$CH_3CD_2CH_2{}^+N(CH_3)=CH_2$ should isomerize to $[CH_3CD^+CH_2D\ CH_3N=CH_2]$ and so expel C_3H_5D and $C_3H_4D_2$ in the ratio of 1:5 (β-D and β-H transfer, respectively). This expectation was subsequently verified by experiment (actual ratio 1:4.3); in contrast, a 1,2-elimination, without isomerization of the propyl cation, predicts exclusive C_3H_5D loss.

2. Demonstration of Reversible Hydrogen Exchange between the Components of an INC. This method is exemplified by the fragmentation of ionized ethers (section B2a); in this case, the abnormally low KE releases that accompany dissociation provide additional evidence supporting the involvement of INCs.[23]

3. Verification That the Longevialle Criterion Is Satisfied. This technique is probably the most powerful. It is illustrated by the long-range hydrogen transfers that are observed between substituents on steroid structures.[4,28,29] Such processes cannot be understood without supposing that detachment of one substituent occurs, followed by mutual rotation of the ionic and neutral components, thus facilitating hydrogen transfer between groups which were originally locked apart.

Future research will probably be directed toward refining existing methods and developing new criteria for demonstrating the intermediacy of INCs. It is especially desirable to devise means whereby the circumstances in which INCs are encountered can be predicted.

D. Conclusion and Prognosis

The advent of INCs has initiated a debate that has led to a thorough revision of many aspects of mass spectrometry. Moreover, it is clear that the chemistry displayed by INCs is inherently of wide interest in other fields, notably physical organic chemistry and theoretical chemistry. There is good reason to believe that INCs will provide a valuable unifying concept for interpreting the diverse reactions of ions which are encountered in many scientific disciplines.

I wish to record my appreciation of Dr. D. H. Williams, whose enthusiasm first aroused my interest in the chemistry of isolated ions, and whose wisdom ensured that the potential of new concepts such as INCs were properly exploited. It was a pleasure, as well as a privilege, to be associated with Dudley and his group. Other scientists also made important contributions to the development of INC-mediated mechanisms in Cambridge. Professors H. Schwarz and C. Wesdemiotis and Drs. B. J. Stapleton, J. R. Kalman, and J. Wendelboe deserve special mention. I thank Professors A. G. Harrison, A. Maccoll, K. R. Jennings, and P. J. Derrick, with whom I have enjoyed more recent collaborations. Finally, I gratefully acknowledge the financial support given to me by SERC (Research Studentship, 1974-1977, and Advanced Fellowship, 1985-1991) and Sidney Sussex College, Cambridge (Research Fellowship, 1977-1981).

Sponge Sterols: Origin and Biosynthesis†

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Received June 17, 1991 (Revised Manuscript Received October 14, 1991)

The pioneering work of Bergman, begun in the late 1940s, pointed toward the existence of a variety of sterols in marine sponges.[1] Not until the 1970s, however, was the potential hinted at by his work realized. The characterization of a bewildering variety of novel sterols[2] was made possible by the development of sophisticated analytical techniques necessary for the separation and structure elucidation of these sterols. These techniques, notably gas chromatography alone or coupled with mass spectrometry, high-field NMR, and HPLC, heralded the renaissance of the sterol field that continues today. This renaissance has branched in three distinct directions.

The initial discovery of sterols with structures having few or no terrestrial counterpart prompted a continuing search for novel sterols.[2] As very recent examples, we cite the novel cyclopropane-containing sterols šormosterol (**1b**)[3] and 29-norhebesterol (**2b**);[4] a multiply alkylated sterol, sutinasterol (**3f**);[5] and the cyclopropene-containing sterol (23*S*)-23*H*-isocalysterol (**4b**)[6] (Charts I and II).

The discovery of such novel sterols—by now well in excess of 100—prompted the obvious question of their biosynthesis. The answer to this question was delayed until satisfactory protocols were established for feeding the sterol precursors to the sponges and keeping them alive for the period of incorporation.[7] There are many

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† Biosynthetic Studies of Marine Lipids. 41. Part 40: Giner, J.-L.; Djerassi, C. *Acta Chem. Scand.*, submitted for publication.

(1) (a) Bergman, W. *J. Mar. Res.* **1949**, *8*, 137. (b) Bergman, W. In *Comprehensive Biochemistry*; Florkin, M., Manson, H. S., Eds.; Academic Press: New York, 1962; Vol. 3, p 103.

(2) (a) Djerassi C. *Steroids Made it Possible.* In the series *Profiles, Pathways, and Dreams Autobiographies of Eminent Chemists*; Seeman, J. I., Ed.; American Chemical Society: Washington, DC, 1991; pp 114-138. (b) Ikekawa, N. Structures, biosynthesis and function of sterols in invertebrates. In *Sterols and Bile Acids*; Danielsson, H., Sjövall, J., Eds.; Elsevier: Amsterdam, 1985; pp 199-230.

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0001-4842/91/0124-0371$02.50/0

