Table XII. Calculated and Observed Bijvoet Differences

h	k	l	ESF[a] obsd	ESF[a] calcd
$\bar{3}$	2	3	2.70	2.11
2	1	2	2.68	2.04
9	9	0	-0.34	-1.81
$\bar{1}$	4	1	-0.99	-1.64
1	5	4	2.66	1.63
$\bar{8}$	2	5	-0.81	-1.52
1	7	5	1.48	1.50
1	5	3	-0.20	-1.45
3	5	9	-1.63	-1.42
$\bar{11}$	7	6	-0.23	-1.42
11	6	2	-0.58	-1.37
$\bar{3}$	10	1	1.13	1.37
$\bar{13}$	1	6	-2.06	-1.37
$\bar{9}$	7	6	2.64	1.36
2	3	4	2.82	1.35
0	10	0	0.74	1.34
$\bar{1}$	8	8	-1.14	-1.30
0	12	0	1.33	1.27
$\bar{1}$	7	8	-0.44	-1.23
2	9	0	2.28	1.22
6	5	2	-0.07	-1.21
$\bar{6}$	3	4	3.49	1.21
$\bar{3}$	3	2	-0.38	-1.20
7	4	0	1.43	1.17

[a] ESF = $[F^2(+) - F^2(-)]/\sigma(F^2)$, where $F^2(+) = F^2(hkl)$ and $F^2(-) = F^2(h\bar{k}\bar{l})$.

$K\alpha_1$ radiation. All X-ray measurements were carried out on an Enraf-Nonius CAD-4 diffractometer equipped with a liquid N_2 low-temperature device.

Crystal data: briareolide B, $C_{26}H_{36}O_{11}$, M_r = 524.6, monoclinic, $P2_1$, a = 12.606 (2) Å, b = 10.460 (3) Å, c = 10.304 (2) Å, β = 107.68 (1)°; V = 1294.5 Å^3, Z = 2, D_{calcd} = 1.345 gm cm^{-3}, $F(000)$ = 560, μ(Cu Kα) = 7.9 cm^{-1}.

The intensity data of all the unique reflections within 2θ range 0-150° were collected at 163 ± 2 K by using Cu Kα radiation and a θ-2θ scan technique with a variable scan width of (0.90 + 0.20 tan θ)°. A total of 2818 unique reflections were recorded, of which 2771 reflections were considered "observed" on the basis of $I > 2\sigma(I)$. The intensities were corrected for Lorentz and polarization factors, but no absorption correction was made. The structure was solved by direct methods and the use of the program MULTAN80[28] and refined by a full-matrix least-squares routine SHELX86[29] in which the quantity $\sum w(F_o - F_c)^2$ is minimized, where $w = 1/\sigma^2(F_o)$. All the hydrogen atoms were located from a difference Fourier map, and their parameters were refined. The non-hydrogen atoms were given anisotropic thermal parameters. The refinement converged to a final R = 0.0295, R_w = 0.0363 for 2771 observed reflections [$I > 2\sigma(I)$], S = 1.4, Δ/σ 0.04, electron density in the final difference map ±0.2 e/Å^3. The absolute configuration of briareolide B was determined by a Bijvoet method[30,31] using the anomalous dispersion of Cu radiation by oxygen and carbon atoms. Intensities of 24 most enantiomer sensitive Friedel pairs were measured repeatedly (15 times each) at low temperature. The calculated and observed weighted Bijvoet differences for these pairs of reflections are compared in Table XII. The intensity differences of all 24 pairs are in agreement with the absolute configuration shown in this report.

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Supplementary Material Available: Bond distances, bond angles, torsion angles, hydrogen atom parameters, and anisotropic thermal parameters for 11 and ^{13}C NMR spectra of 3-14 and 16-18 (21 pages). Ordering information is given on any current masthead page.

(28) Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declerq, J.-P.; Woolfson, M. M. *MULTAN80. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*; University of York: York, England, 1980.

(29) Sheldrick, G. M. *SHELX76. Program for Crystal Structure Determination*; University of Cambridge: Cambridge, England, 1986.

(30) Bijvoet, J. M.; Peerdeman, A. F.; van Bommel, A. J. *Nature* **1951**, *168*, 271.

(31) van der Helm, D.; Hossain, M. B. In *Patterson and Pattersons*; Glusker, J. P., Patterson, B. K., Rossi, M., Eds.; Oxford University Press: New York, 1987; p 482.

Biosynthetic Studies of Marine Lipids. 33.[1] Biosynthesis of Dinosterol, Peridinosterol, and Gorgosterol: Unusual Patterns of Bioalkylation in Dinoflagellate Sterols

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The biosynthesis of the 23-methylated dinoflagellate sterols gorgosterol (**1-N**), dinosterol (**2-K**), and peridinosterol (**3-K**) was demonstrated experimentally using cell-free extracts of the dinoflagellates *Cryptothecodinium cohnii*, *Peridinium foliaceum*, and the cultured zooxanthella symbiont of *Cassiopea xamachana*. In assays of sterol methyltransferases using [^{3}H]-*S*-adenosylmethionine, radiochemical conversions were demonstrated by reverse phase HPLC of the Δ^{24} sterol side chain (**5**) to the 24-methylene side chain (**4**); of the sequence 24(*R*)-4α-methylergost-22-enol (**9-K**) to dinosterol (**2-K**) to 4α-methylgorgostanol (**1-K**); and, in *P. foliaceum*, of 24-(*R*)-4α-methylergost-22-enol (**9-K**) to peridinosterol (**3-K**). Methylation of the 24(*R*)-methyl Δ^{22} side chain (**9**) in *P. foliaceum* is believed to involve more than one SAM–sterol methyltransferase based on changes in the ratios of the products (**2** and **3**) with changes in the conditions of the assay. Furthermore, deuterium substitution of the sterol substrate (**9-K**) at C-23 did not significantly alter the product ratio. A hypothesis is put forward that the attenuation of gorgosterol (**1-N**) production in aposymbiotic zooxanthellae is linked to an increase in dimethylpropiothetin biosynthesis via a decrease in *S*-adenosylmethionine concentration.

Almost half a century has passed since the discovery of gorgosterol (**1-N**)[2] and 20 years since the elucidation of the structure of this unique cyclopropyl sterol[3] marked our entry into the field of marine sterol chemistry.[4] A bios-

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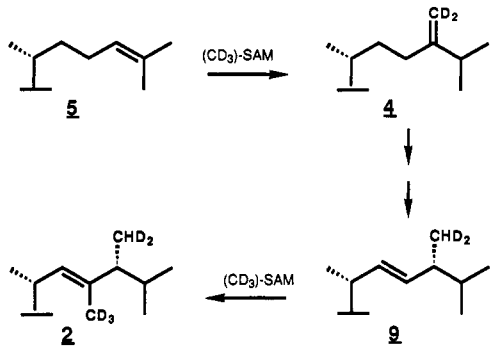
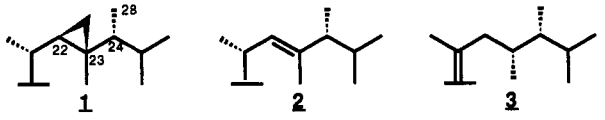
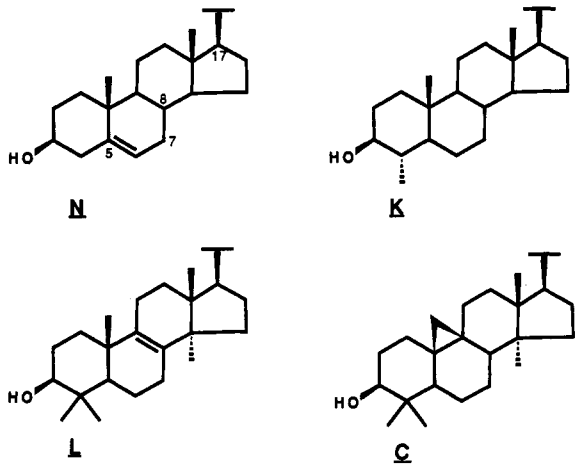


