Biosynthetic Studies of Marine Lipids. 24.[1] Experimental Demonstration of an Unprecedented Cyclopropane → Cyclopropane Rearrangement in the Biosynthesis of the Sponge Sterol Petrosterol

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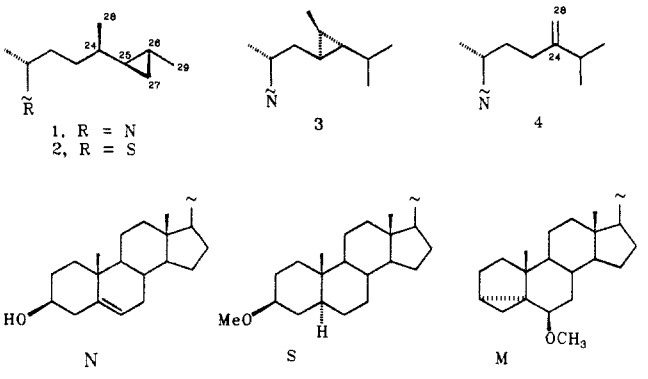
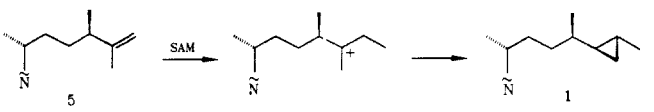
Abstract: The marine sterol petrosterol (**1**) is shown to be biosynthesized from 24-methylenecholesterol (**4**) via an unprecedented cyclopropane → cyclopropane rearrangement presumably involving a protonated species of dihydrocalysterol (**3**). Such rearrangement was confirmed by feeding [28-^{14}C]-24-methylenecholesterol (**4**) to the sponge *Cribrocalina vasculum* and demonstrating that the ^{14}C label, originally at C-28 in **4**, has migrated to C-24 in **1**. Acid-catalyzed cyclopropane ring opening of **1**—a key step in the degradation scheme for locating the label—was studied in detail and shown to be accompanied by an unexpected rearrangement process.

Petrosterol (**1**)[2,3] is an important member of an intriguing group of marine sterols with a cyclopropane ring in the side chain. It was first isolated from the Mediterranean sponge *Petrosia ficiformis*[4] and later from other marine sponges such as *Petrosia hebes*[5] and *Halicondria* sp.[3] Its structure and stereochemistry have been established by X-ray analysis[2] as well as by synthesis.[6,7] Since it is the principal sterol in the sponge, petrosterol is believed to assume cholesterol's role in the cell membrane.[8,9]



Biosynthetically, it seemed plausible that petrosterol is biosynthesized by *S*-adenosylmethionine (SAM) methylation of epicodisterol (**5**, Scheme I).[10] Feeding [26-^{14}C]-radiolabeled epicodisterol (**5**) to *P. ficiformis*, however, resulted in no incorporation of radioactivity, whereas [28-^{14}C]-24-methylenecholesterol (**4**) served as an excellent precursor of petrosterol.[11] In another experiment with the sponge *Calyx nicaeenis*, **4** was also shown to be an excellent precursor of the isomeric cyclopropane sterol dihydrocalysterol (**3**).[12] Prompted by the results of these incorporation experiments and considering the stereochemical relationship[13] between dihydrocalysterol and petrosterol, we suggested[13] that petrosterol arises from **4** via rearrangement of a protonated dihydrocalysterol species, **6** (Scheme II). A crucial feature of this rearrangement is that the ^{14}C label (originally at C-28 in **4**) should end up at C-24 in **1** (Scheme II). It is thus possible to test this proposed biosynthetic scheme experimentally by determining the location of the label in **1**.

Scheme I



Scheme II

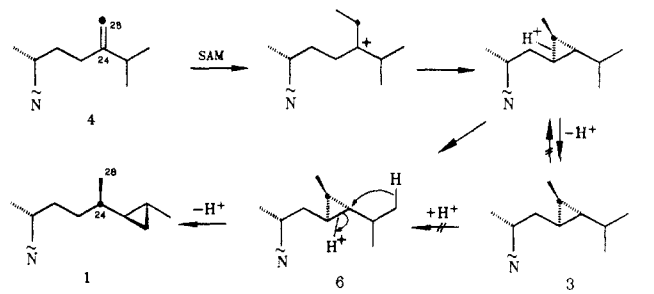


Table I. Results of the Feeding Experiments in *C. vasculum*

	precursor fed[a]			
	[28-^{14}C]-**4**[b]		[3α-^{3}H]-**3**[c]	
sterol recovered	**1**	**3**	**1**	**3**
radioactivity,[d] dpm	463 000	101 000	5000	672 000
wt,[d] mg	12.3	3.0	8.3	2.1
spec act.,[d] dpm/mg	38 000	42 000	600	320 000
incorp,[e] %	26.2[f]	7.1	0.8	89.5

[a] Administered amount of each precursor was 40 μCi. [b] Recovered activity was 4.1% of the administered amount. [c] Recovered activity was 1.7% of the administered amount. [d] After purification to constant specific activity. [e] Based on recovered radioactivity. [f] 58% of the recovered radioactivity was associated with (24*S*)-24-ethylcholesterol (clionasterol). The unchanged precursor (**4**) accounted for only 1.9% of the recovered activity.

(1) Part 23. Hahn, S.; Lam, W.-K.; Wu, I.; Silva, C. J.; Djerassi, C., submitted for publication.
(2) Mattia, C. A.; Mazzarella, L.; Puliti, R.; Sica, D.; Zollo, F. *Tetrahedron Lett.* **1978**, 3953-3954.
(3) Ravi, B. N.; Kokke, W. C. M. C.; Delseth, C.; Djerassi, C. *Tetrahedron Lett.* **1978**, 4379-4380.
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(11) Proudfoot, J. R.; Catalan, C. A. N.; Djerassi, C.; Sica, D.; Sodano, G. *Tetrahedron Lett.* **1986**, *27*, 423-426.
(12) Margot, C.; Catalan, C. A. N.; Proudfoot, J. R.; Sodano, G.; Sica, D.; Djerassi, C. *J. Chem. Soc., Chem. Commun.* **1987**, 1441-1442.
(13) Proudfoot, J. R.; Djerassi, C. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1283-1290.

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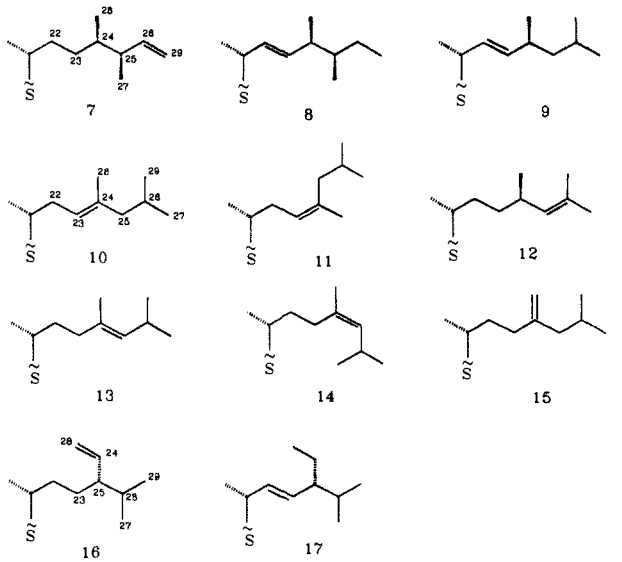
Chart I. Acid-Catalyzed Isomerization Products of Petrosterol (**1**)[a]



[a]The numbering system corresponds to that of the carbons in the side chain of petrosterol (**1**) to illustrate the rearrangements involved.