Chart I

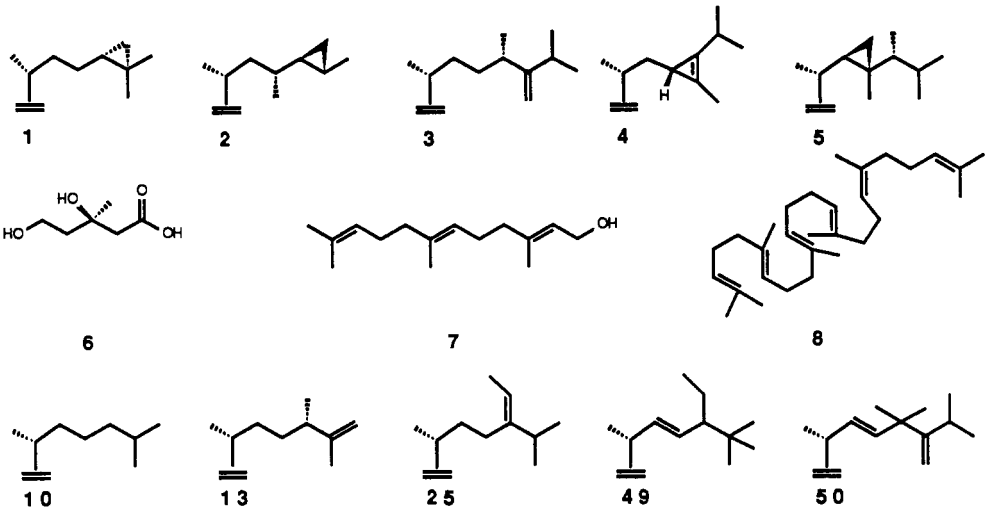
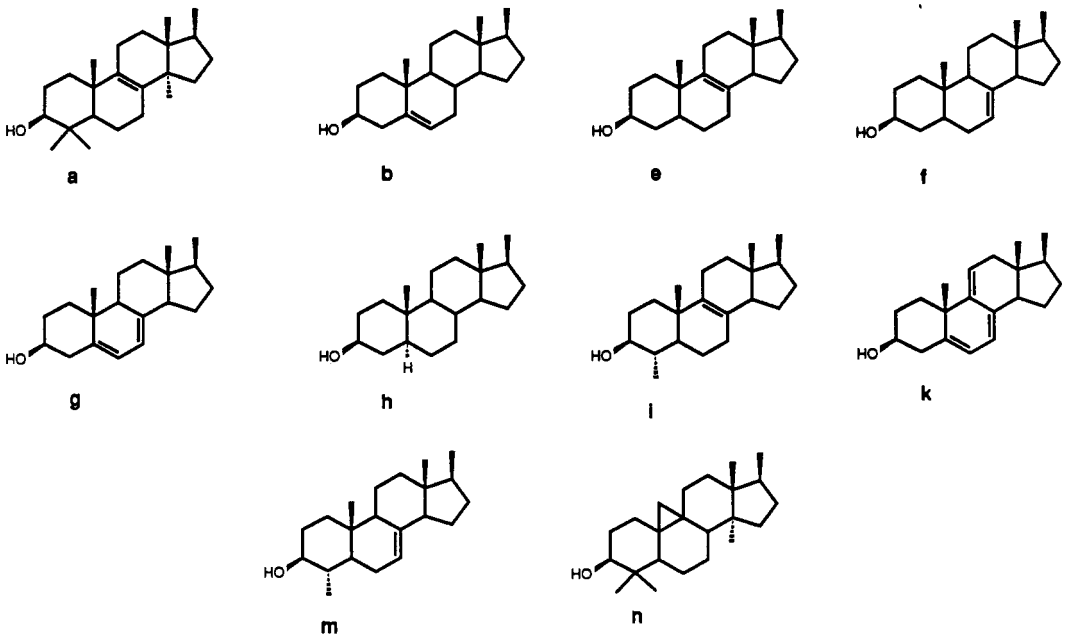


Chart II



reasons why biosynthetic studies of marine organisms, notably of filter feeders, are difficult to perform, and these have been well reviewed by Garson.[8] Once techniques were developed in our laboratory,[7] the results of these experiments revealed the richness of sponge sterol biochemistry. With the exception of some seminal studies performed by Minale, Sodano, and their co-workers in Italy,[9,10] most of this work has been performed in our laboratory and constitutes the subject of this Account.

The third aspect concerns the function of these novel sterols. In many ways this is the most compelling, but least developed area of study, in which the interplay with equally novel sponge phospholipids needs to be taken into consideration. These latter studies, mostly conducted in our laboratory, have been reviewed recently in this journal.[11]

Origin of Sponge Sterols

Sponges are simple cell aggregates adapted to filter-feeding in the aqueous environment.[12] This simple nature belies their ability to colonize virtually all aquatic environments: from the intertidal zone of coastal waters to the deepest marine trenches, from the tropics to the poles, and from near the surface of a seasonal freshwater pond to the geothermal vents in Lake Bikal. It is equally surprising that these simple organisms possess such a diverse sterol metabolism.

Goad pointed out that there were four ways that sterols could be obtained by a sponge:[13] exclusively de novo, exclusively by diet, by modification of dietary sterols, and through de novo biosynthesis on the part of a symbiont. Insects obtain their sterols exclusively by modification of dietary sterols,[14] since they are in-

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(9) (a) Minale, L.; Sodano, G. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2380–2384. (b) De Rosa, M.; Minale, L.; Sodano, G. *Experientia* **1975**, *31*, 408–410.
(10) (a) Minale, L.; Sodano, G. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1888–1892. (b) De Rosa, M.; Minale, L.; Sodano, G. *Experientia* **1975**, *31*, 758–759.
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capable of de novo synthesis. Mollusks obtain their sterols from the diet and also seem to be capable of de novo sterol biosynthesis.[15] The unusual cyclopropane-containing sterol gorgosterol (**5b**),[16] found in gorgonians and soft corals, was shown to originate from the symbiont zooxanthella and not from the soft coral or the gorgonian.[17] Since sponges are filter feeders and are known to have symbionts,[12] none of these possibilities can be excluded a priori.

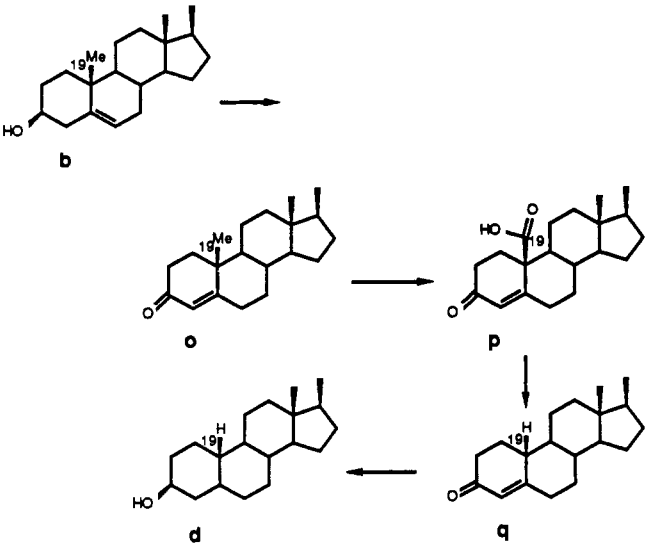
In fact, it was only very recently that the question of the origin of sponge sterols was resolved. The early work on this question employed radiolabeled forms of the water-soluble isoprenoid precursors acetate and mevalonate (**6**).[9b,10b,18a-c] However, the experiments yielded results which suggested that some sponges made their sterols de novo and others did not.[9b,10b,18a-c] Sponges of the same order, family, and even genus could be capable or incapable of de novo sterol biosynthesis, which complicated the matter further. Furthermore, it seemed that sponges containing novel sterols were not capable of de novo sterol biosynthesis, while those possessing "common" sterols were.[13,15]

