Biosynthetic Studies of Marine Lipids. 37.[1] Enzymatic Desaturation of 24(S)-Methylcholesterol to 23,24-Methylenecholesterol, Norficisterol, and Norhebesterol. Further Evidence for a Unified Biosynthesis of Marine Sterols with Unique Side Chains

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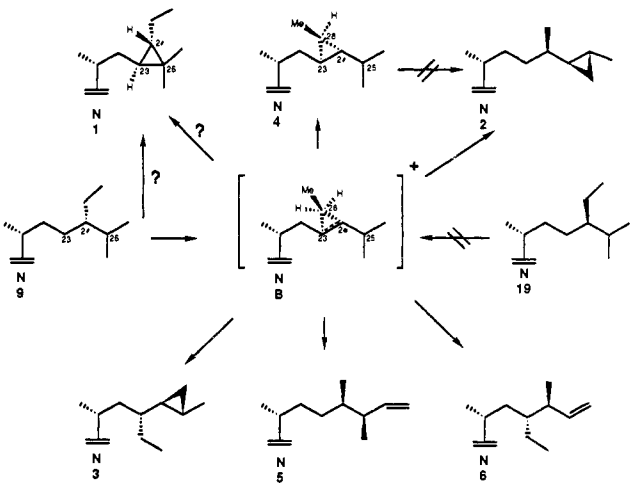
Abstract: The enzymatic desaturation of 24(S)-methylcholesterol to 23,24-methylenecholesterol, 29-norhebesterol, and norficisterol was demonstrated in the sponge, *Petrosia ficiformis*, by feeding the [3-^{3}H]-labeled analogue. [3-^{3}H]-24(R)-Methylcholesterol and [3-^{3}H]-24(R)-ethylcholesterol were not metabolized, though comparable amounts of each of the three precursors were isolated from the sponge. [3-^{3}H]Cholesterol was converted to 24-methylenecholesterol and clionasterol, presumably by its desaturation to desmosterol and subsequent biomethylation. The structure and stereochemistry of 29-norhebesterol was proven by partial synthesis. The possible role of a similar enzymatic process in the biosynthesis of cyclopropene-containing sterols is also considered.

Introduction

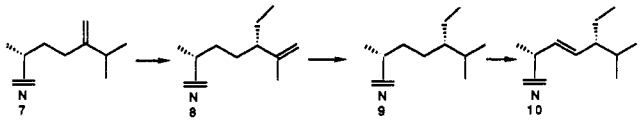
Marine sponges of the order Haplosclerida[2] contain an apparently disparate ensemble of structurally unusual C_{29} sterols with cyclopropane-containing side chains[3] (**1**–**4**) and acyclic olefinic side chains (**5**,[4] **6**,[5,6c]) of a type that has hitherto never been observed in terrestrial organisms. Formally, the latter can be considered to be the products of the acid-catalyzed ring opening of their isomeric cyclopropanes.[6] Based in part on our demonstration that all of the novel side chains possess the same absolute configuration at the relevant centers, we proposed that this set of sterol side chains was biosynthesized by a highly stereospecific rearrangement of a protonated cyclopropane (Scheme I).[7] This hypothesis was substantiated by the degradation of biosynthetically radiolabeled petrosterol (**2**).[8] In a related experiment we showed that dihydrocalysterol (**4**) was not converted to petrosterol (**2**).[8a] While these experiments established the operation of a highly stereospecific rearrangement in the biosynthesis of petrosterol (**2**) as well as the common origin of petrosterol (**2**) and dihydrocalysterol (**4**), it provided no insight into the immediate precursor of the proposed protonated cyclopropane.

Experiments with a cell-free extract of the marine sponge *Cribrochalina vasculum* showed the product of the biomethylation of 24-methylenecholesterol (**7**) to be clerosterol (**8**), rather than the predicted cyclopropanes (**2** and **4**).[9] In green algae, 24-methylenecholesterol (**7**) is biomethylated to clerosterol (**8**), which is reduced to clionasterol (**9**) and then desaturated to poriferastrol (**10**) (Scheme II).[10] The same sequence has been shown to occur in two sponges of the order Haplosclerida, except that sponges are, ironically (porifera = sponge), unable to introduce the Δ^{22} double bond that converts clionasterol (**9**) to poriferasterol (**10**).[1] The inability to introduce the Δ^{22} double bond is common to all sponges studied, except those of the order Halichondrida.[1] In yeast, the Δ^{22}-desaturase has been proposed to be a cytochrome P-450 enzyme and to produce no isolable intermediate.[11] A 23-hydroxy (**11**) sterol was isolated from a yeast mutant incapable of introducing the Δ^{22} double bond, suggesting that it may be an intermediate in the desaturation process.[12] Experiments in our laboratory with yeast microsomes and radiolabeled 23-hydroxy (**11**) sterols, however, showed that the putative 23-hydroxy (**11**) precursors were not converted to the Δ^{22} compounds, while various sterols with saturated side chains were desaturated, suggesting that the Δ^{22} desaturation is not a 2-step hydroxylation/dehydration process.[13] This led to our proposal that the Δ^{22} desaturation

