Biosynthetic Studies of Marine Lipids. 9.[1] Stereochemical Aspects and Hydrogen Migrations in the Biosynthesis of the Triply Alkylated Side Chain of the Sponge Sterol Strongylosterol

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Abstract: The biosynthesis of strongylosterol, a product of triple bioalkylation of the cholesterol side chain, was studied in the Indo-Pacific sponge *Strongylophora durissima*. Out of four plausible biosynthetic pathways, the operative sequence was shown to proceed via codisterol and 24(28)-dehydroaplysterol, which means that methylation of C-26 at the terminus of the side chain occurs prior to methylation at C-24. The incorporation experiments were carried out with the following 10 radiolabeled precursors: [24-^{14}C]desmosterol, [28-^{14}C]-24-methylenecholesterol, [26-^{14}C]codisterol, [26-^{14}C]epicodisterol, [24-^{3}H](epi)codisterol, [26-^{14}C]clerosterol, [26-^{14}C]epiclerosterol, [6-^{3}H]-24(28)-dehydroaplysterol, [6-^{3}H]-24(28)-dehydroepiaplysterol, [28-^{14}C]-24-methylene-25-dehydrochlolesterol. There was no stereoselectivity in the chirality of C-25 but high selectivity at C-24. As demonstrated by tritium (^{3}H) labeling, each alkylation was accompanied by 1,2-hydrogen migration.

Sponges are noteworthy among the animal phyla in possessing unconventional sterols, with unusual nuclei and elongated, branched, or cyclopropane-containing side chains.[2] Although their function in the cell membranes of these lower invertebrates is not yet well understood,[3] considerable progress toward an understanding of their biosynthesis has recently been achieved[1,4-6] as a result of our recent shift in emphasis from isolation and structure proof of new marine sterols to the elucidation of their biosynthesis. A knowledge of the operative biosynthetic pathways should provide invaluable information on the origin of these sterols, which is required for further application of sterol-based chemosystematics to this very specious group (>5000 spp), for which conventional morphological characters are not always useful.[7]

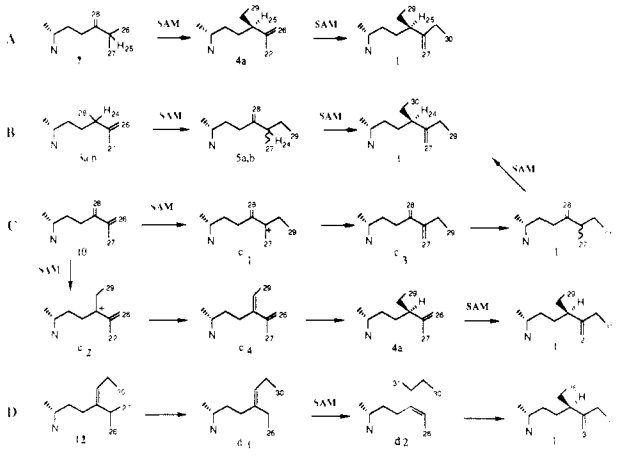
Side-chain alkylation does not occur in sterols of higher animals. In terrestrial plants, single and double alkylation is common,[8] while triple alkylation has been encountered only once[9] and quadruple bioalkylation never. We are now in a position to examine these last two processes in sponge sterols, since examples of such multiple alkylation have been observed by us on numerous occasions.[10]

Representative of the many sponge sterols with unusual side chains, generated by triple or quadruple biomethylation, is the generic structure (Figure 1) which is produced by superposition of several naturally encountered substitution patterns.[10] The circles in this model structure refer to extra carbons introduced into the conventional cholesterol side chain. These represent our biosynthetic target.

Side-chain extension at C-26 was first demonstrated[4] successfully in the California sponge *Aplysina fistularis*, where it was shown that epicodisterol (**3a**), but not its C-24 epimer codisterol (**3b**), is readily converted into 25-dehydroaplysterol (**2**). Recently,[1,6] we established the course of the stereoselective triple alkylation in the biosynthesis of 24-isopropylcholesterols (**11**) in the Great Barrier Reef sponge *Pseudoaxinyssa* sp.

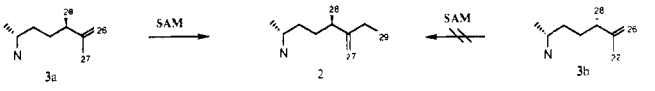
The observed conversion of epicodisterol (**3a**) into 25-dehydroaplysterol (**2**) prompted us to undertake an examination of the biosynthesis of its next higher homologue, (24*R*)-24-ethyl-27-methylcholesta-5,25-dien-3β-ol (strongylosterol) (**1**), which was first isolated by Bartolotto et al.[11] as the virtually exclusive sterol in the Papua New Guinea sponge *S. durissima*; its stereochemistry was established[12] in our laboratory. In addition we encountered[13] in the same sponge two structurally intriguing trace sterols (**8** and **9**) arising from quadruple alkylation (Chart I).

Scheme I[a]



[a] The numbering of the side-chain carbon atoms in the side chain of the sterols in this scheme is based on the biosynthetic order of introduction of each subsequent carbon atom.

Scheme II



Based on biosynthetic precedent,[8] four possible routes to strongylosterol (**1**) have been postulated in the literature.[10a,b,14,15]

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(1) For preceding paper, see: Stoilov, I. L.; Back, T. G.; Thompson, J. E.; Djerassi, C. *Tetrahedron* **1986**, *42*, 4147-4155.
(2) Ikekawa, N. In *Sterols and Bile Acids*; Danielson, H., Sjovall, J., Eds.; Elsevier: Amsterdam, 1985; pp 199-230.
(3) (a) Bloch, K. E. *CRC Crit. Rev. Biochem.* **1983**, *14*, 47-92. (b) Carlson, R. M. K.; Tarchini, G.; Djerassi, C. In *Frontiers of Bioorganic Chemistry and Molecular Biology (IUPAC)*; Anachenko, S., Ed.; Pergamon: Oxford, 1980; pp 211-224.
(4) Catalan, C. A. N.; Thompson, J. E.; Kokke, W. C. M. C.; Djerassi, C. *Tetrahedron* **1985**, *41*, 1073-1084.
(5) Proudfoot, J. R.; Catalan, C. A. N.; Djerassi, C.; Sica, D.; Sodano, G. *Tetrahedron Lett.* **1986**, *27*, 423-426.
(6) Stoilov, I. L.; Thompson, J. E.; Djerassi, C. *Tetrahedron* **1986**, *42*, 4156-4160.
(7) (a) Bergquist, P. R.; Hofheinz, W.; Oesterhelt, G. *Biochem. Syst. Ecol.* **1978**, *8*, 423-435. (b) Bergquist, P. R.; Wells, R. In *Marine Natural Products*; Scheuer, P. J., Ed.; Academic: London, 1983; Vol. 5, pp 1-50.