Figure 1. Dinosterol biosynthesis as suggested by methionine-d_3 feeding experiment.[7]

ynthetic precursor to the gorgosterol side chain (1) was proposed[3b] which was subsequently isolated from free-swimming dinoflagellates as dinosterol (**2-K**).[5] Experimental verification of this scheme has been extremely difficult, causing one researcher to abandon "any systematic attempt to find a preparation capable of forming gorgosterol, despite the lure of this sterol's biosynthetic uniqueness".[6]





Originally isolated from gorgonians, gorgosterol (**1-N**) was later shown to be the product of the unicellular dinoflagellate algae (zooxanthellae) that live symbiotically inside the cells of these marine invertebrates.[7] When symbiotic dinoflagellates were isolated and cultured outside of their hosts, the production of gorgosterol (**1-N**) ceased in almost every case, leading to speculation that this sterol was somehow involved in the symbiotic process and was thus subject to regulation.[8] Nevertheless, gorgosterol (**1-N**) and its saturated 4α-methyl analogue (**1-K**) were isolated from an aposymbiotic zooxanthella[8b] as well as from two free-swimming dinoflagellates, *Peridinium foliaceum* and *Peridinium balticum*.[9] Also isolated from the latter two species was peridinosterol (**3-K**), an isomer of dinosterol (**2-K**) with a rare $\Delta^{17(20)}$ double bond.[9]

Table I. *C. cohnii* Cell-Free Extract Experiment (1×10^6 cpm ^{3}H-SAM)

substrate	cpm	product
–	271 391	–
5-K	2858 2608	4-K
4-K	355 379	–
6-K	277 261	–
7-K	251 279	–
8-K	311 387	–
9-K	1628 1980	2-K

Knowledge of the biosynthesis of these unusual sterols with their unique pattern of 23-methylation has been greatly restricted by technical difficulties. Feeding experiments with d_3-methionine in *Cryptothecodinium cohnii* showed that, while the 23-methyl group of dinosterol (**2-K**) bore three deuterium atoms, the 24-methyl group only incorporated two, indicating the intermediacy of a 24-methylene side chain (4, Figure 1).[10] However, further feeding experiments were precluded by the inability of the alga to take up labeled sterols from the medium. Attempts to solve this problem by extracting the biosynthetic enzymes from the algal cells (cell-free extracts) were also met with failure.[6] Thus, until now, no successful experimental entry into the biosynthetic origin of the unique cyclopropane-containing side chain of gorgosterol (**1-N**) has been uncovered, in spite of the great interest in this structural feature as a geochemical marker.[11]

(1) Part 32. Giner, J.-L.; Djerassi, C. *J. Am. Chem. Soc.* **1991**, *113*, 1386–1393.
(2) Bergmann, W.; McLean, M. J.; Lester, D. *J. Org. Chem.* **1943**, *8*, 271–282.
(3) (a) Hale, R. L.; Leclercq, J.; Tursch, B.; Djerassi, C.; Gross, R. A., Jr.; Westheimer, A. J.; Gupta, K. C.; Scheuer, P. J. *J. Am. Chem. Soc.* **1970**, *92*, 2179–2180. (b) Ling, N. C.; Hale, R. L.; Djerassi, C. *J. Am. Chem. Soc.* **1970**, *92*, 5281–5282.
(4) (a) Djerassi, C.; Theobald, N.; Kokke, W. C. M. C.; Pak, C. S.; Carlson, R. M. K. *Pure Appl. Chem.* **1979**, *51*, 1815–1828. (b) Djerassi, C. *Ibid.* **1981**, *53*, 873–890. (c) Withers, N. W. in *Marine Natural Products, Chemical and Biological Perspectives, Vol. 5*; Scheuer, P. J., Ed.; Academic Press: New York, 1983; pp 87–130.
(5) (a) Shimizu, Y.; Alam, M.; Kobayashi, A. *J. Am. Chem. Soc.* **1976**, *98*, 1059–1060. (b) Finer, J.; Clardy, J.; Kobayashi, A.; Alam, M.; Shimizu, Y. *J. Org. Chem.* **1978**, *43*, 1990–1992.
(6) Anderson, D. G. *Comp. Biochem. Physiol.* **1985**, *81B*, 423–428.
(7) Ciereszko, L. S.; Johnson, M. A. W.; Schmidt, R. W.; Koons, C. B. *Comp. Biochem. Physiol.* **1968**, *24*, 899–904.
(8) (a) Kokke, W. C. M. C.; Fenical, W.; Bohlin, L.; Djerassi, C. *Comp. Biochem. Physiol.* **1981**, *68B*, 281–287. (b) Withers, N. W.; Kokke, W. C. M. C.; Fenical, W.; Djerassi, C. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 3764–3768; correction, p 6390.
(9) (a) Withers, N. W.; Kokke, W. C. M. C.; Rohmer, M.; Fenical, W. H.; Djerassi, C. *Tetrahedron Lett.* **1979**, 3605–3608. (b) Swenson, W.; Tagle, B.; Clardy, J.; Withers, N. W.; Kokke, W. C. M. C.; Fenical, W. H.; Djerassi, C. *Tetrahedron Lett.* **1980**, *21*, 4663–4666.
(10) Withers, N. W.; Tuttle, R. C.; Goad, L. J.; Goodwin, T. W. *Phytochemistry* **1979**, *18*, 71–73.
(11) (a) Moldowan, J. M. *Geochim. Cosmochim. Acta* **1984**, *48*, 2767–2768. (b) Wunsche, L.; Gulacar, F. O.; Buchs, A. *Org. Geochem.* **1987**, 215–219. (c) Summons, R. E.; Volkman, J. K.; Boreham, C. J. *Geochim. Cosmochim. Acta* **1987**, *51*, 3075–3082.

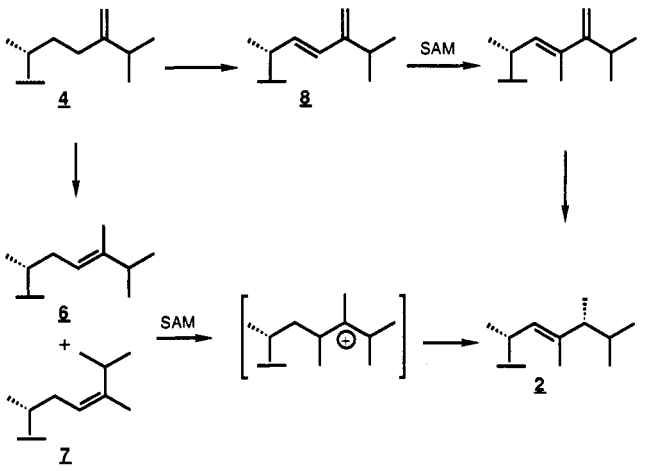
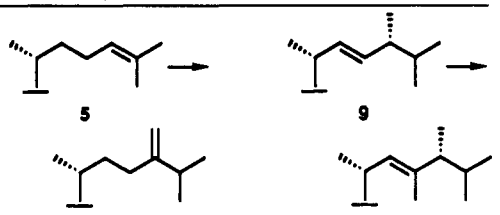


Figure 2. Alternative pathways of dinosterol (2) biosynthesis.