Scheme I. Unified Biosynthesis of C_{29} Sterols Found in Haplosclerid Sponges



Scheme II. Biosynthesis of Poriferasterol (**10**) in Green Algae



proceeds by a process equivalent to a C-23 hydride removal mediated by a cytochrome P-450 to yield the C-23 carbonium

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(1) Part 36: Silva, C. J.; Djerassi, C. *Comp. Biochem. Physiol. B.*, in press.
(2) van Soest, R. W. M. *Stud. Fauna Curacao Other Caribb. Isl.* **1980**, *62*, 1–177.
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(8) (a) Doss, G. A.; Proudfoot, J. R.; Silva, C. J.; Djerassi, C. *J. Am. Chem. Soc.* **1990**, *112*, 305–310. (b) Proudfoot, J. R.; Catalan, C. A. N.; Djerassi, C.; Sica, D.; Sodano, G. *Tetrahedron Lett.* **1986**, *27*, 423–426.
(9) Giner, J.-L.; Djerassi, C. *Tetrahedron Lett.* **1990**, *31*, 5421–5424.

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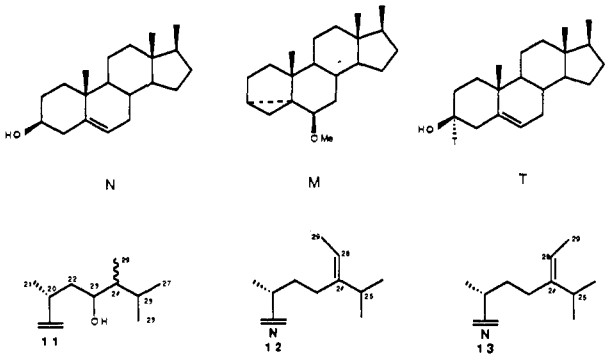
ion, followed by C-22 proton abstraction to yield the Δ^{22} double bond.[13] The 23-hydroxy (**11**) sterol, isolated from the yeast mutant, is consistent with the trapping of this C-23 carbonium ion by a hydroxyl group.[13]



These considerations led us to try feeding the sponge *Petrosia ficiformis* radiolabeled clionasterol (**9**), a C_{29} sterol with a saturated side chain, whereupon we discovered that **9** was efficiently desaturated to the predicted[7] sterols petrosterol (**2**), dihydrocalysterol (**4**), ficisterol (**6**), and 26-dehydroaplysterol (**5**).[14] Each had similar specific activities, which indicates a common origin for these sterols. Fucosterol (**12**) was also radiolabeled, but it had a lower specific activity. This may be due to its biosynthesis by a different process or dilution by dietary fucosterol (**12**). The isofucosterol (**13**) isolated from this experiment was not radioactive. The biosynthesis of these (**2**, **4**–**6**, **12**) sterols is consistent with the action of an errant Δ^{22}-desaturase which instead functions efficiently as a cyclopropanase.[14]

Though we have no experimental evidence for the nature of the enzyme responsible for this transformation, iron-dependent enzymes have been shown to be involved, via an oxidative cyclization, in the biosynthesis of clavaminic acid,[15] isopenicillin N,[16] and deacetoxycephalosporin C.[16] These enzymes are not cytochrome P-450s and have not been suggested to involve carbonium ions.[15,16] In addition to these examples, the biosyntheses of biotin,[17] lipoic acid,[17] and coronamic acid[18] have features that are consistent with an oxidative cyclization, though this has not been shown experimentally. The biosynthesis of isopenicillin N and deacetoxycephalosporin C is proposed to occur via a metal-bound intermediate[16] that can homolytically cleave to yield a free radical.[16] In the errant Δ^{22}-desaturase, a heterolytic cleavage of an analogous metal-bound intermediate could account for the formation of the C-23 carbonium ion whose rearrangement leads to the biosynthesis of these C_{29} (**2**, **4**–**6**) sterols.

Sponges of the order Haplosclerida possess a homologous set of C_{28} sterols—23,24-methylenecholesterol (**14**)[19] and norficisterol (**16**)[5]—whose biosynthesis was proposed to originate from the analogous C_{28} protonated cyclopropane (Scheme III).[7] It is likely that these sterols are also the products of such an errant desaturase. Given the established stereochemistry of these novel C_{28} (**14** and **16**) and C_{29} (**1**–**6**) sponge sterols, their biosynthetic precursors are derived from 24-methyl (**17**) and 24-ethyl (**9**) sterols with the