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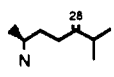
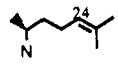
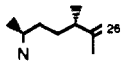
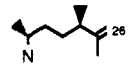
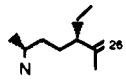
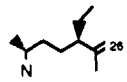
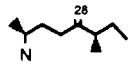
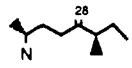
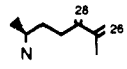
Table I. Incorporation of Labeled Precursors in *Strongylophora durissima*

labeled precursor		total activity incorporated, [μCi]	sponge dry wt, g	recovered radioactivity in strongylosterol 2σ*, %[c]		recovered radioactivity in precursor 2σ*, %[c]	
7, 	[28-^{14}C]	20.0	14.7	300[a] 0.0006[b]	24.2	4210600[a] 9.5[b]	0.3
6, 	[24-^{14}C]	16.7	14.1	101010[a] 0.63[b]	3.3	500[a]	21.4
3b, 	[26-^{14}C]	20.0	11.9	3420180[a] 7.8[b]	0.4	3400[a]	10.8
3a, 	[26-^{14}C]	20.0	12.3	151860[a] 0.34[b]	1.6	1620[a]	16.9
4b, 	[26-^{14}C]	20.0	10.7	cold		1580200[a] 3.6[b]	0.8
4a, 	[26-^{14}C]	20.0	11.4	350[a]	23.7	3960400[a] 9.0[b]	0.4
5b, 	[6-^{3}H]	20.1	13.7	11288300[a] 25.6[b]	0.1	2600[a]	12.5
5a, 	[6-^{3}H]	20.1	14.0	12030200[a] 27.0[b]	0.1	1800[a]	16.1
10, 	[28-^{14}C]	21.0	12.8	2900[a] 0.019[b]	12.1	1060[a]	19.7
mevalonate	[2-^{14}C]	17.8	15.0	8600[a] 0.049[b]	6.4		
methionine	[CH_3-^{14}C]	50.0	10.8	86120[a] 0.172[b]	3.5		

[a] Total radioactivity recovered in dpm. [b] Percent of incorporation based on total radioactivity administered to the sponge. [c] Real error calculated, Beckman LS7500 Liquid Scintillation System Manual (1979), p 46.

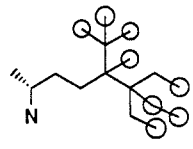
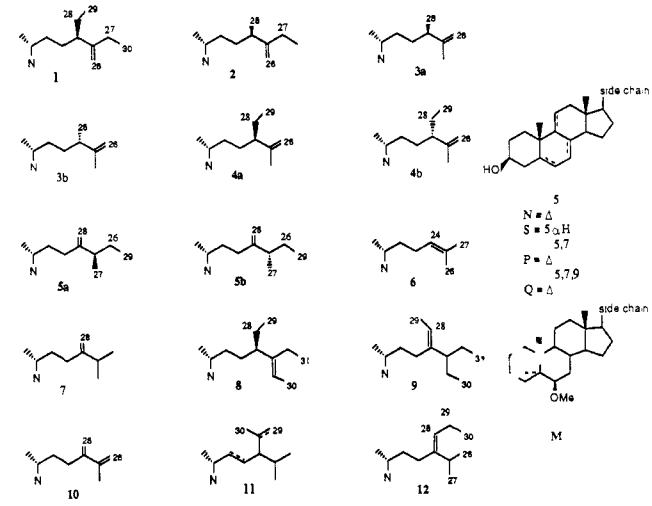


Figure 1.

By synthesizing appropriately labeled precursors, we have now shown that only one of these hypothetical pathways is operational. Although a simple repetitive multialkylation, along the lines established for plants, was predicted[16] for the biosynthesis of marine sterols with unusual side-chain alkylation patterns, unexpected complex rearrangements in marine sterol biosynthesis have recently been encountered.[5] Therefore, experimental verification with radiolabeled precursors is required in order to establish the operative pathway among the four different hypothetical routes. The results described below demonstrate the existence of two successive "push–pull" mechanisms in the biosynthesis of strongylosterol (**1**), each involving a stereospecific 1,2-hydrogen migration.

Chart I



Results and Discussion

The four hypothetical biosynthetic pathways (A, B, C, and D) postulated in Scheme I for strongylosterol (**1**) were tested by duplicate incorporation experiments with the following 10 radioactive-labeled precursors: [24-^{14}C]desmosterol (**6**), [28-^{14}C]-24-methylenecholesterol (**7**), [26-^{14}C]codisterol (**3b**), [26-^{14}C]epicodisterol (**3a**), [24-^{3}H](epi)codisterol (**3a,b**), [26-^{14}C]-clerosterol (**4b**), [26-^{14}C]epiclerosterol (**4a**), [6-^{3}H]-24(28)-

(8) (a) Lederer, E. *Q. Rev., Chem. Soc.* **1969**, *23*, 453–481. (b) Nes, W. R.; McKean, M. L. *Biochemistry of Steroids and Other Isopentenoids*; University Park: Baltimore, MD, 1977.
(9) Kikuchi, T.; Kadota, S.; Suehara, H.; Namba, T. *Chem. Pharm. Bull.* **1982**, *30*, 370–373.
(10) (a) Djerassi, C.; Theobald, T.; Kokke, W. C. M. C.; Pak, C. S.; Carlson, R. M. K. *Pure Appl. Chem.* **1979**, *51*, 1815–1828. (b) Djerassi, C. *Pure Appl. Chem.* **1981**, *53*, 873–890. (c) Djerassi, C. In *Natural Products and Drug Development*; Krogsgaard-Larsen, P., Brøgger-Christensen, S., Kofod, H., Eds.; *Alfred Benzon Symposium 20*; Munkgaard: Copenhagen, 1984; pp 167–176.
(11) Bartolotto, M.; Braekman, J.; Paloze, D.; Tursch, B. *Bull. Soc. Chim. Belg.* **1978**, *87*, 539–543.
(12) Theobald, N.; Djerassi, C. *Tetrahedron Lett.* **1978**, 4369–4372.
(13) Li, L. N.; Djerassi, C. *Tetrahedron Lett.* **1981**, 4639–4642.
(14) Li, L. N.; Sjostrand, U.; Djerassi, C. *J. Org. Chem.* **1981**, *46*, 3867–3870.
(15) Gebreyesus, T. G.; Stoilov, I. L.; Luo, F.-T.; Djerassi, C. *Steroids* **1986**, 447–452.
(16) Goad, L. J. *Pure Appl. Chem.* **1981**, *51*, 837–852.

dehydroaplysterol (**5b**), [6-^{3}H]-24(28)-dehydroepiaplysterol (**5a**), and [28-^{14}C]-24-methylene-25-dehydrocholesterol (**10**).

Route A appears to be a plausible biosynthetic pathway[10a,b] by analogy to the demonstrated[4] side-chain extension of epicodisterol (**3a**) to 25(26)-dehydroaplysterol (**2**) (Scheme II). Unexpectedly, attempts to incorporate the predicted precursor, epiclerosterol (**4a**), which has the same stereochemistry at C-24 as strongylosterol (**1**), were not successful, although the lack of incorporation of its 24-epimer, clerosterol (**4b**), was not surprising. Furthermore, only clerosterol (**4b**), not epiclerosterol (**4a**), has been encountered so far in nature and its biosynthesis confirmed in a unicellular alga.[17] Lack of incorporation of its precursor, [28-^{14}C]-24-methylenecholesterol (**7**), which in some plants[8] is known to be involved in the biosynthesis of clerosterol (**4b**), excludes pathway A entirely.