Recently, we were able to resolve this question by identifying a precursor suitable for settling the question of de novo sterol biosynthesis in sponges.[18d] Our study employed radiolabeled mevalonate (**6**), farnesol (**7**), squalene (**8**), and lanosterol (**9a**), which allowed a comparison with the previous experiments. These experiments showed that water-soluble precursors (e.g., acetate, methionine, and mevalonate) are inherently unsatisfactory because they are poorly incorporated by sponges and/or used more efficiently for other (nonsteroid) biosynthetic purposes. Poor uptake/alternate metabolism, and not the inability to perform de novo sterol biosynthesis, accounted for the low incorporation found in these experiments. With lipid precursors, such as squalene (**8**), we showed that all sponges are capable of performing de novo sterol biosynthesis, though with differing abilities.[18d]

Variety of Sponge Sterols

In the 1970s and 1980s the sterol contents of many sponges were examined,[2,15,19] thus uncovering the variety of sterols found in sponges. This work showed that sponges possess a greater range of sterols than all other organisms combined. The sterols found in sponges differ from their terrestrial counterparts in the occurrence of four structural features: multiply alkylated side chains (found relatively rarely among plants); cyclopropanes and cyclopropenes in the side chain; significant quantities of several different conventional nuclei (e.g., **e**, **f**, **g**, **h**, and/or **i**); and the presence of the *A*-nor (**c**) and 19-nor (**d**) nucleus as a replacement for the conventional steroid template.

Scheme I
Biosynthesis of the 19-Norstanol Nucleus (d)



A perplexing aspect of this sterol diversity is illustrated by five sponges of the *Axinella* genus found in the Mediterranean. *Axinella polypoides* is the only sponge known to possess the 19-nor (**d**),[10,21] nucleus which is exclusively represented among its sterol mixture. *Axinella verrucosa* meets its sterol requirement solely with sterols possessing the *A*-nor (**c**)[9] nucleus. *Axinella damicarnus* is morphologically similar to *A. verrucosa*, yet it contains only saturated 5α-stanols (**h**).[22] *Axinella cannabina*, in contrast, has a sterol mixture composed of six different nuclei (**e**, **f**, **g**, **b**, **h** and **k**).[23]

Source of Nuclear Variation

"Conventional" Nuclei. The different conventional nuclei (e.g., **e**, **f**, **g**, **h**, and/or **i**) found in sponges[23,24] can be thought of as "leaky" blocks in sterol metabolism. In this sense they are not extraordinary, except that their presence suggests that sponges biosynthesize sterols in a slightly different fashion than do plants[25] and animals.[26] For example, sponges seem to remove the 4α-methyl group from the 4α-methyl-$\Delta^{8,9}$ nucleus (**i**) rather than the 4α-methyl-Δ^{7} nucleus (**m**) as has been shown in plants[25] and animals.[26] Sponges possess the curious ability[27] to isomerize cycloartenol (**9n**) (the precursor of sterol biosynthesis in plants) to lanosterol (**9a**) (generally considered the precursor to animal sterols), despite the fact that sponges cyclize squalene

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(16) (a) Bergmann, W.; McMean, M. J.; Lester, D. *J. Org. Chem.* **1943**, *8*, 271–282. (b) Hale, R. L.; Leclerq, J.; Tursch, B.; Djerassi, C.; Gross, R. A. J.; Weinheimer, A. J.; Gupta, K. C.; Scheuer, P. J. *J. Am. Chem. Soc.* **1970**, *92*, 2179–2180. (c) Ling, N. C.; Hale, R. L.; Djerassi, C. *J. Am. Chem. Soc.* **1970**, *92*, 5281–5282.
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(18) (a) Walton, M. J.; Pennock, J. *Biochem. J.* **1972**, *127*, 471–479. (b) De Rosa, M.; Minale, L.; Sodano, G. *Comp. Biochem. Physiol.* **1973**, *45B*, 883–893. (c) Voogt, P. A. *Neth. J. Zool.* **1976**, *26*, 84–93. (d) Silva, C. J.; Wünsche, L.; Djerassi, C. *Comp. Biochem. Physiol.* **1991**, *99B*, 763–773.
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(21) Crist, B. V.; Djerassi, C. *Steroids* **1983**, *43*, 331–343.
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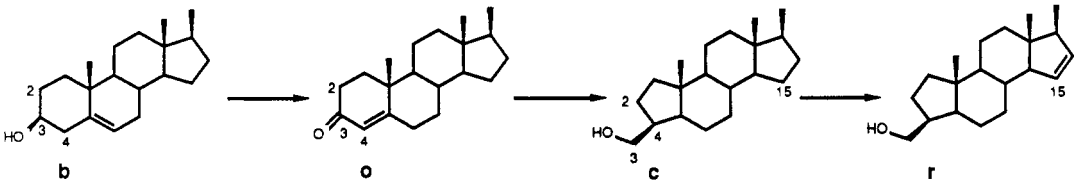
(24) Silva, C.; Djerassi, C. *Comp. Biochem. Physiol., B*, in press.
(25) Goodwin, T. W. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; John Wiley & Sons: New York, 1981; Vol. 1, pp 443–480.
(26) Gaylor, J. L. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; John Wiley & Sons: New York, 1981; Vol. 1, pp 481–545.
(27) Giner, J.-L.; Wünsche, L.; Djerassi, C., to be published.

Scheme II
Biosynthesis of the A-Norstanol Nucleus (c) and the A-Nor-Δ^{15}-stenol Nucleus (r)



(8) exclusively to lanosterol (**9a**).[24,27] Sponges are capable[28] of converting the Δ^5 nucleus (**b**) to the $\Delta^{5,7}$ nucleus (**g**), which is the reverse of the biosynthesis of the Δ^5 nucleus (**b**). This suggests that these blocks not only are "leaky" but also allow reversion to the precursor nucleus.