Scheme III. Unified Biosynthesis of C_{28} Sterols Found in Haplosclerid Sponges

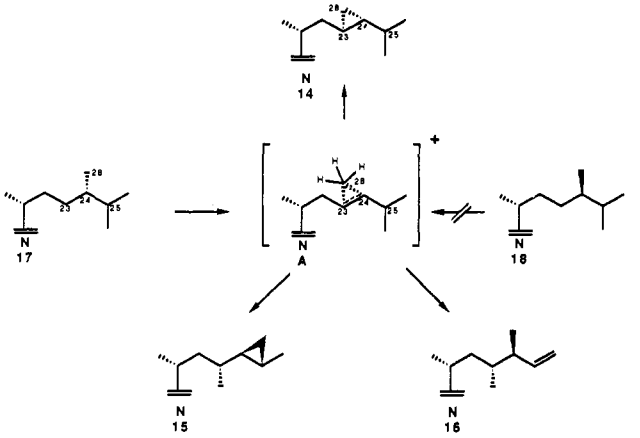


Table I. Results of Feeding Experiments with *Petrosia ficiformis*[a]

	recovered radioactivity (dpm), % recovered, specific activity (dpm/mg); precursors			
sterols	T 20	T 21	T 22	T 23
14			6.8×10^4, 0.15, 8.5×10^5	
15			3.1×10^4, 0.07, 9.6×10^5	
16			7.9×10^4, 0.18, 8.7×10^5	
7				8.7×10^4, 0.20, 8.7×10^5
9				1.0×10^5, 0.22, 4.1×10^4
recd precursor	2.7×10^6, 6.1[a,b]	3.1×10^6, 7.0[a,b]	2.2×10^6, 3.4, 2.2×10^6	1.5×10^6, 3.4[a]

[a] The specific activity was not calculated. [b] This was the only radioactivity recovered. [c] 4.4×10^7 dpm of each precursor fed.

24β stereochemistry. There is no a priori reason that 24-methyl (**18**) and 24-ethyl (**19**) sterols with the 24α stereochemistry might not be converted, in an analogous fashion, to the epimeric cyclopropanes and olefins, though such sterols have so far not been isolated from these sponges.[3] The Δ^{22} desaturation experiments performed with yeast microsomes showed that the yeast's Δ^{22}-desaturase had a strong preference for a particular (coincidentally β) C-24 stereochemistry.[13] Therefore, we then also tested the substrate specificity of the cyclopropanase from the sponge *Petrosia ficiformis* by feeding it [3-^{3}H]-24(*R*)-ethylcholesterol (**20**), [3-^{3}H]-24(*R*)-methylcholesterol (**21**), [3-^{3}H]-24(*S*)-methylcholesterol (**22**), and [3-^{3}H]cholesterol (**23**).

Results and Discussion

These four radiolabeled substrates were prepared in a manner previously described[20] and fed[21a] to the Mediterranean sponge *Petrosia ficiformis*. After an incubation period of 3 weeks, the sponge sterols were isolated and chromatographed on silica gel, silica gel HPLC, and reverse-phase HPLC. The results of these

(10) Goad, T. W. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; John Wiley and Sons: New York, 1981; Vol. 1, pp 443–480.
(11) Hata, S.; Nishino, T.; Komori, M.; Katsuki, H. *Biochem. Biophys. Res. Commun.* **1981**, *103*, 272–277.
(12) Hata, S.; Nishino, T.; Oda, Y.; Katsuki, H.; Aoyama, Y.; Yoshida, Y. *Tetrahedron Lett.* **1983**, *24*, 4729–4730.
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(14) Giner, J.-L.; Silva, C. J.; Djerassi, C. *J. Am. Chem. Soc.* **1990**, *112*, 9626–9627.
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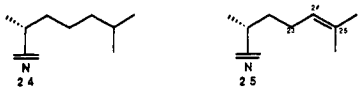
(21) (a) Catalan, C. A. N.; Thompson, J. E.; Kokke, W. C. M. C.; Djerassi, C. *Tetrahedron* **1985**, *50*, 1073–1084. (b) Cho, J.-H.; Thompson, J. E.; Stoilov, I. L.; Djerassi, C. *J. Org. Chem.* **1988**, *53*, 3466–3469.

experiments are summarized in Table I. The experiments in which the sponge was fed [3-^{3}H]-24(*R*)-ethylcholesterol (**20**) and [3-^{3}H]-24(*R*)-methylcholesterol (**21**) yielded radioactivity only in the recovered precursor and, therefore, were not purified beyond the initial reverse-phase HPLC purification. Radioactivity was isolated in 24-methylenecholesterol (**7**) and clionasterol (**9**) in the feeding experiment employing [3-^{3}H]cholesterol (**23**). This is consistent with the desaturation of cholesterol (**24**) to desmosterol[22] (**25**) followed by an initial biomethylation to 24-methylenecholesterol (**7**), which is biomethylated to yield clionasterol (**9**).