Therefore, we examined pathway B (Scheme I), where side-chain elongation (**3a,b** → **5a,b**) proceeds before alkylation at C-28 (**5a,b** → **1**). To our surprise, codisterol (**3b**) was almost completely favored as a precursor (cf. Table I). Since the stereochemistry at C-24 is lost in the intermediate 24(28)-dehydroaplysterol (**5a,b**), one would have assumed that both epimers of codisterol (**3a,b**) might be utilized. Of particular relevance is the observation that in the only other documented[4] stereospecific side-chain extension (**3a** → **2**), it was the other epimer (epicodisterol, **3a**) that was utilized preferentially. Given this remarkable stereospecificity in the second bioalkylation step (**3a,b** → **5a,b**), we expected a similar stereoselectivity in the final step (**5a,b** → **1**). Again to our surprise, this time no stereospecificity was noted, as seen (cf. Table I) from the almost equally high incorporation of [6-^{3}H]-24(28)-dehydroaplysterol (**5b**) and [6-^{3}H]-24(28)-dehydroepiaplysterol (**5a**). The latter sterol had been identified earlier as the main sterol[18] in the Australian sponge *Jaspis stellifera* but was not detected in the sponge *S. durissima*, which may be due to its fast conversion into strongylosterol (**1**). However, traces of one of its presumed biosynthetic precursors, codisterol (**3b**), were found in *S. durissima*.[19] The stereochemical purity of codisterol (**3b**) suggests that its epimer, epicodisterol (**3a**), either is not synthesized or is quickly metabolized further by the sponge. Otherwise, it should have accumulated since we have shown it to be much less efficiently transformed (Table I) into strongylosterol (**1**). As the source of codisterol (**3b**) in the sponge is not known, the possibility of a dietary origin cannot be excluded. Successful incorporation (Table I) of [24-^{14}C]desmosterol (**6**) demonstrated that alkylation at C-24 is operative in the sponge and that desmosterol (**6**) is probably incorporated into strongylosterol (**1**) through the stereoselective production of codisterol (**3b**).

Since 24-methylene-25-dehydrocholesterol (**10**) has been encountered in nature[15] and could, therefore, like codisterol (**3b**), be a precursor along route C, it was tested as well. Thus, the high substrate specificity for codisterol (**3b**), as compared to its double-bond isomer 24-methylenecholesterol (**7**) established (Table I) in the biosynthesis of strongylosterol (**1**), could now be tested in terms of any regioselectivity of the methyltransferase; i.e., is there a preference of alkylation at C-26 vs. C-28 in diene **10**? In actual fact, only poor incorporation of precursor **10** into strongylosterol (**1**) was noted (Table I). This could be due to the formation of a well-stabilized intermediate cation (c_1 or c_3) which blocks the types of hydrogen migrations that appear to accompany each alkylation (e.g., **3a,b** → **5a,b**) on the route to strongylosterol (**1**). Consequently, these negative results eliminate the third biosynthetic pathway C, contrary to our earlier speculation.[15]

The lack of incorporation (Table I) of [28-^{14}C]-24-methylenecholesterol (**7**) also excludes the fourth-postulated[10a,b] pathway D based on 24-propylidenecholesterol (**12**) since the former has been shown[20] to be a biosynthetic precursor of the latter. Consideration of route D was originally prompted[10a,b] by

Scheme III

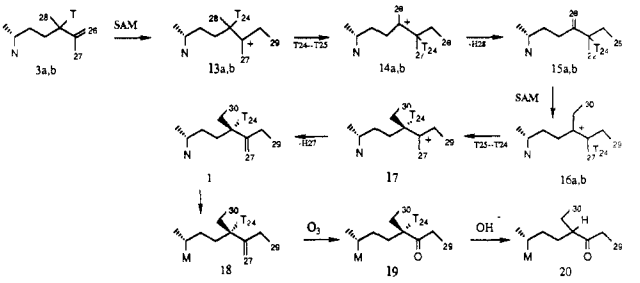
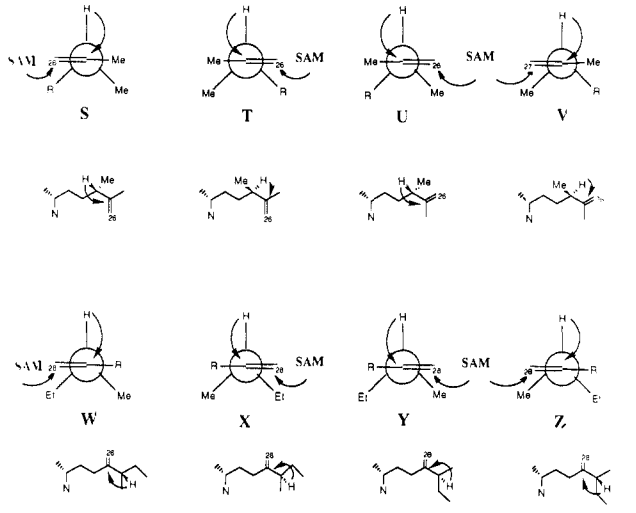


Chart II



the existence in marine organisms of the unique 27-norsterols related to d_1 and the possibility that the known 24-propylidenecholesterol (**12**)[21a,b] might have served as a dietary precursor for the sponge in its further conversion via d_2 to strongylosterol (**1**).

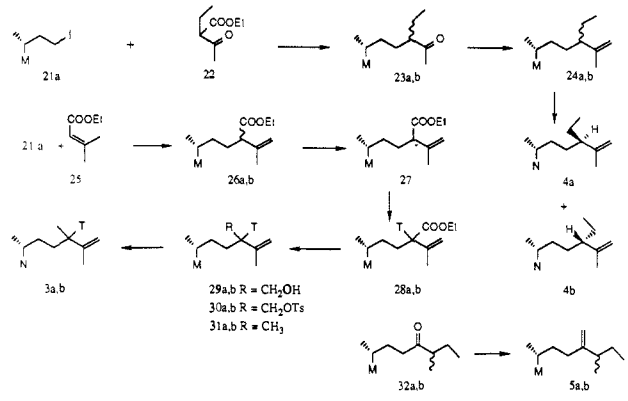
The results summarized in Table I demonstrate complete regioselectivity in pathway B, in that stereoselective bioalkylation at C-26 (**3a,b** → **5a,b**) precedes that at C-28 (**5a,b** → **1**). The observed restricted sequence of alkylation may be termed "sequence-selective" bioalkylation. In other words, the alkylation at C-28 of a C-24(28) double bond can occur only after a C-25(26) double bond (as in codisterol (**3b**)) has already been alkylated, or alternatively, alkylation at C-28 is prohibited unless an additional methyl group is already present at C-26 (**5a,b**), while alkylation at C-26 is prohibited by such an analogous extra methyl group at C-28 (**4a**).

The detailed mechanism of the triple *S*-adenosylmethionine (SAM) bioalkylation from desmosterol (**6**) to strongylosterol (**1**), in particular the twice-occurring migration of the hydrogen atom attached to C-24 in path B of Scheme I, was finally confirmed by incorporation of [24-^{3}H](epi)codisterol (**3a,b**) (Scheme III). Proof that the same proton is involved in a double migration (H-24 → C-25 in **14a,b** and H-25 → C-24 in **17**) was obtained by chemical degradation experiments of the isolated radiolabeled strongylosterol (**1**). Its specific activity (10320 dpm/mg) remained constant during transformation to the *i*-methyl ether **18** and subsequent ozonolysis to the 27-norketone **19**, but all of the radioactivity was lost in the subsequent base-exchange reaction to **20** (Scheme III).