Table II. Sterol Nucleus Preferences in SAM–Sterol Methyltransferase Reactions in the *C. cohnii* Cell-Free Extract (1×10^6 cpm ^{3}H-SAM)



sterol nucleus	4	2
	202	147
-	187	226
	2443	354
Δ^5 (N)	2473	357
	665	265
Δ^7	717	210
	1303	
Δ^8	1492	-
	1833	574
Δ^7 4α-Me	1610	516
	2257	957
Δ^0 4α-Me (K)	2001	1085
	227	
(L)	276	-
	266	
(C)	265	-

We have recently succeeded in preparing biosynthetically active cell-free extracts from a number of dinoflagellate species and have used them to demonstrate the intermediacy of lanosterol (**5-L**) rather than cycloartenol (**5-C**) in the sterol biosynthesis of these organisms.[12] This experience has now enabled us to settle the biosynthetic formation of dinoflagellate sterol side chains (**1**, **2**, **3**) by means of cell-free extracts.

Results and Discussion

Cell-free extracts of the heterotrophic dinoflagellate *C. cohnii* have been used to study the biosynthesis of fatty acids[13] and dimethyl sulfide.[14] A cell-free extract capable of catalyzing the transfer of ^{3}H-labeled methyl groups from *S*-adenosylmethionine (SAM)[15] to the sterol side chain was prepared and used to study dinosterol (**2-K**) biosynthesis. The results (Table I) are in agreement with the proposed biosynthetic pathway (Figure 1).[10] Three sterol side chains representing possible alternative routes (Figure 2) were also synthesized and tested—the Δ^{23} isomers (**6-K**, **7-K**) and the $\Delta^{22,24(28)}$ compound (**8-K**)—but did not serve as substrates (Table I). Although the methylation of the desmosterol side chain (**5**) to yield the 24-methylene side chain (**4**) proceeded to roughly the same extent with both the

Table III. *P. foliaceum* and the Zooxanthella of *C. xamachana* Cell-Free Extract Experiments

substrate	cpm[a]	product
P. foliaceum (25×10^6 cpm ^{3}H-SAM)		
-	0	-
5-K	20000	4-K
9-K	19000	2-K + 3-K
3-K	0	-
2-K	190	1-K
zooxanthella of *C. xamachana* (10×10^6 cpm ^{3}H-SAM)		
9-K	3400	2-K
2-K	0	-

[a] After HPLC purification.

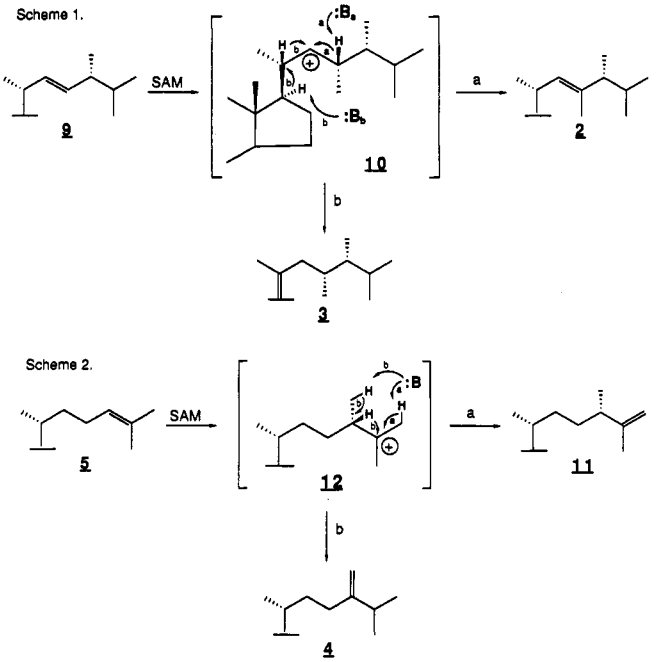


Figure 3. Mechanism of dinosterol (**2-K**) and peridinosterol (**3-K**) biosynthesis.

(12) Giner, J.-L.; Djerassi, C. *Biochem. Syst. Ecol.* In press.
(13) Sonnenborn, V.; Kunau, W.-H. *Biochim. Biophys. Acta* **1982**, *712*, 523–534.
(14) Ishida, Y. *Mem. Coll. Agr., Kyoto Univ.* **1968**, *94*, 47–82; *Chem. Abstr.* **1969**, *70*, 65472u.
(15) Usdin, E., Borschardt, R. T., Creveling, C. R., Eds. *Biochemistry of S-Adenosylmethionine and Related Compounds*; MacMillan Press: London, 1982.

Table IV. Ratio of Dinosterol (2-K) to Peridinosterol (3-K) in *P. foliaceum* Cell-Free Extract Experiments (Experiments Grouped Together Are Directly Comparable)

	2-K/3-K
–SAH hydrolase	0.92
+SAH hydrolase	1.47
4.6 μM ^{3}H-SAM; 0.4 mM **9-K**	1.47
13.8 μM ^{3}H-SAM; 0.4 mM **9-K**	0.81
616 μM ^{3}H-SAM; 0.4 mM **9-K**	0.45
4.6 μM ^{3}H-SAM; 0.04 mM **9-K**	1.78
4.6 μM ^{3}H-SAM; 0.4 mM **9-K** (23-deuterio)	1.92
Tween 80	2.27
Lubrol PX	0.81
Δ^0 4α-Me nucleus (**9-K**)	1.26
Δ^5 nucleus (**9-N**)	3.22

Δ^5 (**N**) and 4α-methyl Δ^0 (**K**) sterol nuclei, the Δ^5 (**N**) sterol nucleus was a poorer substrate in the conversion of the brassicasterol side chain (**9**) to the dinosterol side chain (**2**) (Table II).

In addition to dinosterol (**2-K**), the enzymatic methylation of 4α-methylergost-22-enol (**9-K**) in the cell-free extract of *P. foliaceum* (Table III) also gave peridinosterol (**3-K**). Both side chains can be drawn as originating from a common reaction intermediate (**10**, Figure 3, Scheme 1). A similar case has been described for the biosynthesis of 24-methylenecycloartenol (**4-C**) and cyclolaudenol (**11-C**) in *Trebouxia*, a green alga (Figure 3, Scheme 2).[16] In experiments with deuterium-labeled precursors it was shown[17] that these arise from a single enzyme, having as the slow kinetic step the alkylation of the double bond to give a carbonium ion (**12**). A kinetic deuterium isotope effect in the fast step—deprotonation of the carbonium ion (**12**)—caused, in this case, changes in the product ratio. In our *P. foliaceum* extract, however, deuterium substitution of 4α-methylergost-22-enol (**9-K**) at C-23 caused only a slight change in the ratio of dinosterol (**2-K**) to peridinosterol (**3-K**) (Table IV). Significant changes in the product ratio (Table IV) resulted from changes in the concentrations of both of the substrates (sterol and SAM), in the sterol nucleus of the sterol substrate, in the detergent used to solubilize the sterol substrate in the enzyme assay, and—through the addition of *S*-adenosylhomocysteine hydrolase[18]—in the concentration of *S*-adenosylhomocysteine (SAH, the product of SAM-methyltransferases and a powerful inhibitor of these enzymes).[18,19] This is the first clear evidence for the involvement of two different SAM–sterol methyltransferases in an organism. Previous evidence for multiple enzymes has rested on kinetic measurements on poorly defined enzymatic systems.[20] Although *P. foliaceum* is a symbiotic association of a dinoflagellate and an endosymbiotic alga of Chrysophyte affinities,[21] it is unlikely that the two different enzymes come from the two different algae since the production of 23-methylated sterols is restricted to dinoflagellates.