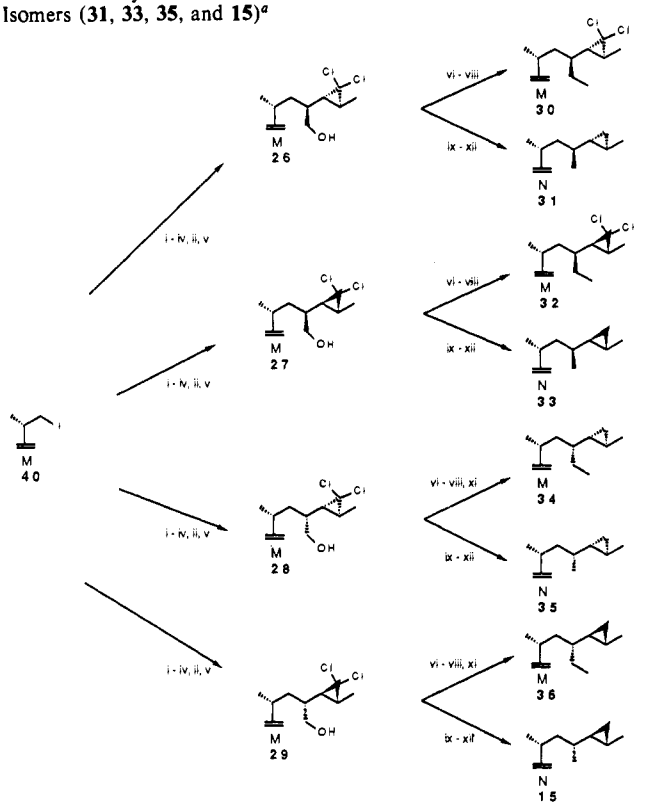


The initial reverse-phase HPLC purification of the sterols from the feeding experiment with [3-^{3}H]-24(*S*)-methylcholesterol (**22**) yielded three radioactive fractions: the recovered precursor (**17**), norficisterol (**16**), and a fraction containing 23,24-methylenecholesterol (**14**) and an unknown sterol. The new sterol was noticed in the fraction containing 23,24-methylenecholesterol (**14**). Repeated fractionation on reverse-phase HPLC yielded two radioactive fractions with similar specific activities: the known 23,24-methylenecholesterol (**14**) and a second unknown sterol. Each of these fractions was chromatographed to constant specific activity on reverse-phase HPLC using three different solvent systems (the recovered precursor was purified on one solvent system). With the exception of recovered precursor, each fraction was hydrogenated and reinjected on reverse-phase HPLC and all of the radioactivity coeluted with the respective hydrogenated sterol.

The unknown sterol had ^{1}H NMR resonances characteristic of a conventional Δ^5 sterol nucleus (e.g., 1.006 ppm, s, 3 H, C-19; 0.682 ppm, s, 3 H, C-18; 3.353 ppm, bm, 1 H, C-6; 3.55–3.45 ppm, bm, 1 H, C-3). The mass spectral molecular ion of the hydrogenated sterol corresponded to the molecular formula $C_{28}H_{48}O$, which is consistent with a saturated nucleus (resulting from the reduction of the Δ^5 double bond, as indicated by the ^{1}H NMR) and a side chain with nine carbons and one site of unsaturation that was unaffected by our hydrogenation conditions. The ^{1}H NMR spectrum displayed no olefinic resonances, other than that of the Δ^5 double bond, but rather signals characteristic of a cyclopropane (0.5–0.4 ppm, bm, 1 H; 0.2–0.1 ppm, bm, 3 H) with general features of cyclopropyl resonances found in the spectra of petrosterol (**2**)[23] and hebesterol (**3**),[6b] albeit with differing chemical shifts. Additional methyl resonances (0.810 ppm, d, J = 6.3, 3 H; 0.897, d, J = 6.7, 3 H; 1.001, d, J = 6.2, 3 H) were consistent with a C-21 doublet, a C-24 and C-23 doublet, and a cyclopropyl methyl doublet. The spectral data and biosynthetic considerations suggested the structure of 29-norhebesterol (**15**), which was predicted to exist[7a] but had so far not been isolated.

The structure and absolute stereochemistry of this compound was confirmed by a synthesis (Scheme IV), which also provided relevant stereochemical information. Four isomers of 24,25-(dichloromethylene)-23-(hydroxymethyl)-3α,5-cyclo-27-nor-5α,6β-cholestane (**26–29**) were prepared. Each of these was converted to a known 24,25-(dichloromethylene)-23-ethyl-3α,5-cyclo-27-nor-5α,6β-cholestane (**30** or **32**)[6b] or 24,25-methylene-23-ethyl-3α,5-cyclo-27-nor-5α,6β-cholestane (**34** or **36**),[6b] which permitted us to assign the absolute stereochemistry at C-23, C-24, and C-25 of the four 24,25-(dichloromethylene)-23-(hydroxymethyl)-3α,5-cyclo-27-nor-5α,6β-cholestanes (**26–29**) by transformation to the corresponding 29-norhebesterol epimers (**31**, **33**, **35**, or **15**). This natural compound had a ^{1}H NMR spectrum that matched, in all respects, that of synthetic (23*R*, 24*R*, 25*S*)-29-norhebesterol (**15**), which is the stereostructure we predicted earlier[7a] on biosynthetic speculations.

Scheme IV. Synthesis and Structure Proof of Four 29-Norhebesterol Isomers (**31**, **33**, **35**, and **15**)[a]



[a] i. Ethyl *trans*-3-pentenoate, 10% HMPA/THF, LDA, −78 °C to room temperature. ii. LAH, ether. iii. Ac_2O, pyridine. iv. $HCCl_3$, 50% NaOH/water, benzyltriethylammonium chloride (cat). v. Purification by preparative TLC. vi. PCC, CH_2Cl_2. vii. CH_2=PPh_3, THF. viii. H_2, 5% Rh/C, iPrOH. ix. TsCl, pyridine. x. $NaBH_4$, DMSO, 90 °C. xi. Li/NH_3, −33 °C. xii. 10% aqueous dioxane, cat. pTsOH, 100 °C.