19-Norstanols. The biosynthesis of the 19-nor (**d**) nucleus was first examined by Minale, Sodano, and their collaborators,[10b] who showed that cholesterol (**10b**) served as a starting material for elimination of the angular methyl group. The intimate details of this reaction have now been established in our laboratory as shown in Scheme I,[29] the key intermediate being the Δ^4-3-keto 10-carboxylic acid (**p**).

***A*-Norstanols.** The biosynthesis of the *A*-nor (**c**) nucleus involves a similar oxidation of the Δ^5 nucleus (**b**) to the Δ^4 3-ketone (**o**).[9] In a mechanism that has yet to be elucidated, the bond between carbons 2 and 3 is broken and re-formed between carbons 2 and 4 (Scheme II). Some sponges also contain *A*-norsterols with a Δ^{15}-position (**r**), a double-bond location that has never been observed before in naturally occurring sterols. As demonstrated[30] in the sponge *Phakellia aruensis*, the Δ^{15}-double bond is introduced after the ring contraction to **c**, rather than before.

Biosynthesis of Novel, Multiply Alkylated Sterol Side Chains

The biosynthesis of the multiply alkylated side chains appears to be a fairly straightforward exercise in the iterative addition of carbons to double bonds through the action of *S*-adenosylmethionine (SAM). Nevertheless, we encountered interesting examples of extreme stereoselectivity, of low stereoselectivity, and of unexpected intermediates. The first of these multiply alkylated side chains to be studied was 25,27-dehydroaplysterol (**11b**) (Scheme III), which, in the sponge *Aplysina fistularis*,[7] is biosynthesized by the biomethylation of epicodisterol (**12b**) but not of codisterol (**13b**). Further methylation to the trace sterol verongulasterol (**14b**) was also demonstrated as well as the saturation of 25,27-dehydroaplysterol (**11b**) to aplysterol (**15b**). The biosynthesis of the sterols in the sponge *A. fistularis*, therefore, is based on a straightforward extension of established biosynthetic schemes.

The biosyntheses (Scheme III) of xestosterol (**16b**),[31] mutasterol (**17b**),[32] aplysterol (**15b**),[7] and verongulasterol (**14b**)[7] share a similar C-25 carbonium ion (**A**) precursor, which undergoes proton elimination to yield the 25,27-double bond (11 and 18) or 24,25-double bond (19). These intermediates (11, 18, and 19) are biomethylated to yield verongulasterol (**14b**), xestosterol (**16b**), and mutasterol (**17b**). Though these four, seemingly disparate, sterols (**14b–17b**) share a common intermediate (**A**), the biosynthesis (Scheme III) of mutasterol (**17b**) and xestosterol (**16b**) is not dependent upon the C-24 stereochemistry of the precursors, since, contrary to the biosynthesis of 25,27-dehydroaplysterol (**11b**), both codisterol (**13b**) and epicodisterol (**12b**) are transformed to these sterols.

The biosynthesis of strongylosterol (**20b**) is summarized in Scheme IV.[33] Although **20b** can be biosynthesized from the biomethylation of either of the C-25 epimers of 24(28)-dehydroaplysterol (**21b**), only codisterol (**13b**), but *not* epicodisterol (**12b**), is utilized in the preceding biomethylation step to yield 24(28)-dehydroaplysterol (**21b**) despite the fact that the latter's C-24 stereochemistry is lost in the formation of the C-24(28) double bond. This is an example of an iterative sequence of biomethylations with an unexpected stereochemical requirement. In addition we demonstrated[33] that epiclerosterol (**22**) cannot be utilized by the sponge.

The biosynthesis of 22-dehydro-24-isopropylcholesterol (**23b**) is another example of the iterative biomethylation found in sponges (Scheme V).[34] Instead of alkylation at C-26, the sponge biomethylates at C-28 of 24-methylenecholesterol (**24b**) and fucosterol (**25b**) or isofucosterol (**26b**), to yield 24-isopropenylcholesterol (**27b**). Though the proposed intermediate (**B**) in this process is achiral, the hydride migration was shown to occur only from C-28 to C-24 and not from C-25 to C-24 (Scheme V). The subsequent steps, also demonstrated by labeling experiments, involve saturation to 24-isopropylcholesterol (**28b**) and subsequent introduction of the Δ^{22}-double bond to yield 22-dehydro-24-isopropylcholesterol (**23b**). These two highly alkylated sterols represent over 98% of the total sterols in this sponge.

Biosynthesis of Cyclopropane- and Cyclopropene-Containing Sterols[35]

Cyclopropane-Containing Side Chains.[35] The presence of cyclopropane-containing sterols (**29b**, **30b**, **31b**, and **2b**) in sponges is noteworthy; even more surprising is the presence of the cyclopropene ring (**4b**, **32b**, **33b**, **34b**). Again, these sterols are found in great abundance in a few sponges of the other Haplosclerida. The structure of one of the most abundant of these sterols, petrosterol (**30b**), suggests an iterative biosynthesis from epicodisterol (**12b**) (Scheme VI) in a manner analogous to that of 25,27-dehydroaplysterol (**11b**)