An extract capable of converting 4α-methylergost-22-enol (**9-K**) to dinosterol (**2-K**) was also prepared from the cultured zooxanthella symbiont of the jellyfish *Cassiopea xamachana*[22] (Table III) but did not convert dinosterol (**2-K**) to 4α-methylgorgostanol (**1-K**). Demonstration of the conversion of dinosterol (**2-K**) to 4α-methylgorgostanol (**1-K**), however, was achieved using a cell-free extract of *P. foliaceum*, albeit at only very low levels. This result was reproducible and, in control experiments carried out without added sterol substrate (dinosterol (**2-K**)), but in which all other components of the incubation were kept the same, absolutely no radiolabeled 4α-methylgorgostanol (**1-K**) could be detected. The identity of the product was determined by co-purification with "cold" 4α-methylgorgostanol (**1-K**) by reverse-phase HPLC using two different solvent systems (Figure 4). The slightly shorter retention time of the radiolabeled product is due to a tritium isotope effect.[23]

The low yield of 4α-methylgorgostanol (**1-K**) suggested to us a physiological explanation for the attenuation of gorgosterol (**1-N**) production in zooxanthellae in the aposymbiotic state.[8] Kinetic measurements of other SAM–sterol methyltransferases have shown a two-stage binding scheme in which sterol binds to the SAM–enzyme complex (Figure 5).[17b,24] In the aposymbiotic state the cells are exposed to a higher osmotic strength environment (seawater). A typical osmotic buffer used by marine algae is dimethylpropiothetin,[25] the degradation of which to di-

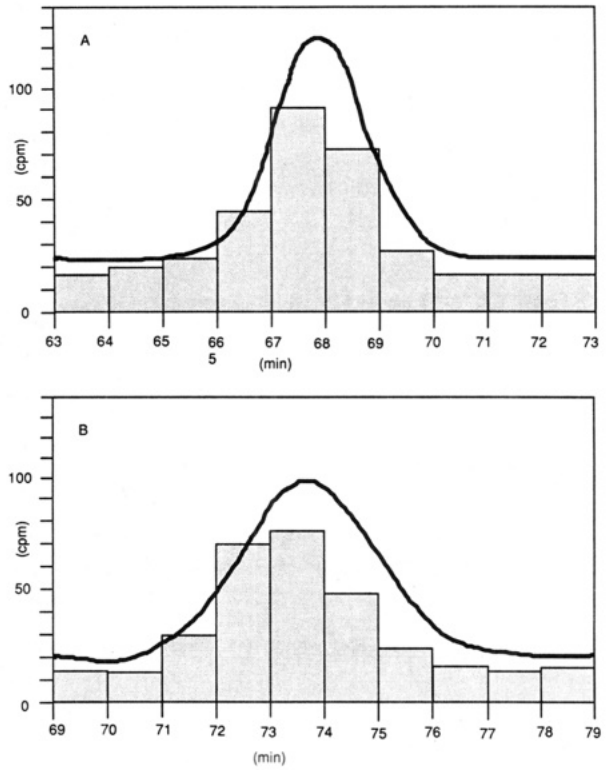


Figure 4. HPLC of radioactive 4α-methylgorgostanol (**1-K**) from ^{3}H-SAM and 4α-methylergost-22-enol (**9-K**) with *P. foliaceum* cell-free extract. (A) 4 mL/min MeOH. (B) 4 mL/min CH_3CN/MeOH/EtOAc (3:1:1).

$$\text{Enz} \longrightarrow \text{Enz}\cdot S_1 \longrightarrow \text{Enz}\cdot S_1 S_2 \longrightarrow \text{Enz}\cdot P_1 P_2$$

Figure 5. Enzyme binding scheme (S_1 = SAM; S_2 = sterol; P_1 = SAH; P_2 = sterol).

(16) Wojciechowski, Z. A.; Goad, L. J.; Goodwin, T. W. *Biochem. J.* **1973**, *136*, 405–412.
(17) (a) Mihailovic, M. M. Doctoral Thesis, Swiss Federal Institute of Technology, Zurich, 1984. (b) Giner, J.-L.; Djerassi, C. Manuscript in preparation.
(18) Deguchi, T.; Barchas, J. *J. Biol. Chem.* **1971**, *246*, 3175–3181.
(19) Brochardt, R. T. *J. Med. Chem.* **1980**, *23*, 347–357.
(20) (a) Bailey, R. B.; Thompson, E. D.; Parks, L. W. *Biochim. Biophys. Acta* **1974**, *334*, 127–136. (b) Rahier, A.; Narula, A. A. S.; Benveniste, P.; Schmitt, P. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 20–25.
(21) Withers, N.; Haxo, F. T. *Plant Sci. Lett.* **1975**, *5*, 7–15.
(22) Trench, R. K.; Coley, N. J.; Fitt, W. K. *Ber. Dtsch. Bot. Ges.* **1981**, *94*, 529–545.
(23) van Hook, W. A. *J. Liquid Chromatogr.* **1985**, *8*, 2699–2704.
(24) Oehlschlager, A. C.; Angus, R. H.; Pierce, A. M.; Pierce, H. D., Jr.; Srinivasan, R. *Biochemistry* **1984**, *23*, 3582–3589.

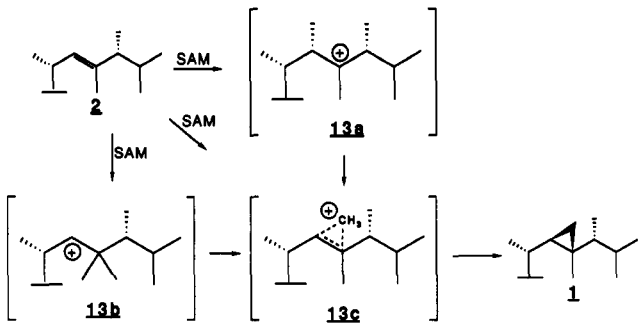


Figure 6. Possible intermediates to gorgosterol (1-N).

methyl sulfide is responsible for the characteristic smell of these organisms.[14] An increase in dimethylpropiothetin biosynthesis,[14,26] leading to lower levels of SAM, is expected to lead to a drop in gorgosterol (1-N) production. Alternatively the SAM–sterol methyltransferase may be inhibited by the concomitant rise in SAH concentration. Whether gorgosterol (1-N) has any physiological importance in symbiotic zooxanthellae is at this time unclear.

The detailed mechanism of the enzymatic formation of gorgosterol (1-N) is still uncertain. Does the methylation of the dinosterol side chain (2) occur at C-22 or C-23? Although alkylation at C-22 would give a more stable tertiary carbonium ion (13a, Figure 6),[5b] the alkylation of 4α-methylergost-22-enol (9-K) gives an intermediate with a secondary carbonium ion (10, Figure 3) without problems. It is therefore likely that this reaction proceeds in the same manner as do the other sterol side chain methylation reactions unique to dinoflagellates, with attack at C-23 (13b, Figure 6). If, however, instead of an open carbonium ion intermediate (13a,b), the protonated cyclopropane intermediate (13c, Figure 6) is generated directly in the methyl transfer reaction, then the point is moot. Such nonclassical species have long been considered possible intermediates in enzymatic C-alkylations.[27] Whether or not they are formed directly is at this point a matter of conjecture.