In each of these four feeding experiments, a significant quantity of radioactivity was recovered in the precursor, indicating that the precursor was incorporated by the sponge; however, the only precursors metabolized were [3-^{3}H]cholesterol (**23**) and [3-^{3}H]-24(*S*)-methylcholesterol (**22**) (Table I). [3-^{3}H]-24(*S*)-Methylcholesterol (**22**) possesses the same C-24 stereochemistry as clionasterol (**9**), which was shown to be the progenitor of the unusual C_{29} sterols (**2**, **4–6**) (Scheme I) in *Petrosia ficiformis*.[14] The results summarized in Table I explain the absence in this sponge of analogous cyclopropane-containing sterols originating from precursors (**20** and **21**) with the 24*R* stereochemistry, since the 24*R* compounds are not desaturated by the sponge. A strong preference for a specific C-24 stereochemistry (coincidentally β) was observed in Δ^{22}-desaturase experiments with yeast microsomes,[13] which supports our assertion that the enzyme from *Petrosia ficiformis* is an errant Δ^{22}-desaturase.[14]

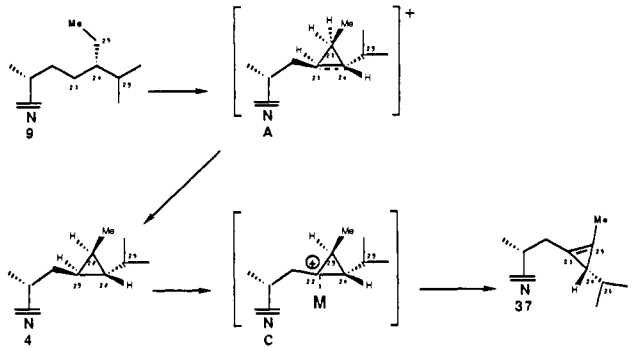
The specific activities of the products isolated from the [3-^{3}H]-24(*S*)-methylcholesterol (**22**) feeding experiment (Table I) are similar. This is consistent with their common origin[7a] from the stereospecific rearrangement of the protonated cyclopropane (A) (Scheme III) in analogy to the biosynthesis of their C_{29} homologues (Scheme I).[14] The specific activity of the products isolated from the [3-^{3}H]cholesterol (**23**) experiment, in contrast, are consistent with a precursor/product relatinship.[1] The encounter of radioactivity in 29-norhebesterol (**15**) is further demonstrated of the unified biosynthesis of these C_{28} and C_{29} sterols. Although the biosynthesis of hebesterol (**3**), which occurs in *Petrosia hebes* and *Cribrochalina vasculum*[24] but not in *Petrosia ficiformis*,[4] has not been proven experimentally, we feel justified

(22) (a) Fagerlund, U. H. M.; Idler, D. R. *Can. J. Biochem. Physiol.* **1961**, *39*, 505–509. (b) Fagerlund, U. H. M.; Idler, D. R. *Can. J. Biochem. Physiol.* **1961**, *39*, 1347–1355.

(23) (a) Sica, D.; Zollo, F. *Tetrahedron Lett.* **1978**, 837–838. (b) Ravi, B. N.; Kokke, W. C. M. C.; Delseth, C.; Djerassi, C. *Tetrahedron Lett.* **1978**, 4379–4380.

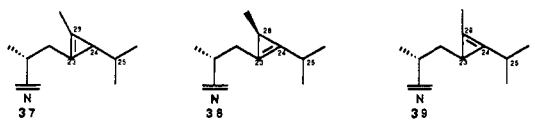
(24) Ha, T. Unpublished results from this laboratory.

Scheme V. Proposed Conversion of Dihydrocalysterol (**4**) to 24*H*-Isocalysterol (**37**) via a Metal-Stabilized (?) Cyclopropyl Cation (C)



in considering hebesterol (**3**) to be the product of the desaturation of clionasterol (**9**) by analogy with the now established biosynthesis of its C_{28} analogue, 29-norhebesterol (**15**).

Two sponges of the order Haplosclerida (*Calyx nicaeensis* and *Calyx podatypa*) possess cyclopropene-containing sterols (**37–39**) as the major components of their sterol mixtures.[3] These sterols (**37–39**) are biosynthesized by the desaturation of dihydrocalysterol[25] (**4**) to 24*H*-isocalysterol (**37**) followed by its isomerization to the other cyclopropene-containing sterols (**38** and **39**).[20] Thus,



with the exception of nicasterol (**1**),[7b] which is found in trace amounts, all of the novel C_{29} sterols found in these sponges are either dihydrocalysterol (**4**) or derived from it (**37–39**). There are two striking similarities between the formation of these C_{29} cyclopropene-containing sterols (**37–39**) and the C_{28} and C_{29} (**14–16** and **2–6**) sterols discussed previously.