(28) John, V.; Stoilov, I. L.; Djerassi, C.; Karuso, P.; Poiner, A.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 1642–1647.
(29) Rabinowitz, M. H.; Djerassi, C. *J. Am. Chem. Soc.*, in press.
(30) Malik, S.; Stoilov, I. L.; Djerassi, C. *Tetrahedron Lett.* **1988**, *29*, 4081–4084.
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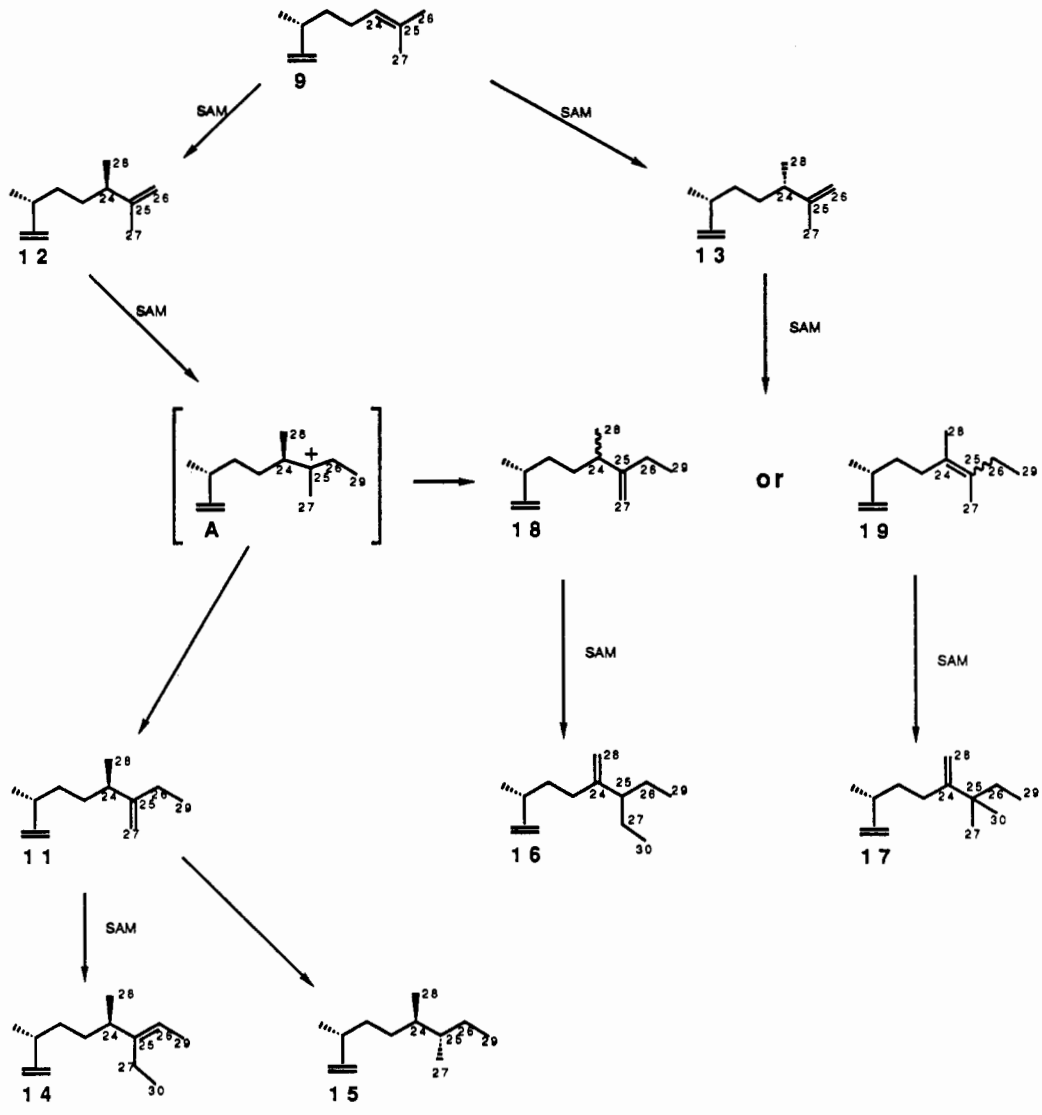
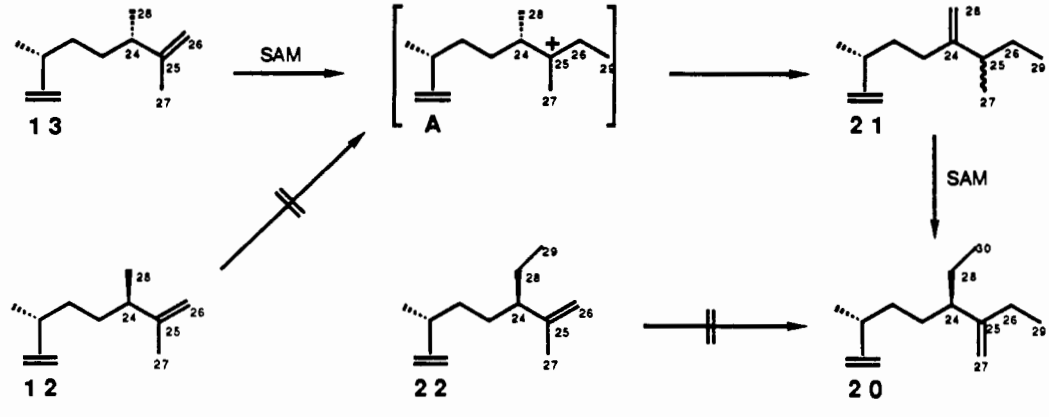
(32) Kerr, R. G.; Kerr, S. L.; Djerassi, C. *J. Org. Chem.* **1991**, *56*, 63–66.
(33) Stoilov, I. L.; Thompson, J. E.; Cho, J.-H.; Djerassi, C. *J. Am. Chem. Soc.* **1986**, *108*, 8235–8241.
(34) (a) Stoilov, I. L.; Thompson, J. E.; Djerassi, C. *Tetrahedron* **1986**, *42*, 4147–4155. (b) Stoilov, I. L.; Bach, T. G.; Thompson, J. E.; Djerassi, C. *Tetrahedron* **1986**, *42*, 4156–4160.
(35) Djerassi, C.; Doss, G. A. *New J. Chem.* **1990**, *14*, 713–719.