Summary

The biosynthesis of three unique 23-methylated dinoflagellate sterol side chains (1–3) has been demonstrated using cell-free extracts. The sequence of biological methylation reactions (5 → 4 → 9 → 2 → 1) in the biosynthesis of gorgosterol (1-N) is exactly as we postulated 20 years ago when the structure of gorgosterol was first established.[3b] Subsequently, many marine sterols have been isolated in this laboratory and in others pointing to biological methylation of the Δ^{22} double bond at C-23 in dinoflagellates.[4,5,9] It is likely that some of these sterols arise from the same enzyme through an incomplete specificity for the substrate or through the partitioning of a common high energy intermediate (e.g. Figure 3, Scheme 2). In the case of dinosterol and peridinosterol (2-K, 3-K), however, we have shown that more than one enzyme is acting in the methylation of their common intermediate 4α-methylergost-22-enol (9-K). This may be the first clear-cut evidence for multiple SAM–sterol methyltransferases in a single organism. Whether or not yet another enzyme is responsible for the methylation leading to the gorgosterol side chain (1), the recurrent pattern of C-23 methylation renders it likely that these enzymes are very closely related.

The often drastic drop in gorgosterol (1-N) levels in zooxanthellae, that have been separated from their host and cultured in seawater,[8] is proposed to be due to a diversion of SAM to the biosynthesis of the osmotic regulator dimethylpropiothetin.[14,25,26] This regulation could be due to either reduced levels of SAM, or to increased levels of SAH, or both. Whether this phenomenon has physiological consequences, perhaps relating to the symbiotic relationship, is an intriguing question.

Experimental Section

All compounds were purified by silica gel TLC and by reverse-phase HPLC. HPLC chromatography was carried out on Altex Ultras ODS 5-μm columns (10 mm i.d. × 25 cm) in series. The HPLC solvent was MeOH (3–4 mL/min) in all cases.

To analyze the products of enzymatic experiments "cold" carriers were added, HPLC separations were carried out collecting 60- and 30-s fractions, the fractions were allowed to evaporate and were counted by liquid scintillation spectrometry after the addition of scintillation fluid. HPLC retention times (MeOH, 4 mL/min): 4α-methylergosta-24(28)-enol (4-K), 42 min; peridinosterol (3-K), 49 min; dinosterol (2-K), 54 min; 4α-methylgorgostanol (1-K), 68 min. As a second solvent system for the identification of radiolabeled 4α-methylgorgostanol (1-K), CH_3CN/MeOH/EtOAc (3:1:1, 4 mL/min), was used (t_R = 73.5 min).

Desmosterol (5-N) and cholesta-7,24-dienol were synthesized as previously described.[28] Cholesta-8,24-dienol was extracted from yeast.[29] Lanosterol was obtained commercially and purified by HPLC. Cycloartenol was prepared from γ-oryzanol (Tsuno Rice Fine Chemicals Co., Ltd., Wakayama, Japan) by saponification and HPLC purification. 4-Demethyl-5-dehydrodinosterol (2-N) was synthesized as previously described.[30] Dinosterol, peridinosterol, and 4α-methylgorgostanol (1-K) were extracted from *P. foliaceum*.[9] Brassicasterol (9-N) and ergosta-7,22-dienol were prepared from ergosterol by lithium/ethylamine reduction.[31]

4α-Methylergosta-7,24(28)-dienol[32] was prepared from ergosta-7,24(28)-dienol (114 mg) by the sequence: PCC oxidation to the ketone (101 mg, 89%), deprotonation with LDA (0.7 mmol) in 10 mL of THF (room temperature), treatment of the resulting enolate with methyl iodide (1 mL), and $NaBH_4$ reduction of the crude mixture. The monomethylated sterols were isolated by silica gel TLC (eluent: hexanes/ether, 1:1) (73 mg, 73%) and were separated by HPLC to give the 4α-methyl sterol (60%, t_R 57 min) and the 2α-methyl isomer (40%, t_R 53 min). 2α-Methylergosta-7,24(28)-dienol: ^1H NMR (400 MHz) δ ($CDCl_3$) 5.159 (m, 1 H, C7), 4.713 (s, 1 H, C28), 4.658 (s, 1 H, C28), 3.145 (m, 1 H, C3), 1.027 (d, J = 7.0 Hz, 3 H, C26 or 27), 1.022 (d, J = 7.0 Hz, 3 H, C26 or 27), 0.981 (d, J = 6.4 Hz, 3 H, 2-Me), 0.953 (d, J = 6.5 Hz, 3 H, C21), 0.805 (s, 3 H, C19), 0.535 (s, 3 H, C18); low-resolution mass spectrum, m/z (relative intensity) 412 (M^+, $C_{29}H_{48}O$, 19) 397 (13), 328 (25), 285 (100), 69 (68), 55 (72). 4α-Methylcholesta-7,24-dienol and 4α-methylergosta-7,22-dienol[32] were prepared in the same way.

4α-Methylergost-22(*E*)-en-3β-ol[33] (9-K) was synthesized from ergosterol by the following sequence of reactions: Oppenauer oxidation to ergosta-4,7,22-trien-3-one,[34] acid-catalyzed isomerization to ergosta-4,6,22-trien-one,[35] Birch reduction to ergosta-

(25) (a) Dickson, D. M.; Wyn Jones, R. G.; Davenport, J. *Planta* **1980**, *150*, 158–165. (b) Larher, F.; Hamelin, J.; Stewart, G. R. *Phytochemistry* **1977**, *16*, 2019–2020.
(26) Greene, R. C. *J. Biol. Chem.* **1962**, *237*, 2251–2254.
(27) (a) Arigoni, D. *Ciba Found. Symp.* **1978**, *60*, 243–261. (b) Lederer, E. *Q. Rev. Chem. Soc.* **1969**, *23*, 453–481.
(28) Giner, J.-L.; Margot, C.; Djerassi, C. *J. Org. Chem.* **1989**, *54*, 2117.
(29) Taylor, U. F.; Kisic, A.; Pascal, R. A., Jr.; Izumi, A.; Tsuda, M.; Schroepfer, G. J., Jr. *J. Lipids Res.* **1981**, *22*, 171–177.
(30) Giner, J.-L.; Margot, C.; Djerassi, C. *J. Org. Chem.* **1989**, *54*, 369–373.
(31) Barton, D. H. R.; Lusinchi, X.; Magdzinski, L.; Ramirez, J. S. *J. Chem. Soc., Chem. Commun.* **1984**, 1236–1238.
(32) Sucrow, W.; Littmann, W.; Raduchel, B. *Chem. Ber.* **1977**, *110*, 1523–1531.
(33) Dow, W. C.; Gebreyesus, T.; Popov, S.; Carlson, R. M. K.; Djerassi, C. *Steroids* **1983**, *42*, 217–230.
(34) Shepherd, D. A.; Donia, R. A.; Campbell, J. A.; Johnson, B. A.; Holysz, R. P.; Slomp, G., Jr.; Stafford, J. E.; Pederson, R. L.; Ott, A. C. *J. Chem. Soc.* **1955**, *77*, 1212–1215.
(35) Blanke, E.; Krieger, B. *Chem. Abstr.* **1967**, *67*, P44030x.