Dihydrocalysterol (**4**) has the proper C-24 stereochemistry to be a substrate for this errant Δ^{22}-desaturase. The desaturation of clionasterol (**9**) and 24(*S*)-methylcholesterol (**17**) to their respective cyclopropanes (**4** and **14**) is consistent with the absraction of a C-28 hydrogen from the α-face of the protonated cyclopropanes B and A. The biosynthesis of 24*H*-isocalysterol (**37**) occurs by syn elimination of the C-23 and C-28 hydrogens from the α-face of the cyclopropane (Scheme V).[20,25] These stereochemical features of the biosynthesis of cyclopropene-containing sterols are consistent with the action of this errant Δ^{22}-desaturase. The intermediate compatible with the proposed mechanism of action of this enzyme would be a highly unstable cyclopropyl cation (C) (Scheme V), which suggests that either the steric constraints of the active site prevent its rearrangement or this intermediate may be stabilized by the metal involved in the cyclic desaturation. Such a cyclopropyl cation has been proposed to account for the isomerization of 24*H*-isocalysterol to the other cyclopropene-containing sterols (**38** and **39**) found in the sponge *Calyx nicaeensis*.[3] Thus, it appears that the inability of these sponges to biosynthesize poriferasterol (**10**) (porifera = sponge) is responsible for the biosynthesis of cyclopropane-containing sterols (**2**, **4**, **14**, **15**) as well as cyclopropene-containing sterols (**37–39**).

Experimental Section

The synthesis of the radiolabeled precursors [3-^{3}H]-24(*R*)-ethylcholesterol (**20**), [3-^{3}H]-24(*R*)-methylcholesterol (**21**), [3-^{3}H]-24(*S*)-methylcholesterol (**22**), and [3-^{3}H]cholesterol (**23**) is described elsewhere.[20] After the radiolabel was introduced, each of these precursors was purified by reverse-phase HPLC (Beckman Ultrasphere 5-μ column, 1.0 cm (i. d.) × 25 cm; eluant, methanol). *Petrosia ficiformis* was collected near the Bay of Naples at a depth of 5–15 m. These precursors were fed to the sponge *Petrosia ficiformis* in a manner described previously.[21a] The sponges were dried in Italy and sent to Stanford, where they were extracted with dichloromethane/methanol (1:1) and chromatographed on an open silica gel column to yield a crude mixture of sterols. This mixture was rechromatographed on a silica gel HPLC (Spectral Physics pump SP8810, Waters R403 differential refractometer, Beckman Ultrasil 10-μ column 25 cm × 1 cm i. d.; eluant, ethyl acetate/hexanes (94:6)) to afford a mixture of the pure Δ^5 sterols. These sterols were repeatedly rechromatographed to a constant specific activity on reverse-phase HPLC (Waters solvent delivery system 6000A, Waters R403 differential refractometer, Beckman Ultrasphere 5-μ column, 1.0 cm (i. d.) × 25 cm) using three different solvent systems (methanol, 40 mM silver nitrate in aqueous methanol (2% water), and ethyl acetate/methanol/acetonitrile (4:4:11)). Afterward, each fraction was hydrogenated (1 mL of ethyl acetate, 5 mg of PtO_2, stir at room temperature for 18 h) and all of the radioactivity coeluted with the hydrogenated fraction on reverse-phase HPLC. The radioactivity was determined with a Beckman LZ 7500 liquid scintillation counter. Low-resolution mass spectra were obtained with a Hewlett-Packard Model 5995 GC/MS in the direct inlet mode. All ^{1}H NMR spectra were recorded on a Nicolet NT 300WB (300 MHz) instrument and referenced to the residual $HCCl_3$ signal (7.260 ppm).

Unless noted, all reagents were obtained from commercial sources. All liquids were distilled before use, except where noted. The tetrahydrofuran (THF) was distilled over sodium wire, and the diethyl ether used was the commercially available anhydrous ether. The workup following lithium aluminum hydride reductions consisted of quenching the reaction with ethyl acetate followed by adding enough water to precipitate the aluminum salts as a white precipitate and filtering the supernatant liquid through a small amount of silica gel. The supernatant was concentrated and used directly for the next step.

Synthesis of Four (23*S*,24*R*,25*R*)-24,25-(Dichloromethylene)-23-(hydroxymethyl)-3α,5-cyclo-27-nor-5α,6β-cholestanes (26–29). The 22-iodide[27] (**40**) (1.005 g, 2.2 mM) was condensed with ethyl *trans*-3-pentenoate (0.9 g, 7.0 mM) in the manner previously described[26] to yield a mixture of the trans esters (928 mg, 90% yield). The esters were reduced to the corresponding alcohols with excess lithium aluminum hydride in ether. After workup, the alcohols were acetylated (acetic anhydride (3 mL), pyridine (5 mL), overnight) and then diluted with water and extracted with ether. The ether layer was dried with sodium carbonate, concentrated in vacuo, and chromatographed on silica gel (eluant, 2% ethyl acetate in hexanes) to yield the two acetates as a colorless oil. The acetates, chloroform (5 mL), benzyltriethylammonium chloride (20 mg), and 50% aqueous sodium hydroxide (10 mL) were vigorously stirred for 24 h in a 50-mL flask sealed with a rubber stopper.[28] The reaction mixture was diluted with water and extracted with ether. The ether layer was dried over sodium carbonate, concentrated and chromatographed on silica gel (eluant, 5% ethyl acetate in hexanes) to yield a mixture of the four dichloromethylene acetates as a colorless oil. Excess lithium aluminum hydride was added to a solution of the acetates in ether to yield, after workup, the four epimeric dichlorocyclopropyl alcohols (overall yield 50%), which were separated by preparative thin-layer chromatography.