In this report, we describe the results of feeding [28-^{14}C]-24-methylenecholesterol (**4**) to the sponge *Cribrocalina vasculum* and the degradation of the isolated radiolabeled petrosterol to test the biosynthetic scheme proposed above.

Results

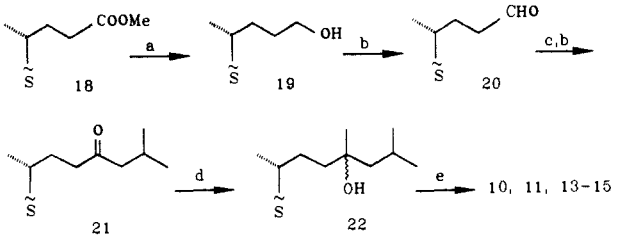
Analysis of the sterol mixture from the Bahamian sponge *C. vasculum* revealed the presence of significant quantities of both petrosterol (**1**) and dihydrocalysterol (**3**), representing 55% and 13% of the total sterols, respectively.[14] Since **3** had previously been found only as a trace sterol in petrosterol-containing sponges,[13] the present finding of significant quantities of both **1** and **3** in the *same* sponge provided a unique opportunity to study the biosynthesis of these presumably biosynthetically related sterols. Analysis of the sterol mixture after administering [28-^{14}C]-24-methylenecholesterol (**4**) to the sponge according to the earlier described procedure[15] showed excellent incorporation of radioactivity in both petrosterol (**1**) and dihydrocalysterol (**3**) (Table I). It is important to note that both sterols exhibited practically the same specific radioactivity (Table I), which is consistent with our proposal that they arise from a single enzyme-bound intermediate, presumably, a protonated dihydrocalysterol (Scheme II).

The nonprotonated form of dihydrocalysterol (**3**) did not serve as a precursor for petrosterol since, when fed to the sponge, [3α-^{3}H]dihydrocalysterol (**3**) was efficiently absorbed but was not metabolized any further (Table I).

The recovery of high amounts of radioactivity in petrosterol (**1**) upon feeding [28-^{14}C]-**4** to *C. vasculum*, prompted us to test our biosynthetic hypothesis by determining the location of the ^{14}C label. The degradation scheme for locating the label relied on acid-catalyzed opening of the cyclopropane ring in **1**. Although this reaction has been studied before[7,16] and then applied[11] to an incubate of lower radioactivity in the Mediterranean sponge *P. ficiformis*, we decided to reexamine it more closely in order to optimize the radiochemical yields of the degradation scheme and to confirm unambiguously the migration of the label from C-28 in **4** to C-24 in **1**.

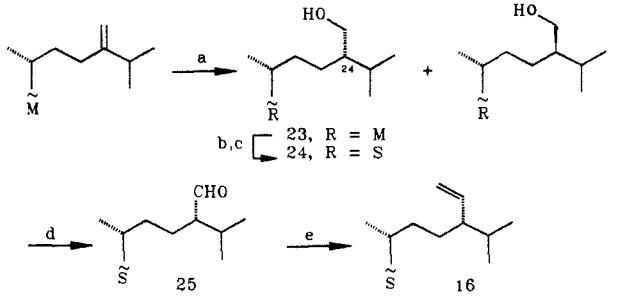
Acid-Catalyzed Cyclopropane Ring Opening of Petrosterol. Reaction of petrosterol (in the form of its 5α-dihydro-3-methyl ether, **2**) with a 5% solution of trifluoroacetic acid in benzene

Scheme III[a]



[a]Reagents: (a) $LiAlH_4$, ether; (b) Pyridinium chlorochromate, CH_2Cl_2; (c) *i*-BuMgBr, THF; (d) MeLi, THF, -78 °C; (e) TsOH (catalytic), dioxane, reflux.

Scheme IV[a]



[a]Reagents: (a) BH_3–THF, then NaOH–H_2O_2; (b) MeOH–dioxane (1:1), TsOH (catalytic), reflux; (c) H_2, PtO_2 (catalytic)/EtOAc; (d) pyridinium chlorochromate, CH_2Cl_2; (e) $Ph_3P^+CH_3I^-$, *n*-BuLi, THF.

resulted in a complex mixture of products, which was extensively fractionated by HPLC to afford 11 alkenes isomeric with petrosterol (Chart I). Five of these alkenes (**7–11**) were similar to those encountered in the acid-catalyzed ring opening of petrosterol acetate.[7] The structures of the new compounds (**12–17**) were determined by spectroscopic and synthetic/degradative methods as follows.

The Δ^{25} alkene **12** was easily identified by the appearance in its NMR spectrum of two vinylic methyl signals (δ 1.585 and 1.668 ppm) quite similar to those of desmosterol. There was also one olefinic proton, which appeared as a doublet (J = 9.3 Hz) further split into a septet (J = 1.4 Hz) by the two terminal methyl groups (C-27 and C-29).

The two Δ^{24} alkenes **13** and **14** both showed one olefinic proton as a broad doublet (δ 4.921 and 4.905 ppm) and one vinylic methyl group (δ 1.576 and 1.635 ppm). The assignment of the *E* and *Z* stereochemistry was based on the fact that the vinylic methyl signals of the *Z* isomers were consistently shifted downfield by 0.059–0.122 ppm compared to those of the corresponding *E* isomers.[7,17] The mass spectra of **13** and **14** were closely similar and exhibited a base peak of m/z 330 resulting from a McLafferty rearrangment, which is diagnostic of Δ^{24} unsaturation.[18,19]

The NMR spectrum of the terminal alkene **15** showed two olefinic protons as unresolved multiplets at δ 4.706 and 4.647 ppm—a pattern quite similar to that of 24-methylenecholesterol (**4**)—as well as the expected base peak at m/z 330 in the mass spectrum. The structure was confirmed by ozonolysis to the ketone **21**, which was also obtained by synthesis (Scheme III).

The structures of **13–15** were confirmed by partial synthesis (Scheme III), which also provided some of the "cold carriers" needed for the degradation experiment described below.

The olefins **16** and **17** were completely unexpected since they possess a rearranged side chain. The NMR spectrum of the vinylic product **16** displayed three secondary methyl signals (C-21, C-27, and C-29) and three olefinic protons exhibiting a splitting pattern indicative of a monosubstituted ethylene. Its structure and stereochemistry were unambiguously established by synthesis from

(14) The remainder of the sterol mixture was composed mostly of the conventional sterol (24*S*)-24-ethylcholesterol (clionasterol).
(15) Catalan, C. A. N.; Thompson, J. E.; Kokke, W. C. M. C.; Djerassi, C. *Tetrahedron* **1985**, *41*, 1073–1084.
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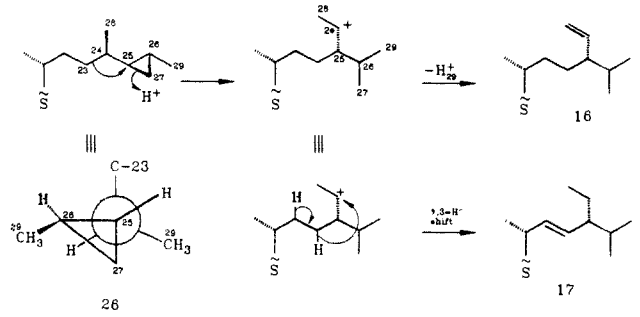
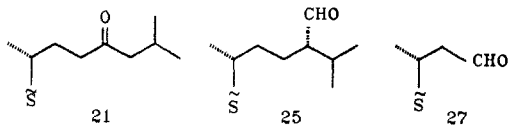
Chart II[a]



[a] The numbering system follows that employed in Chart I.