4,22-dien-3-one,[36] Kirk–Petrow alkylation to 4-[(phenylthio)-methyl]ergosta-4,22-dienone,[37] Birch reduction to 4α-methylergost-22-en-3-one,[32] and, finally, Li(t-BuO)$_3$H reduction to 4α-methylergost-22(*E*)-en-3β-ol (**9-K**).

4α-Methylergosta-22(*E*),24(28)-dien-3β-ol (8-K). The procedure of Fryberg et al. was used.[38] 3β-Acetoxy-4α-methylpregnane-20(*S*)-carboxaldehyde (prepared by ozonolysis[39] from the acetate of 4α-methylergost-22(*E*)-en-3β-ol (**9-K**) or of 4α-methylstigmast-22(*E*)-en-3β-ol)[40] was treated with 1-(triphenylphosphoranylidene)-3-methyl-2-butanone[38] to give 3β-acetoxy-4α-methylcholest-22(*E*)-en-24-one: ^{1}H NMR (400 MHz) δ ($CDCl_3$) 6.706 (dd, 1 H, *J* = 15.6, 9.0 Hz, C22), 6.053 (d, 1 H, *J* = 15.6 Hz, C23), 4.362 (m, 1 H, C3), 2.832 (sept, 1 H, *J* = 6.9 Hz, C25), 2.046 (s, 3 H, OAc), 1.096 (d, 6 H, *J* = 6.9 Hz, C26 and 27), 1.082 (d, 3 H, *J* = 6.6 Hz, C21), 0.838 (s, 3 H, C19), 0.803 (d, 3 H, *J* = 6.3 Hz, C21), 0.684 (s, 3 H, C18); low-resolution mass spectrum, *m/z* (relative intensity) 456 (M^+, $C_{30}H_{48}O_3$, 17) 353 (9), 328 (11), 203 (34), 147 (37), 137 (48), 126 (100), 110 (45), 93 (50). Wittig methylenation of this compound followed by removal of the acetate group with $LiAlH_4$ gave 4α-methylergosta-22(E),24-(28)-dien-3β-ol (**8-K**): HPLC t_R 47 min (MeOH); ^{1}H NMR (400 MHz) δ ($CDCl_3$) 5.929 (d, *J* = 15.7 Hz, 1 H, C23), 5.570 (dd, *J* = 15.7, 8.8 Hz, 1 H, C22), 4.843 (s, 1 H, C28), 4.815 (s, 1 H, C28), 1.076 (d, *J* = 6.8 Hz, 3 H, C26 or 27), 1.062 (d, *J* = 6.8 Hz, 3 H, C26 or 27), 1.040 (d, *J* = 6.6 Hz, 3 H, C21), 0.945 (d, *J* = 6.4 Hz, 3 H, 4-methyl), 0.827 (s, 3 H, C19), 0.681 (s, 3 H, C18); high-resolution mass spectrum, *m/z* (relative intensity) 412.3681 (M^+, $C_{29}H_{48}O$, 3) (calc 412.3702), 397 (4), 316 (16), 301 (14), 287 (100), 161 (31), 123 (37), 107 (33), 81 (50).

4α-Methylergost-22(E)-en-3β-ol (**9-K**) acetate (0.2 g) in 7 mL of THF was treated with 5 mL of 1 M BH_3/THF.[30] After 3 h at room temperature 3 mL of 20% NaOH was added, followed by 2 mL of 30% H_2O_2. The mixture was extracted with ether after another 45 min, and the products were purified by silica gel TLC (eluent: hexanes/ether, 4:1) to give a mixture of 22-hydroxy- and 23-hydroxy-4α-methylergostan-3β-yl acetate (115 mg, 55%). These were separated by normal-phase HPLC (eluent: 6% EtOAc/hexanes, 6 mL/min), and the structures were assigned by comparison of the NMR spectra with sterols bearing the same side chains.[30]

(23*R*,24*R*)-3β-Acetoxy-4α-methylergostan-23-ol (27%): HPLC t_R 13 min; ^{1}H NMR (400 MHz) δ ($CDCl_3$) 4.362 (m, 1 H, C3), 3.695 (m, 1 H, C23), 2.046 (s, 3 H, OAc), 0.945 (d, *J* = 6.4 Hz, 3 H, C28), 0.933 (d, *J* = 6.8 Hz, 3 H, C21), 0.855 (d, *J* = 6.9 Hz, 3 H, C26 or 27), 0.834 (s, 3 H, C19), 0.833 (d, *J* = 6.7 Hz, 3 H, C26 or 27), 0.803 (d, *J* = 6.3 Hz, 3 H, 4-Me), 0.677 (s, 3 H, C18).

(22*R*,24*S*)-3β-Acetoxy-4α-methylergostan-22-ol (31%): HPLC t_R 19 min; ^{1}H NMR (400 MHz) δ ($CDCl_3$) 4.362 (m, 1 H, C3), 3.739 (m, 1 H, C23), 2.046 (s, 3 H, OAc), 0.903 (d, *J* = 6.4 Hz, 6 H, C21 and 24), 0.839 (d, *J* = 6.8 Hz, 3 H, C26 or 27), 0.834 (s, 3 H, C19), 0.804 (d, *J* = 6.4 Hz, 3 H, 4-Me), 0.765 (d, *J* = 6.9 Hz, 3 H, C26 or 27), 0.662 (s, 3 H, C18); low-resolution mass spectrum, *m/z* (relative intensity) 474 (M^+, $C_{31}H_{54}O_3$, 1) 456 (9), 300 (61), 285 (36), 271 (33), 259 (15), 230 (36), 121 (40), 107 (40), 97 (74), 81 (61), 71 (68), 55 (100).

(23*S*,24*R*)-3β-Acetoxy-4α-methylergostan-23-ol (15%): HPLC t_R 21 min; ^{1}H NMR (400 MHz) δ ($CDCl_3$) 4.362 (m, 1 H, C3), 3.597 (m, 1 H, C23), 2.046 (s, 3 H, OAc), 1.035 (d, *J* = 6.5 Hz, 3 H, C28), 0.906 (d, *J* = 6.8 Hz, 3 H, C21), 0.838 (d, *J* = 6.7 Hz, 3 H, C26 or 27), 0.834 (s, 3 H, C19), 0.804 (d, *J* = 6.3 Hz, 3 H, 4-Me), 0.788 (d, *J* = 6.8 Hz, 3 H, C26 or 27), 0.659 (s, 3 H, C18).

(22*S*,24*S*)-3β-Acetoxy-4α-methylergostan-22-ol (28%): HPLC t_R 31 min; ^{1}H NMR (400 MHz) δ ($CDCl_3$) 4.362 (m, 1 H, C3), 3.742 (m, 1 H, C23), 2.046 (s, 3 H, OAc), 0.895 (d, *J* = 6.6 Hz, 3 H, C21), 0.865 (d, *J* = 6.7 Hz, 3 H, C28), 0.836 (s, 3 H, C19), 0.815 (d, *J* = 6.6 Hz, 3 H, C26 or 27), 0.810 (d, *J* = 6.6 Hz, 3 H, C26 and 27), 0.805 (d, *J* = 6.1 Hz, 3 H, 4-Me), 0.655 (s, 3 H, C18); low-resolution mass spectrum, *m/z* (relative intensity) 474 (M^+, $C_{31}H_{54}O_3$, 1), 456 (6), 300 (11), 229 (11), 176 (22), 121 (63), 97 (98), 95 (100), 81 (75), 71 (62), 55 (97).