(23*S*,24*R*,25*R*)-24,25-(Dichloromethylene)-23-(hydroxymethyl)-3α,5-cyclo-27-nor-5α,6β-cholestane (26): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80–3.60 (bm, 2 H, OCH_2), 3.323 (s, 3 H, MeO), 1.307 (d, J = 0 Hz, 3 H, C-26), 1.023 (s, 3 H, C-19), 0.908 (d, J = 6.4 Hz, 3 H, C-21), 0.736 (s, 3 H, C-18); mass spectrum, m/z (relative intensity) 498 (38), 497 (20), 496 (51), 483 (41), 482 (19), 481 (54), 466 (43), 465 (21), 464 (61), 443 (66), 442 (30), 441 (95), 255 (44), 254 (28), 253 (100), 215 (11), 214 (9), 213 (20), 105 (18); high-resolution mass spectrum, m/z (relative intensity) 496.286 308 (M^+, $C_{29}H_{46}O_2Cl_2$) (calcd 496.287 487).

(23*S*,24*S*,25*S*)-24,25-(Dichloromethylene)-23-(hydroxymethyl)-3α,5-cyclo-27-nor-5α,6β-cholestane (27): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67–3.52 (bm, 2 H, OCH_2), 3.320 (s, 3 H, MeO), 1.287 (d, J = 0 Hz, 3 H, C-26), 1.024 (s, 3 H, C-19), 0.992 (d, J = 6.3 Hz, 3 H, C-21), 0.794 (s, 3 H, C-18); mass spectrum similar to **26**.

(23*R*,24*R*,25*R*)-24,25-(Dichloromethylene)-23-(hydroxymethyl)-3α,5-cyclo-27-nor-5α,6β-cholestane (28): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.87–3.65 (bm, 2 H, OCH_2), 3.326 (s, 3 H, MeO), 1.283 (d, J = 5.4 Hz, 3 H, C-26), 1.020 (s, 3 H, C-19), 0.892 (d, J = 6.4 Hz, 3 H, C-21), 0.726 (s, 3 H, C-18); mass spectrum similar to **26**.

(23*R*,24*S*,25*S*)-24,25-(Dichloromethylene)-23-(hydroxymethyl)-3α,5-cyclo-27-nor-5α,6β-cholestane (29): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.75–3.60 (bm, 2 H, OCH_2), 3.326 (s, 3 H, MeO), 1.283 (d, J = 5.5

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Hz, 3 H, C-26), 1.019 (s, 3 H, C-19), 0.950 (d, J = 6.4 Hz, 3 H, C-21), 0.727 (s, 3 H, C-18); mass spectrum similar to **26**.

Assignment of the Absolute Stereochemistry of the Alcohols 26–29 by Conversion to Known Dichloromethylene (30 and 32)[6b] and Cyclopropyl (34 and 36)[6b] i-Methyl Ethers. Each alcohol (**26–29**) (8 mg) was stirred with pyridinium chlorochromate (excess) and dichloromethane (1 mL) for 1 h in a test tube sealed with a rubber septum. The reaction mixture was filtered through Florisil and concentrated to yield the respective aldehydes. Each was stirred with the methyl Wittig reagent (90 mg of methyltriphenylphosphonium bromide, 5 mL of THF, and 0.25 mM *n*-butyllithium) for 30 min. The reaction mixture was diluted with water, extracted with ether, dried over sodium carbonate, concentrated, and purified by preparative TLC to afford the corresponding 23-vinyl compound. Each of the 23-vinyl compounds (5 mg) was stirred in 2-propanol with 5% rhodium on carbon (3 mg) under an atmosphere of hydrogen to afford the known 23-ethyl-24,25-(dichloromethylene)-27-norcholesterol i-methyl ethers (**30** and **32**).[6b] The other two compounds were only known as their i-methyl ethers; hence, the dichloromethylene compounds were dechlorinated to yield the respective i-methyl ethers (**34** and **36**).[6b] The ^{1}H NMR spectrum of each compound was compared with the known sample, enabling us to assign the absolute stereochemistry of the starting dichlorocyclopropyl alcohols (**26–29**).