Table II. Radioactivity of the Ozonolysis Products of the Alkenes **11**, **15**, and **16**



	compound		
	21	**25**[b]	**27**[b]
^{14}C radioactivity,[a] dpm	280	1006	cold[c]
^{3}H radioactivity,[a] dpm	202	723	1157

[a] Calculated from actual cpm values counted after HPLC purification. Tritium radioactivity arose from the [3α-^{3}H]petrosterol added as an internal standard. [b] Since the aldehydes **25** and **27** were not very stable, they were reduced to the corresponding alcohols to allow rigorous purification before counting. [c] cpm value was below background radioactivity (10–25 cpm).

the known[20] 24*S* alcohol **24** (Scheme IV). As shown below, olefin **16** proved to be of crucial importance in locating the radiolabel in the incorporation experiment.

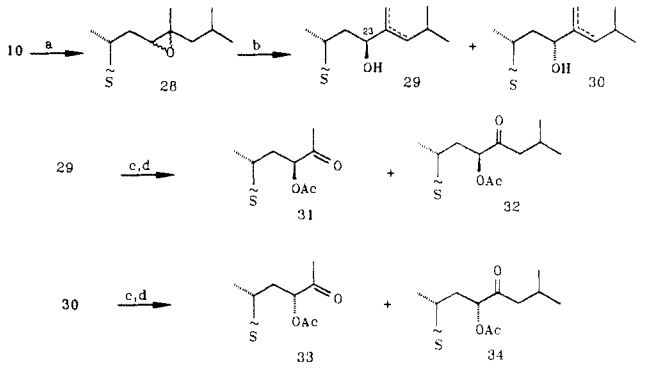
Compound **17** showed one primary (C-28) and three secondary methyl signals (C-21, C-27, and C-29) in addition to two olefinic protons exhibiting a splitting pattern very similar to that of stigmasterol. The stereochemistry at C-24 was established by hydrogenation of the Δ^{22} double bond and comparison of the NMR spectrum of the product with those of authentic samples prepared from the 24*R* and 24*S* sterols, sitosterol and clionasterol.

The mechanism of formation of these rearranged olefins (**16** and **17**) presumably involves C-23 alkyl shift during the cyclopropane ring opening (Chart II). The exclusive formation of the 24*S* isomer is consistent with a concerted migration and ring opening accompanied by inversion of the stereochemistry at C-25. This mechanism is supported by the fact that in the lowest energy conformer[7] of petrosterol (**26**, Chart II), the migrating alkyl group (C-23) is antiperiplanar to the breaking cyclopropane bond (C-25–C-27). It is also in harmony with a similar C-28 methyl shift observed earlier in the acid-catalyzed isomerization of the 24-epimer of petrosterol.[7] The formation of the Δ^{22} compound **17** can be explained (Chart II) by a 1,3-hydride shift (after the alkyl migration), which probably occurs in concert with C-22 proton loss.

Degradation of Radioactive Petrosterol. The ^{14}C-labeled petrosterol isolated from the feeding experiment (415 000 dpm) was mixed with synthetic [3α-^{3}H]petrosterol (300 000 dpm) as an internal standard. The "doubly-labeled" petrosterol ($^{3}H/^{14}C$ ratio = 0.72) was protected as 5α-dihydro-3-methyl ether **2** and subjected to acid-catalyzed ring opening as described above. Ozonolysis of the olefins **15** and **16** yielded the ketone **21** and the aldehyde **25**, respectively, both of which retained the ^{14}C radioactivity (Table II), indicating that the ^{14}C label was not as C-28 of petrosterol and thus a rearrangement of the sidechain carbon skeleton had occurred during the biosynthesis.

(20) Catalan, C. A. N.; Kokke, W. C. M. C.; Duque, C.; Djerassi, C. *J. Org. Chem.* **1983**, *48*, 5207–5214.

Scheme V. Degradation of the Alkene **10**[a]



[a] Reagents: (a) *m*-$ClC_6H_4CO_3H$, CH_2Cl_2, 2 h, 92%; (b) Al(*i*-PrO)$_3$, toluene, reflux, 48 h, 62%; (c) Ac_2O, pyridine, room temperature, 18 h, 95%; (d) O_3, CH_2Cl_2, −78 °C and then Me_2S, 65%.

Table III. Radioactivity of the Ketones **31–34**

	compound			
	31	**32**	**33**	**34**
^{14}C radioactivity,[a] dpm	1470	995	1448	930
^{3}H radioactivity,[a] dpm	1032	667	1038	634

[a] See footnote *a* in Table II.

In order to unambiguously establish that the label is at C-24 as predicted by our proposed biosynthetic scheme (Scheme II), we conducted the following experiments. The olefin **11** was subjected to ozonolysis; the resulting aldehyde **27** (Table II) was devoid of ^{14}C radioactivity, indicating that the label must be at either C-24, C-25, C-26, C-27, or C-29.

Next, the Δ^{23} olefin **10** was degraded as follows (Scheme V).[11] Epoxidation of **10** and base-catalyzed rearrangement[21,22] of the resulting epoxide **28** produced a 1:1 mixture of $\Delta^{24(28)}$ and Δ^{24} (*E* and *Z*) allylic alcohols, **29** and **30** (a total of six isomers). The mixture was separated according to the stereochemistry of C-23 into two fractions, **29** and **30**.[23] Acetylation and ozonolysis of each fraction gave two ketones (**31** and **32** from **29**, and **33** and **34** from **30**). As shown in Table III, as four ketones retained their ^{14}C radioactivity, indicating that the label can only be at C-24 *thus confirming the proposed biosynthetic scheme* (Scheme II).

Discussion

In recent years, a bewildering plethora of new sterols were isolated from the marine environment.[24–26] Of these unusual sterols, cyclopropane- and cyclopropene-containing sterols are unique, with no terrestrial counterparts, thus raising several questions regarding their biological role. We intimated[27] that the cyclopropyl group could be a biosynthetic precursor to allylic methyl groups (e.g., **1** → **35**, Scheme VI) —a process that has

(21) Mori, K.; Sakakibara, M.; Okada, K. *Tetrahedron* **1984**, *40*, 1767–1781.

(22) Terao, S.; Shiraishi, M.; Kato, K. *Synthesis* **1979**, 464–468.