4α-Methylergost-23(*E*)-en-3β-ol (6-K). Phosphorus oxychloride dehydration of (23*R*,24*R*)-3β-acetoxy-4α-methylergostan-23-ol gave the product of anti elimination as previously reported.[30] Deprotection with $LiAlH_4$ gave **6-K**: HPLC t_R 63 min (MeOH); ^{1}H NMR (400 MHz) δ ($CDCl_3$) 5.131 (m, 1 H, C23), 1.539 (s, 3 H, C28), 0.981 (d, *J* = 6.8 Hz, 6 H, C26 and 27), 0.946 (d, *J* = 6.4 Hz, 3 H, 4-methyl), 0.868 (d, *J* = 6.5 Hz, 3 H, C21), 0.821 (s, 3 H, C19), 0.651 (s, 3 H, C18); high-resolution mass spectrum, *m/z* (relative intensity) 414.3879 (M^+, $C_{29}H_{50}O$, 4) (calc 414.3859), 399 (11), 317 (18), 315 (15), 299 (87), 287 (100), 149 (80), 95 (100), 81 (78), 55 (85).

4α-Methylergost-23(*Z*)-en-3β-ol (7-K). Phosphorus oxychloride dehydration of (23*S*,24*R*)-3β-acetoxy-4α-methylergostan-23-ol gave the product of anti elimination as previously reported.[30] Deprotection with $LiAlH_4$ gave **7-K**: HPLC t_R 62 min (MeOH); ^{1}H NMR (400 MHz) δ ($CDCl_3$) 5.022 (m, 1 H, C23), 1.598 (s, 3 H, C28), 0.943 (d, *J* = 6.8 Hz, 9 H, 4-methyl, C26 and 27), 0.883 (d, *J* = 6.4 Hz, 3 H, C21), 0.821 (s, 3 H, C19), 0.647 (s, 3 H, C18); high-resolution mass spectrum, *m/z* (relative intensity) 414.3891 (M^+, $C_{29}H_{50}O$, 7) (calc 414.3859), 399 (10), 330 (11), 317 (22), 315 (15), 299 (85), 287 (100), 203 (26), 177 (24), 149 (28).

(24*R*)-23-Deuterio-4α-methylergost-22(*E*)-en-3β-ol (9-K). The mixture of the 22- and 23-hydroxy sterol acetates was oxidized with pyridinium chlorochromate to the ketones. Separation by HPLC (MeOH) gave (24*S*)-3β-acetoxy-4α-methylergostan-22-one and (24*R*)-3β-acetoxy-4α-methylergostan-23-one.

(24*S*)-3β-Acetoxy-4α-methylergostan-22-one (62%): HPLC t_R 27 min; ^{1}H NMR (400 MHz) δ ($CDCl_3$) 4.362 (m, 1 H, C3), 2.480 (m, 1 H, C20), 2.432 (dd, 1 H, *J* = 17.0, 4.2 Hz, C23), 2.176 (dd, 1 H, *J* = 17.0, 9.0 Hz, C23), 2.044 (s, 3 H, OAc), 1.067 (d, *J* = 6.8 Hz, 3 H, C21), 0.858 (d, *J* = 6.8 Hz, 3 H, C28), 0.834 (s, 3 H, C19), 0.816 (d, *J* = 6.7 Hz, 3 H, C26 or 27), 0.800 (d, *J* = 6.3 Hz, 3 H, 4-Me), 0.795 (d, *J* = 6.4 Hz, 3 H, C26 or 27), 0.664 (s, 3 H, C18); low-resolution mass spectrum, *m/z* (relative intensity) 472 (M^+, $C_{31}H_{52}O_3$, 11) 271 (10), 175 (10), 161 (17), 149 (23), 121 (41), 113 (73), 95 (100), 81 (46), 72 (48), 55 (56).

(24*R*)-3β-Acetoxy-4α-methylergostan-23-one (38%): HPLC t_R 29 min; ^{1}H NMR (400 MHz) δ ($CDCl_3$) 4.362 (m, 1 H, C3), 2.453 (dd, 1 H, *J* = 16.8, 2.7 Hz, C22), 2.295 (dq, 1 H, *J* = 6.9, 6.9 Hz, C24), 2.180 (dd, 1 H, *J* = 16.7, 10.0 Hz, C22), 2.044 (s, 3 H, OAc), 0.970 (d, *J* = 6.9 Hz, 3 H, C28), 0.902 (d, *J* = 6.7 Hz, 3 H, C21 or 26), 0.892 (d, *J* = 6.4 Hz, 3 H, C21 or 26), 0.831 (s, 3 H, C19), 0.831 (d, *J* = 6.7 Hz, 3 H, C27), 0.801 (d, *J* = 6.4 Hz, 3 H, 4-Me), 0.685 (s, 3 H, C18).

Reduction of the 23-ketone with a saturated solution of $NaBD_4$ in i-PrOH at room temperature for 12 h gave a mixture of the 23-deuterio-23-hydroxy compounds. These were converted to their methanesulfonates through treatment with methanesulfonyl chloride and triethylamine in CH_2Cl_2. The crude methanesulfonates were eliminated by heating with KOt-Bu in t-BuOH under reflux for 16 h (treatment with DBU at 120 °C gave only the Zaitchev Δ^{23} olefins **6-K** and **7-K**). The product mixture was purified after extraction by silica gel TLC (eluent: hexanes/ether, 1:1) and HPLC to give 23-deuterio-4α-methylergost-23(*Z*)-en-3β-ol (**7-K**, 21%), 23-deuterio-4α-methylergost-23(*E*)-en-3β-ol (**6-K**, 51%), and (24*R*)-23-deuterio-4α-methylergost-22-en-3β-ol (**9-K**, 28%). Deuterium substitution simplified the NMR spectrum of the Δ^{22} olefinic protons from a multiplet of 2 protons centered at 5.168 (δ) to a doublet of 1 proton (5.141, *J* = 8.2 Hz, C22); low-resolution mass spectrometry showed M^+ = 415 ($C_{29}H_{49}OD$).

4α-Methylcholest-24-en-3β-ol (5-K). Hydrogenation of 3β-acetoxy-4α-methylcholest-22(*E*)-en-24-one over 5% Pd/C in EtOAc gave 3β-acetoxy-4α-methylcholestan-24-one: ^{1}H NMR (400 MHz) δ ($CDCl_3$) 4.361 (m, 1 H, C3), 2.605 (sept, 1 H, *J* = 7.0 Hz, C25), 2.406 (m, 2 H, C23), 2.046 (s, 3 H, OAc), 1.084 (d, 6 H, *J* = 7.0 Hz, C26 and 27), 0.993 (d, 3 H, *J* = 6.4 Hz, C21), 0.831 (s, 3 H, C19), 0.803 (d, 3 H, *J* = 6.4 Hz, C21), 0.636 (s, 3 H, C18); low-resolution mass spectrum, *m/z* (relative intensity) 458 (M^+, $C_{30}H_{50}O_3$, 23), 383 (18), 372 (14), 357 (12), 329 (24), 229 (54), 161 (61), 121 (92), 107 (92), 95 (100), 81 (92), 71 (90).