Synthesis of Four 29-Norhebesterol Isomers (31, 33, 35, 15). A mixture of each alcohol (8 mg), *p*-toluenesulfonyl chloride (16 mg), and pyridine (1 mL) was stirred overnight, then diluted with water, extracted with ether, concentrated, and chromatographed by preparative thin-layer chromatography to afford the respective tosylates. Each tosylate, dimethyl sulfoxide (3 mL), and excess sodium borohydride was stirred for 3 h at 90 °C, then diluted with water, extracted with ether, dried over sodium carbonate, concentrated, and chromatographed to yield the four i-methyl ethers. The chlorine atoms were removed and the i-methyl ethers deprotected by methods previously described to yield the four 29-norhebesterol isomers (**31**, **33**, **35**, **15**).[6b] The natural compound was found, after comparison of ^{1}H NMR spectra, to be the (23*R*,24*R*,25*S*)-29-norhebesterol (**15**).

(23*S*,24*S*,25*R*)-24,25-Methylene-23-methyl-27-norcholesterol (31): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.38–5.34 (bm, 1 H, C-6), 3.60–3.45 (bm, 1 H, C-3), 1.013 (s, 3 H, C-19), 1.010 (d, J = 5.8 Hz, 3 H, C-26), 0.885 (d, J = 6.0 Hz, 3 H, C-28), 0.895 (d, J = 6.4 Hz, 3 H, C-21), 0.720 (s, 3 H, C-18), 0.55–0.45 (bm, 1 H, cyclopropyl), 0.25–0.0 (bm, 3 H, cyclopropyl); mass spectrum, m/z (relative intensity) 398 $C_{28}H_{46}O$ (36.7), 365 (11), 301 (11), 300 (42), 299 (15), 285 (11), 272 (32), 271 (100), 270 (20), 253 (11), 213 (11), 55 (19).

(23*S*,24*R*,25*S*)-24,25-Methylene-23-methyl-27-norcholesterol (33): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.38–5.34 (bm, 1 H, C-6), 3.60–3.45 (bm, 1 H, C-3), 1.011 (s, 3 H, C-19), 1.002 (d, J = 6.4 Hz, 3 H, C-26), 0.888 (d, J = 5.5 Hz, 3 H, C-28), 0.876 (d, J = 6.4 Hz, 3 H, C-21), 0.694 (s, 3 H, C-18), 0.40–0.27 (bm, 2 H, cyclopropyl), 0.20–0.05 (bm, 2 H, cyclopropyl); mass spectrum similar to that of **31**.

(23*R*,24*S*,25*R*)-24,25-Methylene-23-methyl-27-norcholesterol (35): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.38–5.34 (bm, 1 H, C-6), 3.60–3.45 (bm, 1 H, C-3), 1.008 (s, 3 H, C-19), 0.993 (d, J = 6.3 Hz, 3 H, C-26), 0.856 (d, J = 6.5 Hz, 3 H, C-28), 0.827 (d, J = 6.4 Hz, 3 H, C-21), 0.687 (s, 3 H, C-18), 0.45–0.35 (bm, 1 H, cyclopropyl), 0.25–0.05 (bm, 3 H, cyclopropyl); mass spectrum similar to that of **31**.

(23*R*,24*R*,25*S*)-24,25-Methylene-23-methyl-27-norcholesterol (15): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.38–5.34 (bm, 1 H, C-6), 3.60–3.45 (bm, 1 H, C-3), 1.006 (s, 3 H, C-19), 1.002 (d, J = 5.8 Hz, 3 H, C-26), 0.898 (d, J = 6.4 Hz, 3 H, C-28), 0.811 (d, J = 6.4 Hz, 3 H, C-21), 0.683 (s, 3 H, C-18), 0.50–0.40 (bm, 1 H, cyclopropyl), 0.20–0.05 (bm, 3 H, cyclopropyl); mass spectrum similar to that of **31**.

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Biosynthetic Studies of Marine Lipids. 38.[1] Mechanism and Scope of Sterol Side Chain Dealkylation in Sponges: Evidence for Concurrent Alkylation and Dealkylation

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Abstract: A biosynthetic study has been performed to elucidate the precise mechanism of sterol side chain dealkylation in marine sponges. This process resembles that of insects by involving the oxidation of common dietary sterols with unsaturation at C-24(28) [24-methylenecholesterol (**8**), fucosterol (**3**), and isofucosterol (**4**)] to their 24,28-epoxides (**9** and **5**). Loss of formaldehyde (or acetaldehyde) then produces desmosterol (**6**), which is subsequently reduced to cholesterol (**7**). The existence of this pathway in sponges is particularly surprising as these organisms are also capable of the reverse process: *S*-adenosylmethionine-mediated alkylation. The simultaneous operation of these competing processes was demonstrated using doubly labeled sterol precursors.

Introduction

The occurrence of novel sterols with unprecedented structures in sponges is now well-documented.[2] An understanding of the biosynthetic origin of these structures is also emerging.[3a,b] In spite of the tremendous diversity of structures there are certain unifying features in the biosynthesis of these sterols. The use of *S*-adenosylmethionine (SAM) is universal in the construction of the highly alkylated side chains of sponge sterols[3a,b] as well as all phytosterols.[3c] It is also apparent that in sponges of the order Haplosclerida, which characteristically produce unusual sterols

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