(23) Although the correct assignment of the *R* and *S* stereochemistry at C-23 is of no consequence to our conclusion, it was possible to distinguish between the two 23-epimers on the basis of the following observation. The coupling constants between H-23 and the two protons at C-22 in one set of isomers (**31** and **32**) are quite different (ca. 11–12 and 2–5 Hz), whereas in the opposite epimers (**33** and **34**) the difference is averaged out. Conformational analysis of the rotomers around the C-22–C-23 bond showed that in the case of the 23*S* compounds (**31** and **32**) two of the three possible gauche rotomers are sterically hindered. In the most stable conformer, the dihedral angle between H-23 and both hydrogen at C-22 are close to 180 and 60° thus accounting for the large difference in the coupling constants. In the case of the 23*R* compounds (**33** and **34**), there are two equally stable gauche conformers where the difference between the dihedral angles averages out accounting for the similarity of the coupling constants.

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(25) Djerassi, C. In *Alfred Benzon Symposium 20: Natural Products and Drug Development*; Krogsgaard-Larsen, P., Brøgger Christensen, S., Kofod, H., Eds.; Munksgaard: Copenhagen, 1984; pp 164–176.

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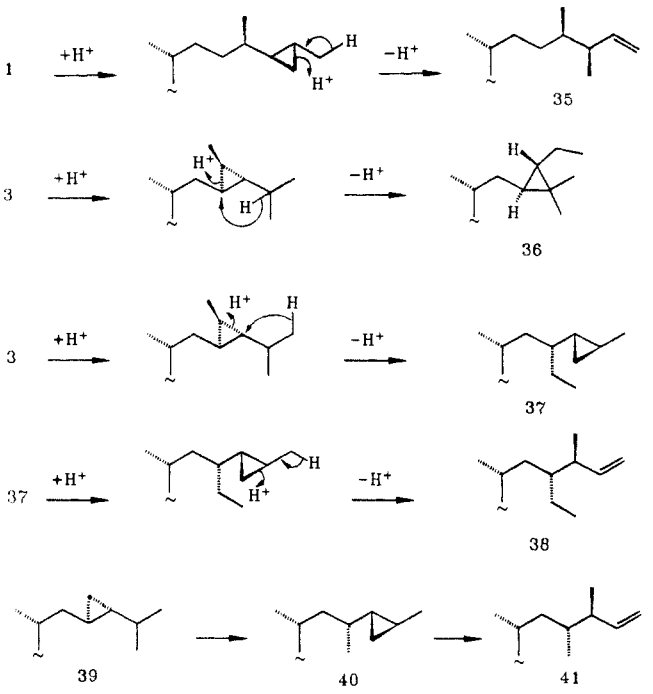
(27) Djerassi, C. *Pure Appl. Chem.* **1981**, *53*, 873–890.

Scheme VI. Possible Role of Cyclopropanes in the Biosynthesis of Marine Sterols



been repeatedly demonstrated by in vitro biomimetic reactions[16,28,29] but so far demonstrated in vivo only in plants in the convesion of cycloeucalenol to obtusifoliol.[30] A notable example of a different cyclopropane intermediate can be found in the biosynthesis of squalene from presqualene pyrophosphate.[31]

In this report we presented experimental support for a different biosynthetic role of a cyclopropane in which its protonated form offers the driving force for the genesis of another cyclopropane (e.g., **6** → **1**, Scheme II). Our findings do not only clarify the biosynthesis of petrosterol (**1**) but may also apply to the biosynthesis of other sterols. Similar rearrangements (Scheme VI) involving protonated cyclopropanes have been proposed[13] to explain the biosynthesis of intriguing sterols such as nicasterol (**36**)[32] and hebesterol[5] (**37**). The existence of the latter was predicted as a missing link in the biosynthesis of the unusual norergostane sterol ficisterol (**38**).[33,34] Similarly, the lower homologue of ficisterol, norficisterol (**41**),[33,34] might be formed by an analogous rearrangement of 23,24-methylenecholesterol (**39**)—a lower homologue of dihydrocalysterol (**3**)—via the yet unknown "norhebesterol" (**40**).

Experimental Section

General Procedures. High-performance liquid chromatography (HPLC) was carried out on a Waters Associates HPLC system (M 6000 pump, UK6 injector, R403 differential refractometer) using two Altex Ultrasphere ODS 5-μm columns (25 cm × 10 mm, i.d.) connected in series. Gas–liquid chromatographic analysis was performed on a Carlo Erba Series 4160 gas chromatograph equipped with 25 m × 0.32 mm SE-54 capillary column and a flame ionization detector. ^{1}H NMR spectra were recorded on a Varian XL-400 spectrometer operating at 400 MHz and were referenced to residual solvent resonance ($CHCl_3$ at 7.260 ppm). Low-resolution mass spectra were recorded on a Ribermag R-10-10 quadrupole instrument. High-resolution mass spectra were recorded on an AEI MS-30 instrument at the Univerisity of Minnesota mass spectrometry service laboratory. Radioactivity was determined with a Beckmann LS 7500 liquid scintillation counter. [^{14}C]methyl iodide and sodium [^{3}H]borohydride were purchased from ICN Biomedicals Inc. THF was dried by distillation over sodium wire.

(24*R*,25*R*,26*R*)-3β-Methoxy-24,26-dimethyl-25,27-cyclo-5α-cholestane (2). A mixture of petrosterol (**1**; 205 mg, 0.5 mmol), Pt_2O (10 mg), and EtOAc (20 mL) was stirred under hydrogen for 20–60 min. (It is important to monitor the progress of the reaction since excessive reaction times lead to opening of the cyclopropane ring.) As soon as the starting material disappeared, the mixture was filtered and the solvent evaporated under reduced pressure. The residue (mainly 5α-petrostanol[5,35]) was then dissolved in dry THF (20 mL), KH (25 mg, 0.62 mmol) was added, and the mixture was stirred for 1 h under argon. Methyl iodide (0.5 mL) was then added and stirring continued for an additional hour. The reaction mixture was carefully quenched with water (1 mL) and evaporated to near dryness. The residue was partitioned between water and ethyl acetate and the organic layer was washed with water, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by chromatography on silica gel (hexane–EtOAc, 95:5) and then fractionated by HPLC (MeCN–EtOAc, 6:4) to give **2** (152 mg, 71%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.332 (s, 3 H, MeO), 3.115 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.997 (d, J = 5.6 Hz, 3 H, C-29), 0.897 (d, J = 6.7 Hz, 3 H, C-21), 0.880 (d, J = 6.7 Hz, 3 H, C-28), 0.785 (s, 3 H, C-19), 0.643 (s, 3 H, C-18), 0.437, 0.594, and 0.05–0.15 (m, 4 H, cyclopropane protons); mass spectrum, m/z (rel intensity) 428 (69, M^+), 413 (6), 402 (8), 381 (7), 371 (10), 330 (23), 315 (20), 287 (100), 274 (9), 255 (15); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.4019.

(24*R*,25*R*)-3β-Methoxy-24,26-dimethyl-5α-cholestane was obtained as a byproduct in the above reaction (22 mg, 11%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.338 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.891 (d, J = 6.6 Hz, 3 H, C-21), 0.855 (t, J = 7.2 Hz, 3 H, C-29), 0.786 (s, 3 H, C-19), 0.744 (d, J = 6.7 Hz, 3 H, C-27/28), 0.720 (d, J = 6.7 Hz, 3 H, C-28/27), 0.641 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 430 (100, M^+), 415 (23), 398 (5), 383 (14), 373 (14), 317 (2), 290 (12), 285 (3), 248 (42), 215 (64); exact mass calcd for $C_{30}H_{54}O$ 430.4172, found 430.4171.