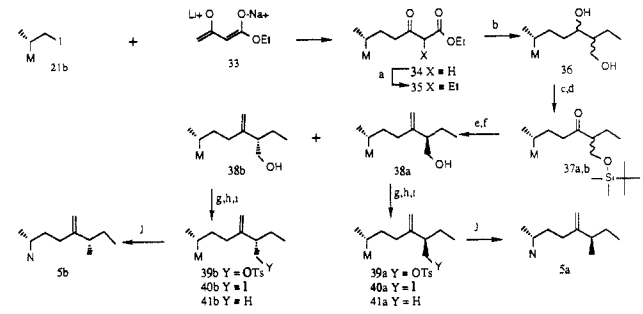
These results obtained from the incorporation of labeled precursors offer some stereochemical insight into the different conformations involved in the individual steps of the bioalkylation. As indicated by Newman projections along the C_{25}–C_{24} bond (Chart II), there are four possible conformations (S, T, U, and V) for codisterol (**3b**) and epicodisterol (**3a**) and four (W, X, Y, and Z) for 24(28)-dehydroaplysterol (**5b**) and 24(28)-dehydro-

(17) Wilkomirski, B.; Goad, L. *Phytochemistry* **1983**, *22*, 929–932.
(18) Theobald, N.; Wells, R. J.; Djerassi, C. *J. Am. Chem. Soc.* **1978**, *100*, 7677–7684.
(19) Catalan, C. A. N., unpublished results in this laboratory.
(20) Kokke, W. C. M. C.; Shoolery, J. N.; Fenical, W.; Djerassi, C. *J. Org. Chem.* **1984**, *49*, 3742–3752.
(21) (a) Idler, D. R.; Safe, L. M.; McDonald, E. F. *Steroids* **1971**, *18*, 545–553. (b) Rohmer, M.; Kokke, W. C. M. C.; Fenical, W.; Djerassi, C. *Steroids* **1980**, *35*, 219–231.

Scheme IV

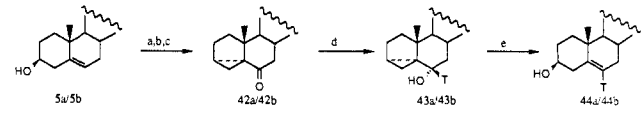


Scheme V[a]



[a] (a) KO-*t*-Bu, EtI, 95%; (b) $LiAlH_4/Et_2O$, 97%; (c) *t*-$BuMe_2SiCl$; (d) PDCr, overall c and d 87%; (e) $Ph_3P{=}CH_2$; (f) $(n\text{-}Bu)_4N^+F^-$; (g) TsCl/Py; (h) NaI/acetone; (i) $NaBH_4$; (j) *p*-TsOH, dioxane, H_2O, 61%.

Scheme VI[a]



[a] (a) TsCl/Py; (b) KOAc–acetone–H_2O; (c) Jones oxidation; (d) $NaBT_4$, EtOH–THF; (e) *p*-TsOH, dioxane, H_2O, overall a–e 25%.

epiaplysterol (**5a**), consistent with the requirement for a perpendicular position of the migrating hydrogen toward the plane of the carbocations formed in each subsequent bioalkylation. Since only strongylosterol (**1**), but not even a trace of its C-24 epimer, is produced, one can state with confidence that in the final (third) bioalkylation (**15a,b** → **16a,b** in Scheme III) only conformers X and Y with α-migration of the C-25 hydrogen to position 24 can be operative, since in conformers W or Z such migration must occur from the β-face. The operative hydrogen migration occurring from the α-face requires that the initiating SAM attack on position 28 (**15** → **16** in Scheme III) occurs from the β-face. The two allowed conformers X and Y correspond to the two epimers **5a** and **5b** of (epi)24(28)-dehydroaplysterol, which are equally well incorporated into strongylosterol (**1**). Therefore the demonstrated lack of stereoselectivity in this bioalkylation could be due to the relatively small difference in steric hindrance of the methyl vs. the ethyl substituent in conformers X and Y.

The high stereoselectivity established for the second alkylation (**3a,b** → **5a,b** in Scheme I) of codisterol (**3b**) could be due to the relatively bigger difference in the steric effect of the methyl group vs. the substituent carrying the remainder of the molecule in conformers S and T or U and V. The hydrogen migration from C-24 to C-25 (**13a,b** → **14a,b** in Scheme II) in codisterol (**3b**) must occur from the β-face, which corresponds to an initiating SAM approach from the α-face. It seems most likely, therefore, that two methyltransferases are involved in the introduction of carbon atoms 29 and 30 in strongylosterol (**1**), since it is unlikely that one and the same enzyme can alkylate from both the α- and β-faces.

Up to 5% of the incorporated activity in the ^{14}C-labeled codisterol (**3b**) and 24(28)-dehydroaplysterol (**5a,b**) experiments is associated with the accompanying 7-dehydrostrongylosterol (**1P**) and 0.1% with its saturated analogue **1S**. These two sterols may be formed by microorganism symbionts present in the sponge.[22]

The high incorporation of radiolabeled codisterol (**3b**), but not epicodisterol (**3a**), indicates that the sponge stereospecifically utilizes one of these epimers and biosynthesizes strongylosterol (**1**) by modifying the codisterol side chain, while potential dietary sterols like 24-methylenecholesterol (**7**) and (epi)clerosterol (**4a,b**) are not utilized as substrates. Whether codisterol (**3b**), which is a known algal sterol, is derived from the sponge's diet or is synthesized by the sponge still remains an unsolved problem. The same question applies to the origin of epicodisterol (**3a**), which is used by the sponge *Aplysina fistularis* in the biosynthesis[4] of 25(26)-dehydroaplysterol (**2**).

Our attempts to study the de novo sterol biosynthesis by incorporation of mevalonate gave very poor results (Table I), in accordance with our previous work[6] and that of others for sponges.[16,23] More detailed incorporation experiments in lower marine animals are needed to study the pathways of any de novo synthesis from mevalonate to desmosterol (**6**), including labeled intermediates of the type that are known to be involved in de novo sterol synthesis in terrestrial organisms.[8b]

Poor incorporation was also noted (Table I) for [*methyl*-^{14}C]-L-methionine. This may be due to difficulties in the uptake of low molecular weight, water-soluble compounds or to the amino acid's much more rapid and efficient utilization for other nonsteroid biosynthetic processes.[6,23]

Synthesis of Labeled Precursors

The sterol precursors used in the current biosynthetic experiments were synthesized as outlined in Schemes IV–VI and then incorporated into separate sponge specimens according to our previously published procedure.[6] The syntheses of radiolabeled 24-methylenecholesterol (**7**), codisterol (**3b**), and epicodisterol (**3a**)[4] as well as of labeled 24-methylene-25-dehydrocholesterol (**10**)[15] have already been described. The synthesis of a 1:1 mixture of codisterol (**3b**) and epicodisterol (**3a**), labeled specifically with tritium at C-24, was accomplished by treating the ester **26a,b**[24,25] (Scheme IV) with 3 equiv of lithium diisopropylamide in THF at −78 °C, followed by quenching with ^3H_2O[24] and converting the 24-ethoxycarbonyl function into a methyl substituent (**31**) via the alcohol **29** and the tosylate **30**.

Radiolabeled clerosterol (**4b**) and its 24-epimer epiclerosterol (**4a**) were prepared (Scheme IV) from the ketone **23a,b**[12] by Wittig condensation with the radioactive phosphorane.[4] The two epimers (**24a** and **24b**) were separated by repeated HPLC, and subsequent deprotection furnished the two labeled epimers (**4a** and **4b**).