Scheme III
Biosynthesis of Aplysterol (15b), Verongulasterol (14b), Xestosterol (16b), and Mutasterol (17b)



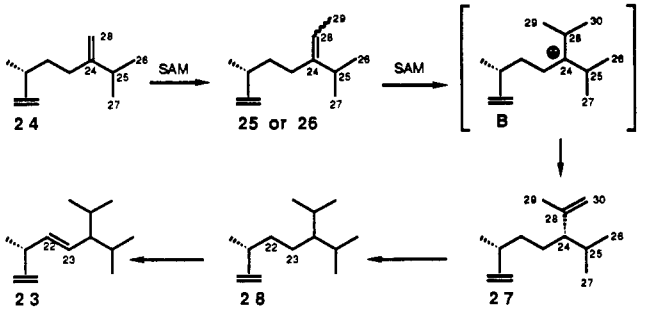
Scheme IV
Biosynthesis of Strongylosterol (20b)



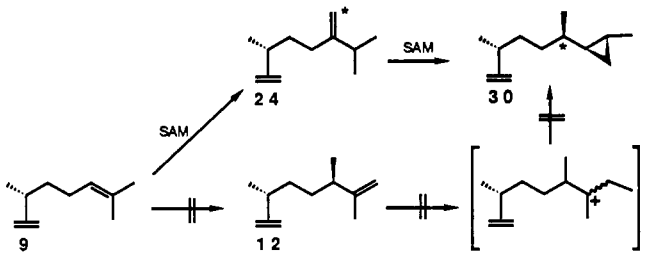
(Scheme III).[36] Yet, when 28-^{14}C-radiolabeled epicodisterol (**12b**) and codisterol (**13b**) were fed to the sponge, no radioactivity was encountered in petrosterol (**30b**), whereas 24-methylenecholesterol (**24b**) was efficiently transformed. On the basis of these results, we concluded that the biosynthesis of petrosterol (**30b**) was not the result of an iterative biomethylation, but rather involved a rearrangement, which was subsequently confirmed by locating the C-28 label of the precursor (**24b**) at C-24 of the product petrosterol (**30b**) (Scheme VI).[36] Such a rearrangement of a protonated cyclopropane (**C** or **D**; Scheme VII or VIII) is consistent with the stereochemistry observed in the novel C_{29} (**29b**, **30b**,

(36) (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.

Scheme V
Biosynthesis of 22-Dehydro-24-isopropylcholesterol (23b)



Scheme VI
Results of Feeding Epicodisterol (12b) and 24-Methylenecholesterol (24b) to the Sponge *Petrosia ficiformis*[a]



[a] The asterisk indicates the position of the ^{14}C label in precursor (**24b**) and product (**30b**).

35b, **36b**) and C_{28} (**2b**, **31b**, **37b**) sterols found in these sponges.[37]

These results and observations provided no insight into the immediate biosynthetic precursor. On the basis of cell-free experiments, we hypothesized that the precursors of these novel C_{29} (**29b**, **30b**, **35b**, **36b**) and C_{28} (**2b**, **31b**, **37b**) sterols were a priori unlikely 24-(*S*)-ethylcholesterol (**38b**) and 24(*S*)-methylcholesterol (**39b**).[38] When these were fed, in radiolabeled form, to the sponge *Petrosia ficiformis*, efficient desaturation was observed to the novel C_{28} (23,24-methylenecholesterol (**31b**), 29-norhebesterol (**2b**), and norficisterol (**37b**)) and C_{29} (dihydrocalysterol (**29b**), petrosterol (**30b**), ficisterol (**35b**), and 26-dehydroaplysterol (**36b**)) sterols respectively.[4,39] The corresponding 24*R* epimers (**41b** or **40b**) were not metabolized. The genesis of these novel cyclopropane-containing sterols is consistent with an errant Δ^{22}-desaturase which performs a cyclic desaturation to yield the observed products.[39]

The cyclopropane-containing sterol šormosterol (**1b**) has been shown to be a product of the initial biomethylation of desmosterol (**9b**) by *S*-adenosylmethionine (SAM) and not the result of a cyclic desaturation.[3,38a,40] Over 20 years ago, Lederer considered this sterol as a possible intermediate in the biosynthesis of 24-methyl sterols (**39b** or **40b**),[41] and experiments performed in our laboratory on the acid-catalyzed ring opening of cyclopropane-containing sterols provided some biomimetic support for this hypothesis.[42] However, when radiolabeled šormosterol (**1b**) was fed to the sponge *Lissodendoryx topsenti*, no in vivo isomerization was encountered.[3] Similarly, radiolabeled dihydrocalysterol (**29b**) was not converted to any other sterols[36a] in *Cribrochalina vasculum*. Thus, cyclopropane-containing sterols are metabolic end products and not intermediates in sterol side chain biosynthesis. The one exception is dihydrocalysterol (**29b**), which is desaturated to the cyclopropene-containing 24*H*-isocalysterol (**33b**) (vide infra).[43]

Cyclopropene-Containing Sterols. The biosynthesis of the unique sterols found in sponges of the genus *Calyx* is summarized in Scheme IX.[43] Earlier work showed that fucosterol (**25b**) is a precursor[44] of calysterol (**32b**), which presumably is reduced to clionasterol (**38b**). We have shown the latter to be desaturated to dihydrocalysterol (**29b**)[39] and thence to 24*H*-isocalysterol (**33b**), which is capable of isomerization to the other cyclopropene-containing sterols (**4b**, **32b**, and **34b**).[43a,c] Finally, the two cyclopropenes **32b** and **33b** can undergo a formal retro-carbene reaction to the corresponding acetylenes (**42b** and **43b**).[43a]

There are three striking similarities between the biosyntheses of dihydrocalysterol (**29b**) and 24*H*-isocalysterol (**33b**). First, the biosynthesis of 24*H*-isocalysterol (**33b**) has been shown to involve the abstraction of hydrogens from C-23 and C-28 of dihydrocalysterol (**29b**) (Scheme IX).[43b,c] The biosynthesis of dihydrocalysterol (**29b**) is consistent with the removal of hydrogen from C-23 and C-28 of 24(*S*)-ethylcholesterol (**38b**) and protonated dihydrocalysterol (**C**) (Scheme VII).[39] Furthermore, the C-28 hydrogen of dihydrocalysterol (**33b**) is removed from the face of the cyclopropane opposite that of the C-28 methyl group (Scheme IX).[43b,c] It is plausible that the proton removed from C-28 of protonated dihydrocalysterol (**C**) is also removed from the face of the cyclopropane opposite that of the C-28 methyl group.[4] Second, the biosynthesis of 24*H*-isocalysterol (**33b**) occurs in sponges that produce dihydrocalysterol (**29b**) virtually exclusively.[4] Last, the stereochemistry at C-24 of dihydrocalysterol (**29b**) is β, which is required for the cyclic desaturation of 24(*S*)-methylcholesterol (**39b**) and 24-(*S*)-ethylcholesterol (**38**).[4,39] Coincidentally, both dihydrocalysterol (**29b**) and calysterol (**32b**) have the same C-28 stereochemistry, though in the process of desaturation the stereochemistry at C-28 is lost.[43b,c] We conclude that it is likely that the C_{29} cyclopropane-containing (**29b** and **30b**) (Scheme VII) and cyclopropene-containing (**4b**, **32b–34b**) (Scheme IX) sterols found in sponges of the order Haplosclerida are bio-