Acid-Catalyzed Ring Opening of Petrostanol Methyl Ether. Petrostanol methyl ether (**2**; 206 mg, 0.5 mmol) was heated under reflux for 6 h with a 5% solution of trifluoroacetic acid in dry benzene (25 mL). The solvent was evaporated under reduced pressure, and the residue was fractionated by HPLC (EtOAc–MeOH, 15:85). Early fractions (R_t < 40 min) contained trifluoroacetates (35%), which were not investigated further. The late fractions (containing the alkenes) were reinjected with the same solvent system and the uncontaminated fractions were further fractionated by repeated injection on HPLC using EtOAc–MeCN, 4:6, as eluent. Mixtures that were still not resolved at this stage were finally fractionated by argentic HPLC (40 mM $AgNO_3$ in EtOAc–MeOH, 1:9, as the mobile phase). The compounds were freed of $AgNO_3$ by evaporating the solvent and partitioning the residue between water and hexane. Eleven compounds were isolated and are described below.

(24*R*,25*R*)-3β-Methoxy-24-methyl-26-methylene-5α-cholestane (7): yield, 24.3 mg (12%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.737 (9-line m, 1 H, C-26), 4.934 (m, 2 H, C-29), 3.336 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.924 (d, J = 6.9 Hz, 3 H, C-27), 0.882 (d, J = 6.4 Hz, 3 H, C-21), 0.785 (d, J = 6.6 Hz, 3 H, C-28), 0.784 (s, 3 H, C-19), 0.639 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 428 (100, M^+), 413 (12), 396 (3), 381 (14), 371 (11), 341 (9), 330 (23), 315 (18), 287 (51), 274 (8), 247 (14), 215 (35); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.4020.

(22*E*,24*S*,25*R*)-3β-Methoxy-24,26-dimethyl-5α-cholest-22-ene (8): yield, 3.1 mg (1.5%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.154 (m, J = 15.8, 8.0 Hz, 2 H, C-22 and C-23); 3.336 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.980 (d, J = 6.5 Hz, 3 H, C-21), 0.872 (d, J = 6.9 Hz, 3 H, C-28), 0.840 (t, J = 7.3 Hz, 3 H, C-29), 0.786 (s, 3 H, C-19), 0.786 (d, J = 6.7 Hz, 3 H, C-27), 0.654 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 428 (84, M^+), 416 (12), 413 (5), 402 (11), 339 (20), 330 (12), 316 (64), 301 (19), 287 (100), 274 (9), 257 (58), 215 (27); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.4040.

(22*E*,24*S*)-3β-Methoxy-24-methyl-26,26-dimethyl-27-nor-5α-cholest-22-ene (9): yield, 2.5 mg (1.2%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.158 (dd, J = 15.2, 8.0 Hz, 1 H, C-23/22), 5.075 (dd, J = 15.2, 7.6 Hz, 1 H, C-22/23), 3.336 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 2.081 (m, 1 H, C-24), 0.976 (d, J = 6.6 Hz, 3 H, C-21), 0.896 (d, J = 6.6 Hz, 3 H, C-28), 0.842 (d, J = 6.7 Hz, 3 H, C-29/27), 0.822 (d, J = 6.5 Hz, 3 H, C-27/29), 0.787 (s, 3 H, C-19), 0.651 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 428 (100, M^+), 413 (6), 396

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(3), 381 (3), 330 (7), 316 (26), 301 (7), 287 (22), 273 (7), 257 (14); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.4014.

(*E*)-3β-Methoxy-24-methyl-26,26-dimethyl-27-nor-5α-cholest-23-ene (10): yield, 16.3 mg (8.2%); ^1H NMR (400 MHz, $CDCl_3$) δ 5.088 (br, t, J = 7.6 Hz, 1 H, C-23), 3.336 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 1.537 (br s, 3 H, C-28), 0.883 (d, J = 6.6 Hz, 3 H, C-21), 0.835 (d, J = 6.6 Hz, 3 H, C-29/27), 0.833 (d, J = 6.5 Hz, 3 H, C-27/29), 0.784 (s, 3 H, C-19), 0.646 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 428 (7, M^+) 413 (1), 330 (12), 316 (10), 315 (11), 287 (100), 285 (43), 255 (7); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.4011.

(*Z*)-3β-Methoxy-24-methyl-26,26-dimethyl-27-nor-5α-cholest-23-ene (11): yield, 6.1 mg (3.1%); ^1H NMR (400 MHz, $CDCl_3$) δ 5.168 (br t, J = 8.8 Hz, 1 H, C-23), 3.337 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 1.661 (br s, 3 H, C-28), 0.879 (d, J = 6.6 Hz, 3 H, C-21), 0.862 (d, J = 6.6 Hz, 3 H, C-29/27), 0.840 (d, J = 6.6 Hz, 3 H, C-27/29), 0.784 (s, 3 H, C-19), 0.641 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 428 (15, M^+) 413 (7), 330 (7), 316 (10), 315 (13), 287 (100), 285 (50), 255 (5); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.4014.

(24*R*)-3β-Methoxy-24-methyl-26,26-dimethyl-27-nor-5α-cholest-25-ene (12): yield, 3.0 mg (1.5%); ^1H NMR (400 MHz, $CDCl_3$) δ 4.873 (d sept, J = 9.3, 1.5 Hz, 1 H, C-25), 3.336 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H. C-3), 2.201 (m, 1 H, C-24), 1.668 (d, J = 1.5 Hz, 3 H, C-29/27), 1.585 (d, J = 1.5 Hz, 3 H, C-27/29), 0.879 (d, J = 6.6 Hz, 6 H, C-21 and C-28), 0.783 (s, 3 H, C-19), 0.630 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 248 (100, M^+), 413 (9), 381 (6), 371 (3), 330 (19), 315 (41), 287 (58), 274 (13), 255 (13), 215 (16); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.4015.

(*E*)-3β-Methoxy-24-methyl-26,26-dimethyl-27-nor-5α-cholest-24-ene (13): yield, 1.1 mg (0.5%); ^1H NMR (400 MHz, $CDCl_3$) δ 4.921 (br d, J = 9.0 Hz, 1 H, C-25), 3.336 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 2.468 (d sept, J = 9.0, 6.7 Hz, 1 H, C-26), 1.576 (d, J = 1.3 Hz, 3 H, C-28), 0.912 (d, J = 6.6 Hz, 3 H, C-29/27), 0.908 (d, J = 6.6 Hz, 3 H, C-27/29), 0.902 (d, J = 6.6 Hz, 3 H, C-21), 0.785 (s, 3 H, C-19), 0.632 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 428 (17, M^+), 413 (5), 388 (6), 381 (3), 371 (1), 357 (2), 330 (100), 315 (55), 287 (30), 255 (6), 247 (15), 215 (20); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.4019.