A mixture of the epimers of 24(28)-dehydroaplysterol (**5a,b**), synthesized according to the earlier described procedure,[18] could not be separated by HPLC either as the free sterols or as their *i*-methyl ethers in a variety of solvent systems including methanol, methanol–acetonitrile–ethyl acetate, and silver nitrate/methanol. Thus, we were unable to label the two sterol epimers with ^{14}C at C-28 via the Wittig method from the corresponding 24-ketones (**32a** and **32b**) and $Ph_3P^{14}CH_3I$ according to our standard method[4] (Scheme IV). Instead, we synthesized the two epimers **5a** and **5b** by parallel synthetic sequences based on an earlier described procedure[1] where the desired separation is achieved readily at the stage of the alcohols **38a** and **38b** (Scheme V). The dianion of ethyl acetoacetate (**33**) was treated with the known[26] 22-iodide

(22) (a) Thompson, J. E., unpublished observation. (b) Wilkinson, C. R. *Proc. R. Soc. London, B* **1984**, *B220*, 509–517.

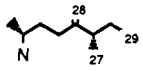
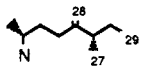
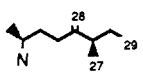
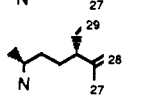
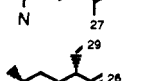
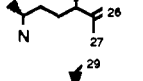
(23) (a) Minale, L.; Sodano, G. In *Marine Natural Products Chemistry*; Faulkner, D., Fenical, W., Eds.; Plenum: New York, 1977; pp 87–109. (b) Rosa, M. D.; Minale, L.; Sodano, G. *Comp. Biochem. Physiol. B* **1973**, *45B*, 883–893.

(24) Rathke, M. W.; Lindert, A. *J. Am. Chem. Soc.* **1971**, *93*, 2318–2320.

(25) Silva, C., unpublished results in this laboratory.

(26) Partridge, J. J.; Faber, S.; Uskokovič, M. *Helv. Chim. Acta* **1974**, *57*, 764–771.

Table II. Selected ^{1}H NMR Data of Natural 24(28)-Dehydroaplysterol (**5a**) and Clerosterol (**4a**) and Their Synthetic Isomers **5a,b** and **4a,b**[c]

sterol		C-18	C-19	C-21	C-26	C-28[a] (C-27)[b]	C-29
5b, 	natural	0.680, s	1.007, s	0.943, d J = 6.5	0.996, d J = 6.7	4.690, s	0.831, t J = 7.4
5b, 	synth	0.680, s	1.007, s	0.943, d J = 6.5	0.996, d J = 6.7	4.690, s	0.831, t J = 7.4
5a, 	synth	0.680, s	1.007, s	0.942, d J = 6.5	1.000, d J = 6.8	4.960, s	0.827, t J = 7.4
4b, 	natural	0.665, s	1.001, s	0.899, d J = 6.5	4.679, d	1.559, s	0.794, t J = 7.4
4b, 	synth	0.665, s	1.001, s	0.899, d J = 6.5	4.679, d	1.559, s	0.794, t J = 7.4
4a, 	synth	0.662, s	1.002, s	0.908, d J = 6.6	4.674, d	1.564, s	0.789, t J = 7.4

[a] Refers to sterols **5a** and **5b**. [b] Refers to sterols **4a** and **4b**. [c] Shifts reported in δ; coupling constants reported in Hz.

(**21b**) to produce exclusively the γ-alkylated product **34** in 87% yield. The substituted β-keto ester **34** was next alkylated to the α-ethyl β-keto ester **35** in tetrahydrofuran by using potassium *tert*-butoxide as base; subsequent lithium aluminum hydride reduction afforded the diol **36** as a diastereomeric mixture. Selective protection of the primary hydroxyl group at C-26, using *tert*-butylsilyl chloride, followed by oxidation of the secondary hydroxyl group at C-24 with pyridinium dichromate afforded the 26-hydroxyl-protected ketone **37** in 87% yield. A Wittig reaction of the protected ketone **37** with methylidenetriphenylphosphorane, followed by treatment with 1 M tetrabutylammonium fluoride in tetrahydrofuran, afforded in 64% yield the alcohols **38a** and **38b**, which were readily separated by normal-phase silica gel HPLC. Both isomers **38a** and **38b** were converted to the free sterols **5a** and **5b** by tosylation, iodide formation, sodium borohydride reduction, and finally deprotection of the *i*-methyl ether. The ^{1}H NMR spectra (Table II) of the two 24(28)-dehydroaplysterol isomers **5a** and **5b** differed in the chemical shifts of the C-27 and C-29 protons, whereby it was shown that **5b** was identical with the natural 24(28)-dehydroaplysterol.

A tritium label was introduced at position C-6 in 24(28)-dehydroaplysterol (**5b**) and its diastereomer (**5a**) by the method of Palmer et al.[27] (Scheme VI), which involved as the key step the sodium borotritide reduction of the 6-oxo-3α,5-cyclosterols **42a** and **42b**. Hydrolysis of the *i*-sterols **43a** and **43b** gave the required [6-^{3}H]-3β-hydroxy-Δ^5-sterols (**44a** and **44b**).

Experimental Section

General. Waters HPLC equipment (M6000 A and M45 pumps, U6K injector, R401 differential refractometers) as well as a Rheodyne Model 7120 and a Valco Model CV-6-UHPa-N60 injector was used for separation of sterol mixtures. The columns used for isolation and further purification were two Altex Ultrasphere ODS columns (10-mm i.d. × 25 cm) connected in series. Retention times are relative to the retention time of cholesterol, coinjected in a separate run with a cold sterol mixture, with the point of injection, rather than the beginning of the solvent peak, used to calculate the relative retention time (RRT) in HPLC. The purity of HPLC fractions was checked by GC using a Hewlett-Packard Model 402 gas chromatograph with FID (3% SP2250 column, 2-mm i.d. × 1.80 m, 260 °C). Low-resolution mass spectra were recorded with a Hewlett-Packard 5995 spectrometer in either DI or GC/MS mode (capillary SE54 column, 15 m, 260 °C). High-resolution mass spectra were recorded on a Finnigan MAT-711 double-focusing mass spectrometer with a direct-inlet sysetm for sample introduction and a PDP-11/45 computer for data acquisition and reduction. ^{1}H NMR spectra (300 MHz) were recorded on a Nicolet NT 300 WB spectrometer. All NMR spectra were recorded in $CDCl_3$ with the solvent peak ($CHCl_3$, 7.259 ppm) as an internal standard. Radioactivity was determined with a Beckman LZ7500 liquid scintillation counter.

Isolation and Purification of Sterols. The sterol fraction of the sponge samples from the incubation experiments was obtained according to our standard procedure.[4] The isolation of strongylosterol (**1**) by HPLC using methanol as the mobile phase was straightforward since **1** is virtually the exclusive sterol of this sponge.[11] Strongylosterol was further purified by HPLC in two different solvent systems to constant activity. Additional attempts of further purification by recrystallization did not change the specific activity and were not used for the rest of the incubation samples. All of the precursors possessed shorter relative retention times in HPLC than strongylosterol, which facilitated their separation and purification.

Synthesis of [24-^{3}H]-(24*R*,*S*)-Codisterol. α-^{3}H Ester 28 and Alcohol 29. The ester **26**[25] (11.4 mg, 0.025 mmol) in 0.2 mL of dry THF was added to lithium diisopropylamide, prepared from isopropylamine (7.6 mg, 0.075 mmol) and *n*-butyllithium (4.8 mg, 0.075 mmol) in 0.2 mL of dry THF at −78 °C. After the mixture stirred for 15 min, the reaction was quenched with ^3H_2O (1.1 μL, 0.05 mmol) added with a syringe. After an additional 15 min of stirring, lithium aluminum hydride (8.5 mg, 0.25 mmol) was added quickly to the reaction mixture and stirring was continued for another 2 h. Ethyl acetate was then added, followed by two drops of water. The mixture was evaporated to dryness in nitrogen, and the residue was triturated with hexane. The hexane extract was evaporated in vacuo and the residue chromatographed over a short column of silica gel (elution with 10% ether in hexane) to afford 9.9 mg (92%) of the 24-^{3}H alcohol **29**.