Reduction of the 24-ketone to the 24-alcohol ($NaBH_4$) followed by phosphorus oxychloride dehydration and deprotection with $LiAlH_4$ gave **5-K**: HPLC t_R 61 min (MeOH); ^{1}H NMR (400 MHz)

(36) (a) Johnson, F.; Newbold, G. T.; Spring, F. S. *J. Chem. Soc.* **1954**, 1302–1306. (b) Daglish, A. F.; Green, J.; Poole, V. D. *Ibid.* **1954**, 2627–2633.
(37) Kirk, D. N.; Petrow, V. *J. Chem. Soc.* **1962**, 1091–1096.
(38) Fryberg, M.; Oehlschlager, A. C.; Unrau, A. M. *Tetrahedron* **1971**, *27*, 1261–1274.
(39) Shu, A. Y. L.; Djerassi, C. *Tetrahedron Lett.* **1981**, *22*, 4627–4630.
(40) Knapp, F. F.; Schroepfer, G. J. *Steroids* **1975**, *26*, 339–357.

δ ($CDCl_3$) 5.087 (m, 1 H, C24), 1.679 (s, 3 H, C26 or 27), 1.598 (s, 3 H, C26 or 27), 0.946 (d, J = 6.4 Hz, 3 H, 4-methyl), 0.916 (d, J = 6.6 Hz, 3 H, C21), 0.822 (s, 3 H, C19), 0.645 (s, 3 H, C18); high-resolution mass spectrum, m/z (relative intensity) 400.3695 (M^+, $C_{28}H_{48}O$, 27) (calc 400.3685), 385 (49), 367 (32), 315 (33), 287 (77), 269 (88), 229 (45), 161 (58), 135 (46), 121 (68), 95 (100), 81 (82).

***Cryptothecodinium cohnii* Cell-Free Extract.** *C. cohnii* cells were cultured as previously described.[41] Eight day old cells from 1.5 L of cultures (8 g) were harvested by centrifugation and suspended with the aid of a glass–Teflon homogenizer with 20 mL of ice-cold 100 mM Na HEPES (*N*-(2-hydroxyethyl)-piperazine-*N*'-2-ethanesulfonic acid hemisodium salt) buffer (pH 7.5), containing 2 mM DTT (dithiothreitol), 10 mM ascorbic acid, 5 mM EDTA, 1 mM $MgCl_2$, 1% (w/v) BSA (bovine serum albumin), 0.5% (w/v) PVP 40 (polyvinylpyrrolidone), to which 0.2 mg of Leupeptin and a solution of 4 mg of PMSF (phenylmethanesulfonyl fluoride) in 0.1 mL of EtOH were added (Buffer A).[42] The cells were broken by passage through a French pressure cell at 8000 psi. The broken cells were centrifuged (3000*g*, 5 min). The yellow lipid layer was removed, and the large beige pellet was discarded. The supernatant was centrifuged again (7600*g*, 10 min) and this supernatant was centrifuged at a higher speed (38700*g*, 80 min). The pellet of the final centrifugation was suspended in 2 mL of Buffer A with a glass–Teflon homogenizer and frozen in liquid N_2 in 0.7-mL aliquots. The cell-free extracts stay active for years when stored in this way.

***Peridinium foliaceum* Cell-Free Extract.** *P. foliaceum* was cultured as previously described.[21] Log-phase cells were harvested by centrifugation and suspended with the aid of a glass–Teflon homogenizer with ice-cold 100 mM Tris-HCl (tris(hydroxymethyl)aminomethane hydrochloride) buffer (pH 7.5) containing 15% (w/v) glycerol. The cells were stored for 5 years at −80 °C. A portion of the frozen cells was thawed and the cells were pelleted (2000*g*, 5 min). The cells (30 g) were suspended in 50 mL (at 0 °C) of a modification of buffer A containing 0.5 mg of Pepstatin, but no $MgCl_2$ (buffer A'). The cells were broken by two passages through a French pressure cell at 8000 psi. The broken cells were centrifuged (2000*g*, 5 min). A portion of the large pellet was suspended in buffer A'. The supernatant was centrifuged (125000*g*, 1 h), and the pellet was suspended in buffer A'. The low-speed pellet was found to contain more SAM–sterol methyltransferase activity than the high-speed pellet, in this case, and was used for the experiments.

Zooxanthellae of *Cassiopea xamachana* Cell-Free Extract. Aposymbiotic zooxanthellae were cultured as previously described.[8a] The cell-free extract was prepared as described for *P. foliaceum* (the high-speed pellet was used).

SAM–Sterol Methyltransferase Assay. Sterol substrates were purified by TLC and HPLC. To solubilize the sterol, Tween 80 (or Lubrol PX for the *C. cohnii* experiments) (10 mg) was mixed with sterol (0.5 mg) in $CHCl_3$. After the solvent was evaporated, the mixture was dissolved in 1 mL of H_2O.

To disposable 1.5-mL plastic centrifuge tubes was added to 10 μL of the cell-free extract, 10 μL of sterol suspension (or 1% Tween 80 or Lubrol PX for the blanks), and 10 μL (10 μCi) of ^{3}H-SAM ([^{3}H]-*S*-adenosylmethionine, Amersham Corp., 72 Ci/mmol). When less ^{3}H-SAM was sufficient to achieve high incorporation of radioactivity into products, a dilution was used. When more ^{3}H-SAM was required to obtain more radioactivity in the products, the components of the reaction were increased proportionately. The labeled SAMs of lower specific activity used to look at their effect on the dinosterol (**2-K**)/peridinosterol (**3-K**) ratio in the *P. foliaceum* cell-free extract were ^{3}H-SAM (New England Nuclear, 12 Ci/mmol) and ^{14}C-SAM (ICN Biomedicals, Inc., 47 mCi/mmol). The effect of *S*-adenosylhomocysteine hydrolase on the dinosterol (**2-K**)/peridinosterol (**3-K**) ratio was tested through the addition of 10 μL of a 1:2 dilution of the enzyme (EC 3.3.1.1., from rabbit erythrocytes, 8 units/mL in 24 mM Tris pH 7.4, containing 1 mM DTT, 1 mM EDTA, and 20% glycerol, Sigma Chemical Co.) with 100 mM Na HEPES.

The components of the enzymatic reaction were mixed on ice, centrifuged briefly and vortexed to ensure mixing, and incubated at 27 °C in a water bath shaker. After 4 h the reaction was extracted with 1 mL of EtOAc containing 0.3 mg of cholesterol (as a marker) by shaking with a reciprocating shaker (Mini-Bead Beater) for 10 s. The phases were separated by centrifugation, the aqueous phase was frozen with dry ice, and the organic layer was drawn off. After evaporation of solvent the extracts were applied in hexanes/ether (9:1) to Pasteur pipette columns containing 5.5 cm (0.75 g) of florisil and eluted with 4.5 mL of the same solvent mixture. The pipette columns were put into new vials and eluted with 6.5 mL of hexanes/ether (1:1) to elute the sterols. After evaporation of the solvent, the sterol fraction could be counted directly, or, after the addition of the appropriate cold carriers, be analyzed by HPLC. In the analysis of radiolabeled 4α-methylgorgostanol (**1-K**) an additional purification by silica gel TLC (eluent: hexanes/ether, 1:1) was carried out prior to HPLC analysis. All experiments were carried out at least in duplicate. The results are summarized in Tables I–IV.

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Supplementary Material Available: ^{1}H NMR spectra for new compounds described in this paper (11 pages). Ordering information is given on any current masthead page.

(41) Tuttle, R. C.; Loeblich, A. R., III *Phycologia* **1975**, *14*, 1–8.
(42) Marsden, W. J. N.; Callow, J. A.; Evans, L. V. *Mar. Biol. Lett.* **1981**, *2*, 353.