(*Z*)-3β-Methoxy-24-methyl-26,26-dimethyl-27-nor-5α-cholest-24-ene (14): yield, 0.5 mg (0.2%); ^1H NMR (400 MHz, $CDCl_3$) δ 4.905 (br d, J = 9.5 Hz, 1 H, C-25), 3.336 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 2.472 (d sept, J = 9.5, 6.5 Hz, 1 H, C-26), 1.634 (d, J = 1.3 Hz, 3 H, C-28), 0.938 (d, J = 6.6 Hz, 3 H, C-21), 0.909 (d, J = 6.6 Hz, 6 H, C-29 and C-27), 0.786 (s, 3 H, C-19), 0.645 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 428 (28, M^+), 413 (6), 381 (4), 371 (4), 330 (100), 315 (45), 287 (40), 255 (6), 247 (12), 215 (20); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.4033.

3β-Methoxy-24-methylene-26,26-dimethyl-27-nor-5α-cholestane (15): yield, 0.5 mg (0.2%); ^1H NMR (400 MHz, $CDCl_3$) δ 4.706 (m, 1 H, C-28), 4.647 (m, 1 H, C-28), 3.338 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.914 (d, J = 6.6 Hz, 3 H, C-21), 0.868 (d, J = 6.6 Hz, 3 H, C-29/27), 0.853 (d, J = 6.6 Hz, 3 H, C-27/29), 0.786 (s, 3 H, C-19), 0.642 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 428 (45, M^+), 413 (12), 381 (5), 330 (100), 315 (39), 287 (73), 274 (5), 255 (8), 215 (15); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.4001.

(24*S*)-3β-Methoxy-5α-stigmast-28-ene (16): yield, 2.4 mg (1.2%); ^1H NMR (400 MHz, $CDCl_3$) δ 5.550 (ddd, J = 17.0, 10.2, 9.0 Hz, 1 H, C-24), 4.980 (dd, J = 10.2, 2.4 Hz, 1 H, C-28), 4.900 (ddd, J = 17.0, 2.3, 0.7 Hz, 1 H, C-28); 3.336 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.895 (d, J = 6.7 Hz, 3 H, C-21), 0.859 (d, J = 6.7 Hz, 3 H, C-27/29), 0.804 (d, J = 6.8 Hz, 3 H, C-29/27), 0.783 (s, 3 H, C-19), 0.630 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 428 (61, M^+), 413 (8), 397 (2), 381 (7), 371 (8), 330 (9), 315 (19), 287 (34), 274 (5), 215 (19), 43 (100); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.4008.

(24*R*)-3β-Methoxy-5α-stigmast-22-ene (17): yield, 0.3 mg (0.1%); ^1H NMR (400 MHz, $CDCl_3$) δ 5.142 (dd, J = 15.1, 8.6 Hz, 1 H, C-22), 5.002 (dd, J = 15.2, 8.9 Hz, 1 H, C-22), 3.338 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 1.002 (d, J = 6.7 Hz, 3 H, C-21), 0.835 (d, J = 6.6 Hz, 3 H, C-27/29), 0.803 (t, J = 7.4 Hz, 3 H, C-28), 0.787 (s, 3 H, C-19), 0.782 (d, J = 6.6 Hz, 3 H, C-29/27), 0.659 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 428 (86, M^+), 413 (16), 396 (100), 381 (29), 357 (13), 354 (17), 329 (18), 303 (14), 287 (28), 275 (12), 255 (24), 213 (16); exact mass calcd for $C_{30}H_{52}O$ 428.4016, found 428.3995.

Synthesis and Dehydration of 3β-Methoxy-24-methyl-26,26-dimethyl-27-nor-5α-cholestan-24-ol (22). 3β-Methoxy-5α-cholan-24-ol (19). To a solution of the ester **18** (404 mg, 1.0 mmol, prepared from the corresponding 3β-OH sterol, the 3β-ol 5α-cholanic acid methyl ester, available from Steraloids Inc., Wilton, NH) in dry ether (20 mL) was added excess $LiAlH_4$ (100 mg). The mixture was stirred under argon for 2 h. Excess $LiAlH_4$ was decomposed by the addition of EtOAc (1 mL) and water (0.5 mL). The mixture was filtered, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by chromatography on silica gel (hexane–EtOAc, 9:1) to yield the pure alcohol **19** (357 mg, 95%): ^1H NMR (400 MHz, $CDCl_3$) δ 3.608 (m, 2 H, C-24), 3.336 (s, 3 H, MeO), 3.118 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.917 (d, J = 6.5 Hz, 3 H, C-21), 0.784 (s, 3 H, C-19), 0.645 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 376 (100, M^+), 361 (16), 344 (10), 329 (15), 319 (14), 262 (10), 248 (35), 215 (54); exact mass calcd for $C_{25}H_{44}O_2$ 376.3341, found 376.3332.

3β-Methoxy-5α-cholan-24-al (20). To a solution of the alcohol **19** (300 mg, 0.79 mmol) in dichloromethane (20 mL) was added pyridinium chlorochromate (200 mg). The mixture was stirred at room temperature for 3 h (TLC monitoring), after which it was filtered through a short column of Florisil. The filtrate was evaporated to give the aldehyde **20** (239 mg, 79%): ^1H NMR (400 MHz, $CDCl_3$) δ 9.762 (t, J = 1.8 Hz, 1 H, C-24), 3.337 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 2.450 and 2.340 (m, 2 H, C-23), 0.906 (d, J = 6.4 Hz, 3 H, C-21), 0.784 (s, 3 H, C-19), 0.644 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 374 (63, M^+), 359 (24), 341 (7), 330 (78), 317 (19), 287 (10), 262 (11), 247 (34), 215 (80); exact mass calcd for $C_{25}H_{42}O_2$ 374.3183, found 374.3174.

3β-Methoxy-26,26-dimethyl-27-nor-5α-cholestan-24-one (21). To the Grignard reagent prepared by reaction of 1-bromo-2-methylpropane (0.55 mL, 5 mmo) with magnesium (115 mg) in THF (5 mL) was added the aldehyde **20** (217 mg, 0.58 mmol) in THF (2 mL) under argon. The mixture was stirred for 1 h after which the reaction was quenched by adding a few drops of water. The mixture was filtered, dried ($MgSO_4$), and evaporated under reduced pressure. The alcohol product was oxidized by pyridinium chlorochromate as described above to afford the ketone **21**, which was purified by chromatography over silica gel (hexane–dichloromethane, 1:1) (205 mg, 82%): ^1H NMR (400 MHz, $CDCl_3$) δ 3.335 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 2.1–2.40 (m, 4 H, C-23 and C-25), 0.900 (d, J = 6.6 Hz, 3 H, C-27/28), 0.903 (d, J = 6.6 Hz, 3 H, C-28/27), 0.884 (d, J = 6.6 Hz, 3 H, C-21), 0.781 (s 3 H, C-19), 0.633 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 430 (30, M^+), 398 (16), 383 (7), 373 (11), 330 (21), 315 (13), 299 (11), 287 (37), 215 (35), 108 (39), 85 (76), 57 (100); exact mass calcd for $C_{29}H_{54}O_2$ 430.3808, found 430.3787.