Tosylate 30. The alcohol **29** (9.9 mg, 0.023 mmol) and freshly recrystallized *p*-toluenesulfonyl chloride (23.8 mg, 0.125 mmol) were stirred in 0.5 mL of dry pyridine for 24 h at 5 °C. The mixture was then diluted with hexane, washed 3 times with aqueous NaCl, dried over $MgSO_4$, and evaporated in vacuo to afford 10.7 mg (80%) of the tosylate **30**.

Reduction of Tosylate 30. The tosylate **30** (10.7 mg) and lithium aluminum hydride (8.5 mg) were stirred for 3 h in 2 mL of dry ether. Ethyl acetate was then added followed by two drops of water. The mixture was evaporated in a stream of nitrogen and the residue triturated with hexane and transferred to a short silica gel column. Elution with 1% ether in hexane afforded 5.2 mg (87%) of the tritiated *i*-methyl ether **31**.

[24-^{3}H](Epi)codisterol (3a,b). The *i*-methyl ether **31** (5.2 mg) from the previous procedure was dissolved in 2 mL of 10% water in dioxane, a crystal of *p*-toluenesulfonic acid was then added, and the mixture was heated for 1 h at 80 °C. The reaction mixture was neutralized by addition of anhydrous sodium carbonate (1 g, which absorbed the water), filtered, and concentrated in a stream of nitrogen. Extraction with hexane and evaporation of the solvent in vacuo followed by purification by chromatography over a short column of silica gel (elution with 25% ether in hexane) afforded 4.2 mg (81%) of the labeled (epi)codisterol as a 1:1 mixture. Although the two epimers are readily separable as their *i*-methyl ethers, such separation was not attempted as it was shown that codisterol was the preferred precursor.

Synthesis of [6-^{3}H]-(25*R*,*S*)-24(28)-Dehydroaplysterol (44a,b). 25-Carboethoxy-6β-methoxy-3α,5-cyclo-27-norcholestan-24-one (34). Sodium hydride (50% oil dispersion, 96 mg, 2.0 mmol) was added to a solution of ethyl acetoacetate (237 μL, 1.9 mmol) in dry tetrahydrofuran

(27) Palmer, M. A.; Goad, L. J.; Goodwin, T. W. *Nouv. J. Chim.* **1978**, *2*, 401–404.

(8 mL) at 0 °C and stirred for 10 min at 0 °C. To the above solution was added *n*-butyllithium (1.2 mL, 1.6 M, 1.92 mmol), and the mixture stirred for 10 min at 0 °C. To a solution of the dianion **33** was added via a syringe the 22-iodide (**21b**) (847 mg, 1.9 mmol) in dry tetrahydrofuran (2 mL). The reaction mixture was then allowed to warm up slowly to room temperature while stirring and then stirred for an additional 90 min. The reaction was quenched with 0.5 mL of H_2O and the mixture concentrated in vacuo to give the γ-alkylation product, which was purified by silica gel column chromatography (eluent, hexane/ether 6:1): yield, 740 mg (87%); ^{1}H NMR (300 MHz) δ 3.320 (3 H, s, OCH_3), 1.019 (3 H, s, C-19), 0.906 (3 H, d, J = 6.5 Hz, C-21), 0.711 (3 H, s, C-18); high-resolution mass spectrum, m/z (relative intensity) 458.3399 (M^+, 59; calcd for $C_{29}H_{46}O_4$ 458.3398), 444.3216 ($C_{28}H_{44}O_4$, 21), 443.3179 ($C_{28}H_{43}O_4$, 64), 426.3133 ($C_{28}H_{42}O_3$, 79), 403.2851 ($C_{25}H_{39}O_4$, 100), 339.2678 ($C_{24}H_{35}O$, 28), 255.2105 ($C_{19}H_{27}$, 24), 253.1942 ($C_{19}H_{25}$, 24), 213.1640 ($C_{16}H_{21}$, 38).

25-Carboethoxy-26-methyl-6β-methoxy-3α,5-cyclo-27-norcholestan-24-one (35). A solution of the β-keto ester **34** (572 mg, 1.25 mmol) and potassium *tert*-butoxide (140 mg, 1.25 mmol) in dry tetrahydrofuran (8 mL) was stirred for 30 min at room temperature. Ethyl iodide (120 μL, 1.5 mmol) was added, and the solution was stirred for 14 h at room temperature. The reaction mixture was concentrated in vacuo to give the α-ethyl β-keto ester **35**, which was purified by silica gel column chromatography (eluent, hexane/ether 6:1): yield, 560 mg (95%); ^{1}H NMR (300 MHz) δ 3.320 (3 H, s, OCH_3), 1.019 (3 H, s, C-19), 0.709 (3 H, s, C-18), the C-21 signal appeared as two doublets (0.909, J = 7.5 and 0.897 Hz, J = 6.4 Hz) due to mixture of 25-epimers; low-resolution mass spectrum, m/z (relative intensity) 486.4 (M^+, 25), 471.4 (20), 454.4 (27), 431.3 (33), 339.2 (18), 296.2 (14), 285.1 (18), 255.2 (30), 253.1 (24), 227.1 (19), 213.1 (39), 201.1 (19), 171.1 (100).

(24(*R*,*S*),25(*R*,*S*))-27-Methyl-6β-methoxy-3α,5-cyclocholestane-24,26-diol (36). Lithium aluminum hydride (85 mg, 2.0 mmol) was added to the α-ethyl β-keto ester **35** (443 mg, 0.91 mmol) in dry ether (15 mL); the reaction mixture was then stirred at room temperature for 30 min, and the excess lithium aluminum hydride was destroyed by addition of ethyl acetate and water. Filtration and evaporation of the solvent in vacuo gave the crude product which was purified by column chromatography over silica gel (eluent, hexane/ether 5:1) to give the diol **36**: 393 mg, 97%; ^{1}H NMR (300 MHz) δ 3.321 (3 H, s, OCH_3), 1.021 (3 H, s, C-19), 0.722 (3 H, s, C-18), with the other peaks overlapping and therefore not assigned.

(25(*R*,*S*))-27-Methyl-26-((*tert*-butyldimethylsilyl)oxy)-6β-methoxy-3α,5-cyclocholestan-24-one (37). Imidazole (161 mg, 3 equiv) and *tert*-butyldimethylsilyl chloride (119 mg) were added to a solution of the diol **36** (342 mg) in dry dimethylformamide (4 mL) and the mixture stirred at room temperature for 3 h, followed by addition of pyridinium dichromate (1 g) and continued stirring at room temperature for 11 h. The reaction mixture was then chromatographed on silica gel (eluent, hexane/ether 10:1) to produce the protected ketone **37**: 373 mg, 87%; ^{1}H NMR (300 MHz) δ 3.318 (3 H, s, OCH_3), 1.020 (3 H, s, C-19), 0.903 (3 H, d, J = 6.5 Hz, C-21), 0.865 (9 H, s, *tert*-butyl), 0.712 (3 H, s, C-18), 0.029 (3 H, s, Si-CH_3), 0.013 (3 H, s, Si-CH_3); low-resolution mass spectrum, m/z (relative intensity) 544.60 (M^+, 1), 501.50 (10), 470.45 (16), 469.45 (43), 377.30 (17), 253.15 (34), 215.10 (35), 213.10 (12), 75.05 (100).

