (**18**) and β-selinene (**19**).[17]



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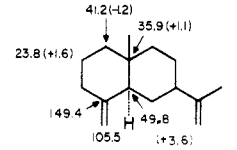

19

In this manner, carbons 1, 2, 5, and 10 were assigned.

Theonellasterol (**12d**) was isolated from the related sponge *T. swinhoei*. This sponge also contained a reasonable amount of conicasterol (**12c**). The presence of this sterol (**12d**) was first detected by low-field ^1H NMR (60 MHz) which indicated methylene signals identical with those of conicasterol (**12c**). Inspection of the high-field ^1H NMR (360 MHz) indicated signals almost identical with those of conicasterol (**12c**) except for the methyl region. The existence of a different side-chain substitution pattern became obvious from the appearance of a triplet at 0.856 ppm, *J* = 7.4 Hz, and the shifting of the C-21, C-26, and C-27 signals. Direct comparison to the known 4α-methyl-24(*S*)-ethylcholest-8(14)-en-3β-ol (**9d**)[2b] confirmed the presence in the side chain of a 24(*S*)-ethyl substituent. This was also supported by the mass spectrum which displayed a parent ion at *m/z* 426 ($C_{30}H_{50}O$) with a subsequent loss of the $C_{10}H_{21}$ side chain to give *m/z* 285.

(14) Wright, J. L. C.; McInnes, A. G.; Shimizu, S.; Smith, D. G.; Walter, J. A.; Idler, D.; Khalil, W. *Can. J. Chem.* **1978**, *56*, 1898–1903.
(15) Crews, P.; Kho-Wiseman, E.; Montana, P. *J. Org. Chem.* **1978**, *43*, 116–120.
(16) Ratcliffe, R.; Rodehorst, R. *J. Org. Chem.* **1970**, *35*, 4000–4002.
(17) Wenkert, E.; Bulkwalter, B. L.; Burfitt, I. R.; Gasic, M. J.; Gottlieb, H. E.; Hagaman, E. W.; Schell, F. M.; Wovkulich, P. M.; Zheleva, A. In "Topics in Carbon-13 NMR spectroscopy"; Levy, G. C., Ed.; Wiley: New York, 1976; Vol. 2, pp 81–124.

Since the rest of the ^1H NMR was virtually superimposable, the nucleus must be identical with that of conicasterol (**12c**). The structure of theonellasterol, therefore, is 4-methylene-24(*S*)-ethylcholest-8(14)-en-3β-ol (**12d**).

Conclusion

To our knowledge conicasterol and theonellasterol represent the first cases of 4-methylene sterols encountered from any source, natural or synthetic. Since these compounds were the major sterols in the marine sponges *Theonella conica* and *Theonella swinhoei*, unaccompanied by conventional sterols, it is likely that they play a special role in cell membrane stabilization.[18] As far as their biosynthesis is concerned, they are likely to be transformation products[2c] of dietary precursors—possibly 4α-methyl- or 4α-hydroxymethyl-substituted sterols.

Experimental Section

General Methods. Analytical TLC was performed on precoated (Analtech, Uniplate) silica gel GF (250 μm) glass plates (10 × 20 cm). The plates were developed in hexane–diethyl ether (1:1) and visualized by treatment with a ceric sulfate solution (25 g in 1 L of 25% sulfuric acid) and charring. Preparative TLC was performed on freshly made $AgNO_3$ plates (20 × 20 cm) developed in hexane–benzene (85:15). For large-scale isolation a medium-pressure LC apparatus was used. The unit employed an FMI RPG-150 pump, a Waters R403 differential refractometer, and various length columns packed with TLC grade silica gel. Hexane–ethyl acetate (10:1) was the usual solvent. For small-scale isolation a Waters high-pressure LC unit (M6000 pump, UK6 injector, R401 differential refractometer) was used with a Whatman ODS-3 reverse-phase column (4.6 mm i.d. × 25 cm) and absolute methanol as elution solvent. Analytical GLC was carried out with a Hewlett-Packard 402A chromatograph on 3% OV-17 on GCQ (Applied Scientific, Inc.) in 4 mm i.d. × 1.5 m, U-shaped, glass columns with a standard 402A flame-ionization detector. The oven temperature was 260 °C with helium used as the carrier gas at a flow of 100 mL/min. ^1H NMR spectra were recorded in $CDCl_3$ on a Varian T-60 (60 MHz) or a Bruker HXS-360 (360 MHZ) spectrometer. Chemical shifts are given in parts per million with Me_4Si as an internal standard; coupling constants are in hertz. ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian FT-80 (20 MHz) instrument. The mass spectra were recorded at 70 eV on a Varian MAT 44 (low resolution) or on a Varian MAT 711 (high resolution) mass spectrometer. Specific rotations were recorded on a Perkin-Elmer 141 polarimeter in chloroform. IR spectra were recorded on a Perkin-Elmer 700A spectrometer in the same solvent. UV spectra were obtained on a Hewlett-Packard 8405A UV/vis spectrophotometer using either methanol or cyclohexane as the solvent. Melting points (uncorrected) were determined on a Thomas-Hoover Unimelt capillary melting point apparatus.