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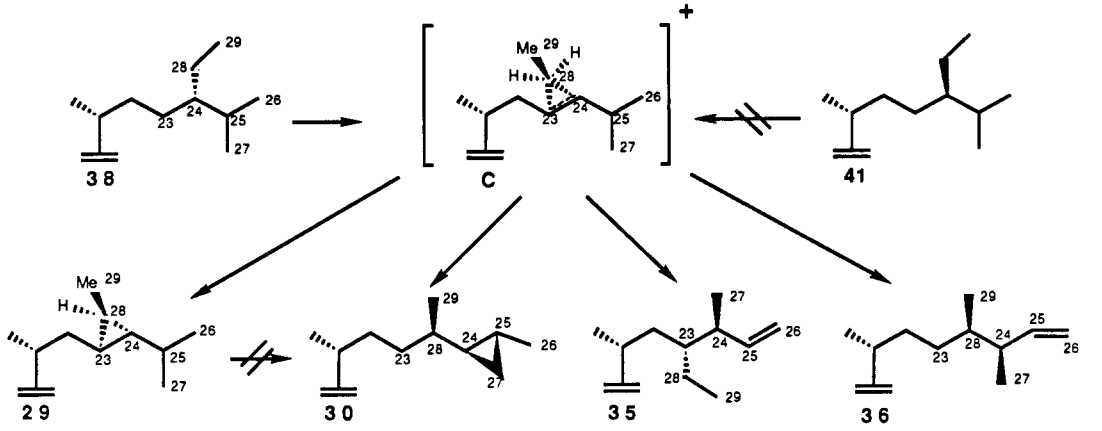
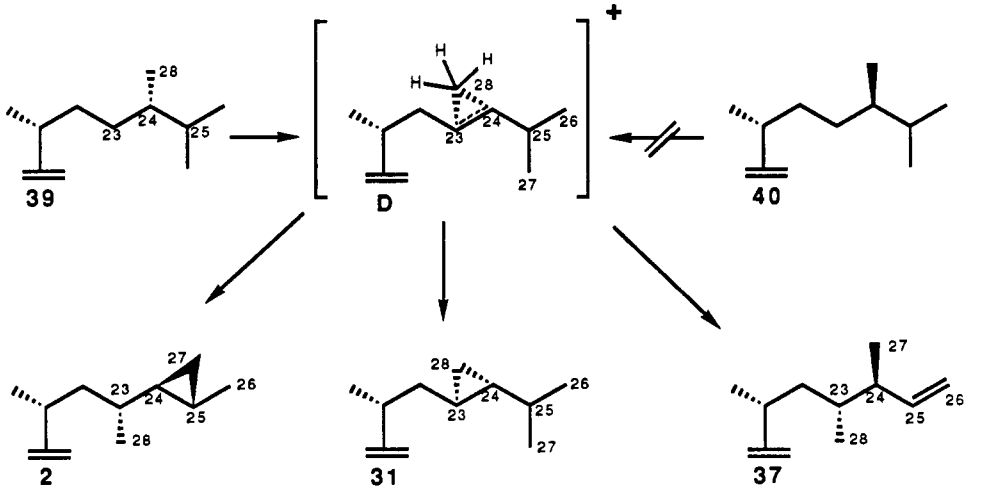
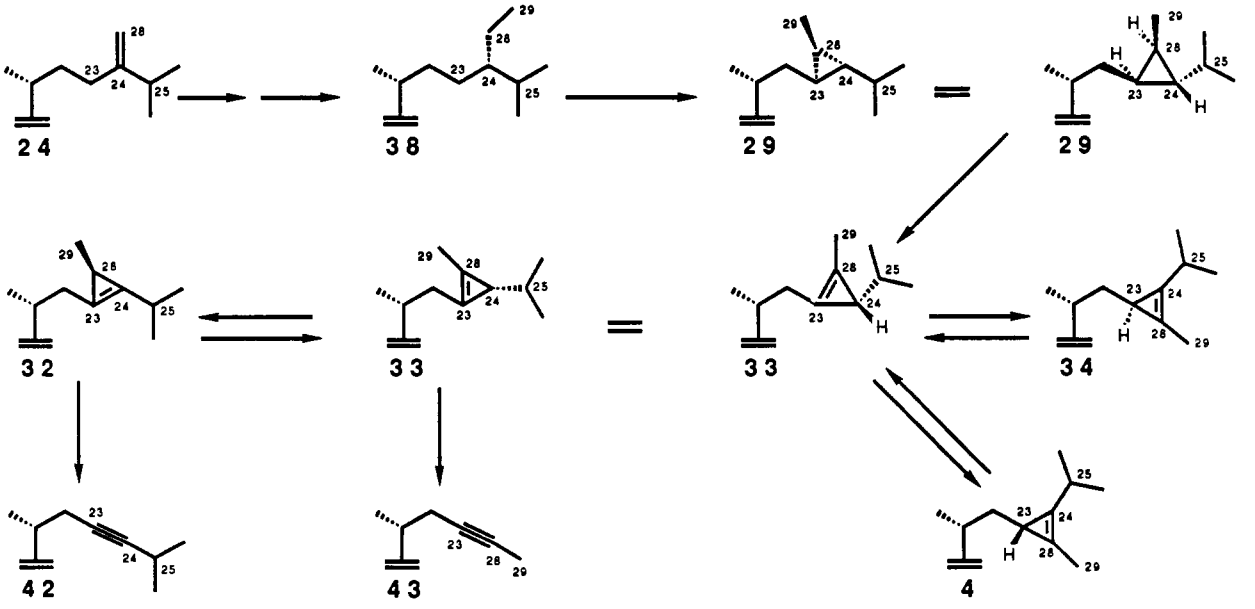
Scheme VII
Unified Biosynthesis of C_{29} Sterols Found in Haplosclerid Sponges