3β-Methoxy-24-methyl-26,26-dimethyl-27-nor-5α-cholestan-24-ol (22). To a stirred solution of the ketone **21** (100 mg, 0.33 mmol) in THF (5 mL) was added MeLi (2.5 mL, 1.5 M solution) at −78 °C under nitrogen. The mixture was allowed to warm to room temperature over a period of 1 h after which it was diluted with water and extracted with ether (3 × 25 mL). The ether layer was washed with water and then brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by chromatography on silica gel using dichloromethane as eluent to afford the alcohol **22** (96 mg, 92%): ^1H NMR (400 MHz, $CDCl_3$) δ 3.335 (s, 3 H, MeO), 3.117 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 1.148 (s, 3 H, C-28), 0.956 (d, J = 6.7 Hz, 3 H, C-29/27), 0.951 (d, J = 6.8 Hz, 3 H, C-27/27), 0.906 (d, J = 6.5 Hz, 3 H, C-21), 0.782 (s, 3 H, C-19), 0.640 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 446 (3, M^+), 445 (4), 428 (43), 413 (15), 397 (35), 389 (31), 371 (22), 357 (16), 346 (6), 339 (16), 330 (73), 315 (60), 299 (14), 287 (53), 257 (66), 215 (36); exact mass calcd for $C_{28}H_{52}O$ (M^+ − 18) 428.4017, found 408.4026.

Dehydration of the Alcohol 22. The alcohol **22** (96 mg, 0.215 mmol) was heated for 5.5 h under reflux with *p*-toluenesulfonic acid (30 mg) in dioxane (5 mL). The solvent was then evaporated under reduced pressure and the residue was purified by chromatography on silica gel (hexane–dichloromethane, 1:1). The mixture of alkenes (82 mg, 89%) was fractionated by HPLC to yield **11**, **10**, **13**, **14**, and **15**; ratio 20:33:30:11:10. Spectroscopic data were identical with those described above.

Ozonolysis of the Steroidal Alkenes. Typical Procedure. To a cooled (−78 °C) solution of the alkene (1–2 mg) in dichloromethane (1–2 mL) containing 1% pyridine was added a slight excess of dichloromethane saturated with ozone at −78 °C following the procedure of Rubin.[36] After 2–5 min, dimethyl sulfide (0.2 mL) was added and the solution was allowed to warm to room temperature. The solvent was evaporated and the residue was purified by HPLC. The yields were typically 55–65%.

3β-Methoxy-5α-cholan-23-al (27) was obtained by ozonolysis of the alkene **11** following the general procedure described above: ^1H NMR (400 MHz, $CDCl_3$) δ 9.733 (dd, J = 1.4, 3.5 Hz, 1 H, C-23), 3.326 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H C-3), 2.142 (ddd, J = 15.9, 1.2, 3.3 Hz, 1 H, C-22), 2.142 (ddd, J = 15.8, 14, 9.3 Hz, 1 H, C-22),

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0.992 (d, J = 6.5 Hz, 3 H, C-21), 0.778 (s, 3 H, C-19), 0.685 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 360 (15, M^+), 345 (7), 316 (100), 301 (13), 287 (3), 247 (10), 230 (4), 215 (27); exact mass calcd for $C_{24}H_{40}O_2$ 360.3027, found 360.3023.

3β-Methoxy-5α-cholan-23-ol was obtained by $NaBH_4$ reduction of **27**: ^1H NMR (400 MHz, $CDCl_3$) δ 3.709 and 3.637 (m, 2 H, C-23), 3.338 (s, 3 H, MeO), 3.119 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.936 (d, J = 6.5 Hz, 3 H, C-21), 0.784 (s, 3 H, C-19), 0.656 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 362 (100, M^+), 247 (18), 330 (9), 315 (16), 305 (16), 248 (23), 230 (6), 215 (48); exact mass calcd for $C_{24}H_{42}O_2$ 362.3182, found 362.3183.

Synthesis and Ozonolysis Products of (24*S*)-3β-Methoxy-5α-stigmast-28-ene (16). (24*S*)-24-Hydroxymethyl-3β-methoxy-5α-cholestane (24) was obtained by refluxing the methyl ether **23**[20] (215 mg, 0.5 mmol) with dioxane–methanol (1:1, 40 mL) in the presence of a catalytic amount of *p*-toluenesulfonic acid (10 mg) for 1 h (TLC monitoring). The resulting Δ^5-3β-MeO alcohol was hydrogenated on PtO_2 in ethyl acetate as described before; overall yield 182 mg (82%): ^1H NMR (400 MHz, $CDCl_3$) δ 3.568 (10-line m, 1 H, C-28), 3.338 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.916 (d, J = 6.6 Hz, 3 H, C-21), 0.893 (d, J = 6.9 Hz, 3 H, C-27/26), 0.882 (d, J = 6.9 Hz, 3 H, C-26/27), 0.785 (s, 3 H, C-19), 0.638 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 432 (100, M^+), 417 (9), 400 (15), 385 (15), 375 (11), 292 (6), 287 (10), 262 (4), 247 (20), 230 (3), 215 (43); exact mass calcd for $C_{29}H_{52}O_2$ 432.3965, found 432.3979.

The same alcohol **24** was obtained by $NaBH_4$ reduction of the aldehyde **25** obtained by ozonolysis of **16**. The spectral data of the 24*R* isomer are given for comparison: ^1H NMR (400 MHz, $CDCl_3$) δ 3.577 (br 3-line m, 1 H, C-28), 3.337 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.908 (d, J = 6.6 Hz, 3 H, C-21), 0.902 (d, J = 6.9 Hz, 3 H, C-26/27), 0.876 (d, J = 6.9 Hz, 3 H, C-27/26), 0.785 (s, 3 H, C-19), 0.643 (s, 3 H, C-18); mass spectrum, similar to that of **24**.

(24*S*)-3β-Methoxy-5α-ergostan-28-al (25) was obtained by pyridinium chlorochromate oxidation of **24** as described before.[20] The same compound was also obtained by ozonolysis of **16** as described above: ^1H NMR (400 MHz, $CDCl_3$) δ 9.603 (d, J = 3.3 Hz, 1 H, C-28), 3.338 (s, 3 H, MeO), 3.118 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.921 (d, J = 6.7 Hz, 3 H, C-21), 0.964 (d, J = 6.5 Hz, 3 H, C-27/26), 0.955 (d, J = 6.7 Hz, 3 H, C-26/27), 0.783 (s, 3 H, C-19), 0.633 (s, 3 H, C-18).

The spectral data for the 24*R* isomer are given for comparison: ^1H NMR (400 MHz, $CDCl_3$) δ 9.567 (d, J = 3.5 Hz, 1 H, C-28), 3.338 (s, 3 H, MeO), 3.118 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 0.913 (d, J = 6.6 Hz, 3 H, C-21), 0.951 (d, J = 6.6 Hz, 6 H, C-26 and C-27), 0.781 (s, 3 H, C-19), 0.635 (s, 3 H, C-18).

(24*S*)-3β-Methoxy-5α-stigmast-28-ene (16) was prepared by Wittig reaction of **25** with methyltriphenylphosphonium iodide according to the published procedure.[20] Spectral data were identical with the material obtained by acid-catalyzed isomerization described above.