Isolation of Conicasterol (12c). This sterol was essentially the sole component of the sterol fraction of *Theonella conica* (Kieschnick) collected from the Red Sea (Gulf of Eilat). The sample was pure by GLC analysis: mp 142–143 °C; $[\alpha]^{25}_D$ +97°; ^1H NMR and ^{13}C NMR, see text: mass spectrum, *m/z* (relative intensity) 412.3737 (M^+, $C_{29}H_{48}O$, 100), 397 (15), 394 (16), 379 (4), 285 (7), 267 (6), 243 (3).

Isolation of Theonellasterol (12d). This sterol was the major component from the Red Sea sponge *Theonella swinhoei* (Gray) (80%) isolated by reverse-phase high-pressure liquid chromatography on a Whatman OS-2 Magnum 9 column with absolute methanol. The other 20% was conicasterol (**12c**), identified by GC/MS. Theonellasterol (**12d**) gave the following: mp 123–124 °C; $[\alpha]^{25}_D$ +12°; ^1H NMR, see text; mass spectrum, *m/e* (relative intensity) 426.3923 (M^+, $C_{30}H_{50}O$, 100), 411 (16), 408 (61), 393 (8), 285 (10), 267 (14), 258 (4).

4-Methylenecholestan-3β-ol (11a). 3β,4β-Epoxy-4α-methylcholestane (**15a**;[8] 8 mg, 0.02 mmol) was treated with 3.2 mmol of lithium diisopropylamide in ether at room temperature for 24 h in order to open the epoxide ring.[9] Analysis of the reaction mixture showed the presence of many side products. Separation by reverse-phase, high-pressure liquid chromatography yielded 0.5 mg (6.3%) of the desired alcohol (**11a**): ^1H NMR (360 MHz) 5.042 (1, br s, *Z* proton of $=CH_2$), 4.628 (1, d, J = 1.2 Hz, *E* proton of $=CH_2$); mass spectrum, *m/z* (relative intensity) 400.3691 (M^+, $C_{28}H_{48}O$, 89), 385 (24), 382 (65), 367 (20), 287 (33), 95 (100).

Hydrogenation of Conicasterol (12c). To a solution of 10 mg (0.02 mmol) of conicasterol (**12c**) in cyclohexane containing with magnetic stirring bar was added a catalytic amount of PtO_2. The mixture was degassed, placed under a hydrogen atmosphere, and allowed to stir for 3 h. The mixture was filtered through Florisil and gave a quantitative yield of 4β-methyl-24(*R*)-methylcholest-8(14)-en-3β-ol (**17c**): ^1H NMR, see text; mass spectrum, *m/z* (relative intensity) 414.3861 ($C_{29}H_{50}O$, 100), 399 (18), 396 (9), 381 (7), 301 (6), 287 (10), 269 (15).

Oxidation of Conicasterol (12c). Conicasterol (3 mg, 0.007 mmol) was oxidized to the α,β-unsaturated ketone **16c** in nearly quantitative yield by employing the methods of Ratcliffe and Rodehorst:[16] ^1HNMR δ 5.823 (1, t, J = 2.1 Hz, *Z* proton of $=CH_2$), 5.074 (1, t, J = 2.1 Hz, *E* proton of $=CH_2$); UV λ_{max} (cyclohexane) 236 nm, ϵ = 1,845;[19] mass spectrum, *m/z* (relative intensity) 410 (M^+, $C_{29}H_{46}O$, 27), 395 (7), 55 (100).

Gd(fod)$_3$ Experiment on Conicasterol (12c). Conicasterol (**12c**, 1 mg) was placed in 0.5 mL of $CDCl_3$ in an NMR tube, and to this solution was added portions of a 1 mg/mL solution of Gd(fod)$_3$ in $CDCl_3$. The addition of 25 μL of this solution was sufficient to show some line broadening of the signals at 4.022, 5.073, and 0.588 ppm. Addition of 50 μL of the above solution caused distinct broadening of the above signals, with the signal at 4.022 ppm disappearing and the signal at 5.073 ppm appearing as a broad bump. This indicated that the (*Z*)-methylene proton was at 5.073 ppm and the C-19 methyl signal was located at 0.588 ppm.

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Registry No. **11a**, 76758-17-3; **12c**, 76758-18-4; **12d**, 76758-19-5; **15a**, 58714-24-2; **16c**, 76758-20-8; **17c**, 76758-21-9.

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