27-Methyl-24-methylene-6β-methoxy-3α,5-cyclocholestan-26-ol (38a,b). To a stirred suspension of methyltriphenylphosphonium iodide (534 mg, 1.32 mmol) in dry ether (8 mL) at 0 °C was added *n*-butyllithium (1.6 M, 0.83 mL). To this ylide solution was then added the protected ketone **37** (360 mg, 0.67 mmol) in ether (2 mL), and the reaction mixture stirred for 6 h at room temperature. After the reaction was quenched with water, ether was added and the Wittig condensation product purified by silica gel chromatography (eluent, hexane/ether 10:1). The silyl protective group was removed by exposure to tetrabutylammonium fluoride (1.0 M, 3 mL) in tetrahydrofuran (7 mL) for 9 h at room temperature to afford a diastereomeric mixture of the 26-hydroxymethyl sterols **38a,b** (187 mg, 64%); this mixture was fractionated by normal-phase HPLC over silica gel (eluent, hexane/ethyl acetate 93:7) to give two compounds in a 1:1 ratio.

Fraction 1. (25*R*)-24-Methylene-26-hydroxy-27-methyl-6β-methoxy-3α,5-cyclocholestane (38b): retention time, 54 min; ^{1}H NMR (300 MHz) δ 4.939 (1 H, s, C-28), 4.821 (1 H, s, C-28), 3.537 (2 H, d, J = 6.4 Hz, C-26), 3.322 (3 H, s, OCH_3), 1.023 (3 H, s, C-19), 0.942 (3 H, d, J = 6.5 Hz, C-21), 0.880 (3 H, t, J = 7.4 Hz, C-29), 0.720 (3 H, s, C-18); high-resolution mass spectrum, m/z (relative intensity) 442.3807 (M^+, 34; calcd for $C_{30}H_{50}O_2$ 442.3813), 427.3578 ($C_{29}H_{47}O_2$, 51), 410.3545 ($C_{29}H_{46}O$, 42), 388.3322 ($C_{26}H_{44}O_2$, 27), 387.8285 ($C_{26}H_{43}O_2$, 100), 328.2770 ($C_{23}H_{36}O$, 62), 313.2541 ($C_{22}H_{33}O$, 23), 296.2515 ($C_{22}H_{32}$, 52), 285.2236 ($C_{20}H_{29}O$, 24), 253.1962 ($C_{19}H_{25}$, 32), 227.1800 ($C_{17}H_{23}$, 20), 213.1652 ($C_{16}H_{21}$, 20).

Fraction 2. (25*S*)-24-Methylene-26-hydroxy-27-methyl-6β-methoxy-3α,5-cyclocholestane (38a): retention time, 60 min; ^{1}H NMR (300 MHz) δ 4.935 (1 H, s, C-28), 4.817 (1 H, s, C-28), 3.541 (2 H, d, J = 6.6 Hz, C-26), 3.322 (3 H, s, OCH_3), 1.023 (3 H, s, C-19), 0.944 (3 H, d, J = 6.5 Hz, C-21), 0.877 (3 H, t, J = 7.4 Hz, C-29), 0.720 (3 H, s, C-18); low-resolution mass spectrum, m/z (relative intensity) 442.40 (M^+, 4), 427.40 (18), 410.40 (10), 387.35 (38), 328.25 (27), 313.25 (14), 296.20 (24), 281.20 (12), 270.20 (10), 255.20 (11), 253.20 (25), 227.20 (16), 213.15 (17), 201.15 (11), 55.10 (100).

24(28)-Dehydroaplysterol Isomers (5a,b). Each individual 26-hydroxy *i*-methyl ether (**38a,b**) (35 mg, 0.08 mmol) was dissolved in dry pyridine (1 mL), and *p*-toluenesulfonyl chloride (100 mg) was added. After 24 h at room temperature, the reaction mixture was diluted with ether and washed with saturated potassium bicarbonate, and the organic layer was separated, dried, evaporated, and purified by short silica gel column chromatography. The tosylate **39** was dissolved in acetone, and sodium iodide (100 mg) was added, followed by heating under reflux for 24 h. The reaction mixture was concentrated and the iodide **40** separated by silica gel column chromatography (eluent, hexane/ether 10:1). The iodide **40** in dry dimethyl sulfoxide (1 mL) was treated with excess sodium borohydride (20 mg) for 13 h at room temperature. The reaction was then quenched with water, ether was added, and the organic layer was separated, dried, and evaporated to give the crude sterol *i*-methyl ether **41**, which was finally purified by silica gel column chromatography (eluent, hexane/ether 10:1). The sterol *i*-methyl ether **41** was dissolved in dioxane/water (4:1, 5 mL) containing *p*-toluenesulfonic acid (3 mg) and heated under reflux for 1 h. Evaporation of the solvents in vacuo, followed by purification of the residue over silica gel (eluent, hexane/ether 4:1), gave 20 mg of the corresponding sterol isomers **5a,b**, which were further purified by reverse-phase HPLC (mobile phase, MeOH).

(25*S*)-24(28)-Dehydroaplysterol (Natural Sterol, 5b). This sterol was derived from the (25*R*)-26-hydroxy *i*-methyl ether **38b**. For ^{1}H NMR data (300 MHz), see Table II: low-resolution mass spectrum, m/z (relative intensity) 412.50 (M^+, 5), 314.25 (98), 312.25 (14), 299.25 (37), 281.25 (40), 271.25 (36), 255.20 (13), 253.25 (13), 231.20 (11), 229.20 (30), 213.20 (21), 55.10 (100).

(25*R*)-24(28)-Dehydroaplysterol (5a). This sterol was obtained from the 26-hydroxy *i*-methyl ether **38a**. For ^{1}H NMR data (300 MHz), see Table II: low-resolution mass spectrum, m/z (relative intensity) 412.60 (M^+, 6), 397.40 (8), 315.25 (24), 314.25 (100), 312.25 (16), 300.25 (16), 299.25 (43), 296.25 (15), 281.25 (45), 271.25 (42), 255.20 (13), 253.20 (13), 229.20 (34), 213.20 (23).

3α,5-Cyclo-6-ketones (42a,b). Each 24(28)-dehydroaplysterol isomer (**5a** and **5b**; 5 mg) was converted into the corresponding tosylate and then converted in the standard manner to the corresponding 3α,5-cyclocholestan-6β-ol. Without further purification, the crude alcohol in acetone (2 mL) was oxidized with Jones reagent by stirring for 15 min at 0 °C. The reaction was quenched by addition of methanol, and the crude *i*-ketone **42** was purified by silica gel column chromatography (eluent, hexane/ether 10:1) and further fractionated by reverse-phase HPLC (mobile phase, methanol).

(25*S*)-24-Methylene-27-methyl-3α,5-cyclocholestan-6-one (42b). This ketone was derived from the isomer **5b**: ^{1}H NMR (300 MHz) δ 4.696 (2 H, s, C-28), 1.003 (3 H, s, C-19), 1.000 (3 H, d, J = 6.8 Hz, C-26), 0.954 (3 H, d, J = 6.5 Hz, C-21), 0.834 (3 H, t, J = 7.4 Hz, C-29), 0.716 (3 H, s, C-18); high-resolution mass spectrum, m/z (relative intensity) 410.3541 (M^+, 27; calcd for $C_{29}H_{46}O$ 410.3551), 313.2507 ($C_{22}H_{33}O$, 68), 312.2453 ($C_{22}H_{32}O$, 100), 298.2280 ($C_{21}H_{30}O$, 37), 297.2224 ($C_{21}H_{29}O$, 78), 269.1897 ($C_{19}H_{25}O$, 48), 229.1581 ($C_{16}H_{21}O$, 22).