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



Scheme IX
Biosynthesis of the Novel Sterols Found in Sponges of the Genus *Calyx*

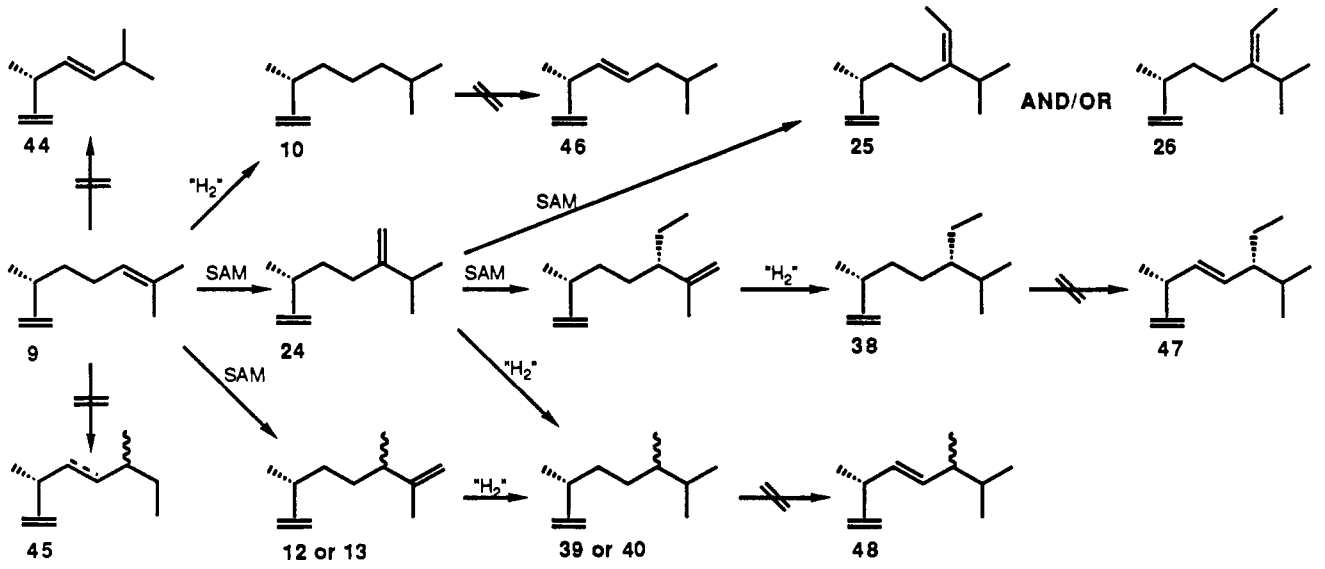


synthesized by the same enzyme.[4]

Origin of Common Sterols[24]

Lost in the flood of novel sterols is the fact that most sponges do not contain novel sterols and instead contain a mixture of "common" sterols (**10b**, **24b–26b**, **38b–40b**, **44b–48b**), "common" in the sense that they are ubiquitous (Scheme X). As the biosynthesis of many of these sterols was known from work on plants[25] and animals,[26] no studies were performed until recently on

Scheme X
Biosynthesis of "Common" Sterols Found in Sponges



the biosynthesis of these "common" sterols in sponges. We have now demonstrated that sponges make cholesterol (**10b**), 24-methylenecholesterol (**24b**), 24-ethylcholesterol (**38b** or **41b**), 24-methylcholesterol (**39b** or **40b**), and fucosterol (**25b**) and/or isofucosterol (**26b**) to differing extents. The relative amounts of these sterols biosynthesized de novo are dependent upon the individual sponge. These sponges share a common inability to biosynthesize sterols possessing the Δ^{22}-double bond, even though all of the sponges possess significant quantities of these sterols (**44b–48b**). Novel sterols possessing the Δ^{22}-double bond (**49b**, **50b**, **23b**) have been found in sponges, but only in the order Halichondrida.[45] Thus it appears that a functional Δ^{22}-desaturase is restricted to a few sponges of a specific order, as is the case with the misfunctional Δ^{22}-desaturase (vide supra).

Dealkylation of Sterol Side Chains[46]

It is surprising that sponges make cholesterol de novo considering that we recently showed them to be capable of dealkylating[46] 24-methylenecholesterol (**24b**) via a 24,28-epoxide to cholesterol (**10b**) in a manner similar to that used by insects,[14] which are *incapable* of de novo sterol biosynthesis. Sponges, in contrast, can undergo both de nova sterol biosynthesis[28] and dealkylation of dietary sterols as demonstrated through the use of doubly labeled sterol precursors.[46c] Some sponges may use the dealkylation of de novo biosynthesized 24-methylenecholesterol (**24b**) as a de facto form of de novo cholesterol (**10b**) biosynthesis. The amount of dealkylation in sponges may be regulated by the rate of de novo cholesterol (**10b**) biosynthesis or dietary absorption. Perhaps the most striking aspect of this metabolism is that sponges are capable of both alkylating sterols *and* dealkylating them.[46c] Such metabolism seems to be a general characteristic of sponges and is found, to our knowledge, in no other organism.[46]

Concluding Remarks

Sponges have a diverse repertoire of sterol metabolism. Such diversity may represent a means of survival for the sponge. While the ability to dealkylate dietary sterols has eliminated the need for de novo sterol biosynthesis in insects, all sponges studied by us are capable of de novo sterol biosynthesis. Alternatively, such metabolism may represent neutral mutations that afford no selective advantage not cause harm. The cyclopropane- and cyclopropene-containing sterols found in sponges of the order Haplosclerida can be viewed as equivalents of clionasterol (**38b**). It is possible that the sterol metabolism found in sponges may be a remnant of prototypical sterol metabolism found in early eucaryotes. The misfunctional Δ^{22}-desaturase found in sponges of the order Haplosclerida may be a failed prototype of a functional Δ^{22}-desaturase that is found in other organisms. The same question can be raised with respect to the ability of sponges to isomerize cycloartenol (**9n**) to lanosterol (**9a**). The fundamental question still remains: Why do sponges have such novel sterols?

Much of this work described herewith was performed at Stanford University by a devoted and skilled group of collaborators, whose names are listed in the footnotes. None of this would have been possible without the generous financial support of the National Institutes of Health.

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