Degradation of the (*E*)-Δ[23]-Alkene 10. 23,24-Epoxy-3β-methoxy-24-methyl-26,26-dimethyl-27-nor-5α-cholestane (28). To a solution of the olefin **10** (11 mg, 0.026 mmol) in dichloromethane (2 mL) was added *m*-chloroperbenzoic acid (10 mg). The mixture was stirred at room temperature for 2 h (TLC monitoring) after which it was diluted with dichloromethane and washed with $NaHSO_3$, $NaHCO_3$, and then brine, dried ($MgSO_4$), and evaporated under a stream of nitrogen. The residue was purified by chromatography on silica gel using 5% EtOAc in hexane as eluent to afford the epoxide **28** (a 1:1 mixture of the (23*R*,24*R*) and (23*S*,24*S*) stereoisomers, 10.1 mg, 91%): ^1H NMR (400 MHz, $CDCl_3$) δ 3.337 (s, 3 H, MeO), 3.119 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 2.709 (m, 1 H, C-23), 1.218 and 1.215 (s, 3 H, C-28), 1.053 and 1.004 (d, J = 6.6 Hz, 3 H, C-21), 0.949 and 0.888 (d, J = 6.6 Hz, 3 H, C-29/27), 0.942 and 0.882 (d, J = 6.6 Hz, 3 H, C-27/29), 0.786 (s, 3 H, C-19), 0.665 and 0.667 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 444 (3, M^+), 429 (5), 402 (4), 387 (57), 344 (17), 329 (6), 316 (22), 301 (7), 287 (67), 248 (23), 57 (99), 43 (100); exact mass calcd for $C_{30}H_{52}O_2$ 444.3967, found 446.3976.

Base-Catalyzed Rearrangement of the Epoxide 28. A mixture of the epoxide **28** (10 mg, 0.0225 mmol) and aluminum isopropoxide (100 mg) in dry toluene (2 mL) was heated under reflux for 48 h (TLC monitoring). The mixture was diluted with ethyl acetate (10 mL), washed with 2 N HCl (2 mL) and then water (2 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was filtered through a short silica gel column (ethyl acetate) and the mixture of products was fractionated by HPLC (MeOH–EtOAc, 9:1) into two fractions (**29** and **30**) according to the stereochemistry around C-23, each fraction containing a 1:1 mixture of the $\Delta^{24(28)}$ and Δ^{24} (*E* and *Z*) compounds; mass spectrum, m/z (rel intensity) 444 (5, M^+), 426 (19), 411 (4), 401 (60), 388 (2), 383 (4), 369 (5), 341 (3), 330 (10), 301 (20), 299 (18), 287 (100); exact mass calcd for $C_{30}H_{52}O_2$ 444.3967, found 446.3965.

Acetylation and Ozonolysis of the Alcohols 29 and 30. Each fraction of the alcohols obtained above was acetylated by reaction with acetic anhydride (0.1 mL) in pyridine (0.3 mL) at room temperature overnight. The mixture was diluted with ethyl acetate (10 mL) and washed with water, 5% $CuSO_4$ solution, and then brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by chromatography on a short silica gel column (hexane–EtOAc, 95:5). Ozonolysis of each of the two acetates prepared above gave two ketones, which were separated by HPLC (methanol): **31** and **32** from **29** and **33** and **34** from **30**.

(23*S*)-23-Acetoxy-3β-methoxy-26,27-dinor-5α-cholestan-24-one (31): ^1H NMR (400 MHz, $CDCl_3$) δ 5.041 (dd, J = 11.6, 2.0 Hz, 1 H, C-23), 3.339 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 2.153 (s, 3 H, OAc/C-25), 2.145 (s, 3 H, C-25/OAc), 0.966 (d, J = 6.4 Hz, 3 H, C-21), 0.788 (s, 3 H, C-19), 0.659 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 446 (0.5, M^+), 414 (1), 404 (2), 386 (2), 330 (3), 311 (2), 299 (9), 215 (13), 287 (19), 255 (2), 116 (64), 43 (100); exact mass calcd for $C_{28}H_{46}O_4$ 446.3396, found 446.3397.

(23*S*)-23-Acetoxy-3β-methoxy-26,26-dimethyl-27-nor-5α-cholestan-24-one (32): ^1H NMR (400 MHz, $CDCl_3$) δ 5.012 (dd, J = 11.8, 2.1 Hz, 1 H, C-23), 3.339 (s, 3 H, MeO), 3.120 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 2.144 (s, 3 H, OAc), 0.966 (d, J = 6.4 Hz, 3 H, C-21), 0.923 (d, J = 6.5 Hz, 3 H, C-28/27), 0.902 (d, J = 6.4 Hz, 3 H, C-27/28), 0.788 (s, 3 H, C-19), 0.659 (s, 3 H, C-18); mass spectrum, m/z (rel intensity) 488 (0.2, M^+), 446 (1), 330 (2), 311 (3), 299 (3), 287 (11), 215 (7), 158 (100), 116 (29), 85 (37), 57 (29), 43 (60); exact mass calcd for $C_{31}H_{52}O_4$ 488.3861, found 488.3860.

(23*R*)-23-Acetoxy-3β-methoxy-26,27-dinor-5α-cholestan-24-one (33): ^1H NMR (400 MHz, $CDCl_3$) δ 4.974 (t, J = 6.6 Hz, 1 H, C-23), 3.337 (s, 3 H, MeO), 3.117 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 2.150 (s, 3 H, OAc/C-25), 2.134 (s, 3 H, C-25/OAc), 1.003 (d, J = 6.3 Hz, 3 H, C-21), 0.782 (s, 3 H, C-19), 0.642 (s, 3 H, C-18); mass spectrum identical with that of **31**.

(23*R*)-23-Acetoxy-3β-methoxy-26,26-dimethyl-27-nor-5α-cholestan-24-one (34): ^1H NMR (400 MHz, $CDCl_3$) δ 4.965 (dd, J = 5.8, 7.2 Hz, 1 H, C-23), 3.337 (s, 3 H, MeO), 3.117 (tt, J = 11.1, 4.7 Hz, 1 H, C-3), 2.125 (s, 3 H, OAc), 0.903 (d, J = 6.4 Hz, 3 H, C-21), 0.920 (d, J = 6.5 Hz, 3 H, C-28/27), 1.003 (d, J = 6.5 Hz, 3 H, C-27/28), 0.782 (s, 3 H, C-19), 0.641 (s, 3 H, C-18); mass spectrum identical with that of **32**.

[3-α[3]H]Petrosterol and [3-α[3]H]dihydrocalysterol were synthesized from petrosterol following the general procedure described earlier[37] and were purified by HPLC (MeCN–EtOAc–MeOH, 22:9:7) before use.

Feeding Experiments and Isolation of Sterols. The precursors were administered to *C. vasculum* collected at a depth of 60 ft near Lucayan Water Way, Grand Bahama, by using the technique described before.[15] The sponge was incubated for 30 days before it was collected for analysis. The air-dried samples were extracted four times with dichloromethane. The extract was concentrated under reduced pressure and was fractionated on an open silica gel column (hexane–ether, 3:1). The sterol fractions (R_f = cholesterol by TLC) were combined and evaporated under reduced pressure. The total sterol mixture was fractionated by HPLC using methanol as solvent. Petrosterol was separated from dihydrocalysterol by HPLC using a mixture of acetonitrile–methanol–ethyl acetate (22:9:7). Overlapping fractions were reinjected as many times as necessary to achieve complete separation. All sterols were purified to constant specific activity by use of at least two different solvent systems.

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