(25*R*)-24-Methylene-27-methyl-3α,5-cyclocholestan-6-one (42a). This ketone was obtained from the isomer **5a**: ^{1}H NMR (300 MHz) δ 4.698 (2 H, s, C-28), 1.004 (3 H, d, J = 6.9 Hz, C-26), 1.004 (3 H, s, C-19), 0.953 (3 H, d, J = 6.5 Hz, C-21), 0.831 (3 H, t, J = 7.4 Hz, C-29), 0.717 (3 H, s, C-18); low-resolution mass spectrum, m/z (relative intensity) 410.40 (M^+, 14), 382.30 (4), 314.25 (14), 313.25 (72), 312.25 (92), 298.30 (37), 297.20 (98), 270.20 (17), 256.20 (15), 243.20 (51), 229.15 (25), 215.15 (11), 55.15 (100).

[6-^{3}H]-24-Methylene-27-methyl-3α,5-cyclocholestan-6α-ol (43a,b). Each 3α,5-cyclocholestan-6-one (**42**; 3 mg) was dissolved in 1 mL of ethanol–tetrahydrofuran (1:1) containing 12.5 mCi of sodium borotritide, and the reaction mixture was stirred at room temperature under argon gas. After 10 h, the reaction was quenched by three drops of water, and [6-^{3}H]-3α,5-cyclocholestan-6α-ol (**43**) was obtained by silica gel column chromatography (eluent, hexane/ether 8:1).

[6-^{3}H]-(25*S*)-24-Methylene-27-methyl-3α,5-cyclocholestan-6α-ol (43b). This compound was obtained from the ketone **42b**: ^{1}H NMR (300 MHz) δ 4.691 (2 H, s, C-28), 0.998 (3 H, d, J = 6.9 Hz, C-25), 0.934 (3 H, d, J = 6.5 Hz, C-21), 0.912 (3 H, s, C-19), 0.832 (3 H, t, J = 7.4 Hz, C-29), 0.685 (3 H, s, C-18); low-resolution mass spectrum, m/z (relative intensity) 412.35 (M^+, 4), 397.25 (21), 394.30 (8), 357.20

(13), 314.20 (43), 299.20 (38), 296.20 (21), 281.15 (16), 271.15 (20), 253.15 (15), 229.15 (13), 213.15 (10), 55.05 (100).

[6-^{3}H]-(25*R*)-24-Methylene-27-methyl-3α,5-cyclocholestan-6α-ol (43a). This compound was obtained from the ketone **42a**: ^{1}H NMR (300 MHz) δ 4.692 (2 H, s, C-28), 1.000 (3 H, d, J = 6.8 Hz, C-25), 0.932 (3 H, d, J = 6.7 Hz, C-21), 0.912 (3 H, s, C-19), 0.828 (3 H, t, J = 7.3 Hz, C-29), 0.684 (3 H, s, C-18); low-resolution mass spectrum, m/z (relative intensity) 412.40 (M^+, 4), 397.40 (25), 394.40 (10), 379.40 (12), 357.35 (15), 314.35 (62), 299.50 (54), 296.30 (31), 281.30 (25), 271.30 (33), 253.20 (26), 245.20 (13), 231.15 (14), 225.15 (19), 213.15 (16), 55.15 (100).

[6-^{3}H]-24(28)-Dehydroaplysterol (44a,b). Each labeled 3α,5-cyclocholestan-6α-ol was dissolved in dioxane/water (4:1, 3 mL), containing *p*-toluenesulfonic acid (3 mg), and heated at 90 °C for 4 h. After the mixture cooled to room temperature, sodium carbonate and hexane were added, and the organic layer was then transferred to a short silica gel column and eluted with hexane/ether (5:1) to afford the tritium-labeled [6-^{3}H]-24(28)-dehydroaplysterol, which was further purified by reverse-phase HPLC (mobile phase, methanol). Identity was confirmed by comparison of the NMR spectra of nonradioactive samples (prepared by sodium borohydride reduction) with synthetic sterols. Thus **44** was obtained from **5** in an overall yield of 25%.

Incorporation Experiments. The sponge specimens were collected along the outer reef slope (25 m) near Madang, Papua New Guinea, and transplanted onto plastic plaques at least 1 month prior to use. The precursors were incorporated into duplicate plaques via 11-h aquarium incubations and returned to the sea for 30 days before being collected according to a previously described method.[6] These experiments were performed in two groups with codisterol, epicodisterol, 24(28)-dehydroaplysterol, and 24(28)-dehydroepiaplysterol incorporations initiated on June 7, 1985 and all others initiated on October 19, 1985. Specimens were air-dried at the collecting site before being shipped to California for analysis.

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Electronic Structure of Piano-Stool Dimers. 3. Relationships between the Bonding and Reactivity of the Organically Bridged Iron Dimers $[CpFe(CO)]_2(\mu\text{-}CO)(\mu\text{-}L)$ (L = CO, CH_2, $C{=}CH_2$, CH^+)[1]

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Abstract: The electronic structures of the title molecules have been investigated via Fenske–Hall molecular orbital calculations. A fragment analysis is used wherein the $[CpFe(CO)]_2(\mu\text{-}CO)$ dinuclear, singly bridged framework is allowed to interact with CO, CH_2, $C{=}CH_2$, or CH^+ as a second bridging ligand. The changes in electronic structure that result from changing the second bridging ligand correlate very well with the changes in the energies and spatial characteristics of the frontier orbitals of the bridging ligands. The diverse chemistry of these dimers with nucleophiles and electrophiles is shown to correlate with the bridging ligand induced changes in electronic structure. The photochemical reactions of these systems, either in the absence of additional ligands or in the presence of alkynes or phosphines, are shown to be "LUMO-controlled". A scheme is proposed to explain the varied photochemical reactions of $[CpFe(CO)]_2(\mu\text{-}CO)_2$ and its bridge-substituted derivatives.

Hydrocarbyl-bridged transition-metal dimer complexes constitute a class of molecules which has been the focus of much recent research in organometallic chemistry.[3] This interest has been fueled by the prospect of such complexes behaving as models for catalytic surface chemistry. Many transition-metal dimer frameworks have demonstrated the ability to support a variety of organic bridging units, and one structural class that has received particular attention is the $[CpML_n]_2(\mu\text{-}L')_m$ (Cp = η^5-C_5H_5) or "piano-stool dimer" system. These structures can be visualized as two $CpML_n$ "piano-stool" complexes bridged through their "legs". Complexes of this type, where L = CO or NO and L′ = an organic moiety, are known for many transition metals and organic units. The electronic versatility of these dimers (achieved by altering either the transition metal or the number and types of ligands L and L′) has succeeded in making these systems very amenable to synthetic and mechanistic investigations.

Perhaps the most synthetically explored piano-stool dimer complexes are those derived from the iron triad having the general formula $[CpM(CO)_2](\mu\text{-}CO)(\mu\text{-}L')$ where M = Fe or Ru and L′ is an organic, formally two-electron donor fragment. The chemistry of these compounds is rapidly expanding and already includes facile cis/trans isomerization,[4] photolytic insertion of small molecules,[5] protonation,[6] hydride abstraction,[7] oxidatively induced

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