Equilibria. For the **3c–4c** system, the same equilibrium content of lactone was determined spectrally from either starting material. Normal procedures could not be used for the **3d–4d** system. Equilibrium spectra were recorded for solutions of **4d** at pH 10–13. The equilibrium concentrations of **4d** were calculated from the previously determined absorbance values of $\mathbf{3d}^-$, $\mathbf{3d}^{2-}$, and **4d**. Values of $K_{app} = [\mathbf{4d}]/[\mathbf{3d}]_{tot}$ were then calculated for each pH. We define $K_{eq} = [\mathbf{4d}]/[\mathbf{3d}] = K_{app}/f_{AH}$, where f_{AH} is the fraction of neutral **3d** at the given pH value, and $f_{AH_2} = [H^+]^2/([H^+]^2 + K_1[H^+] + K_1K_2)$. Thus, $\log K_{app} = \log f_{AH} + \log K_{eq}$; a plot of $\log K_{app}$ vs. $\log f_{AH_2}$ was fitted to a slope of 1, and $\log K_{eq}$ was obtained as the intercept (Figure 5).

Kinetic Measurements. Kinetic measurements were performed as previously described,[2,21] in media containing 20% (by volume) of purified dioxane; acid and base concentrations are final values. All measurements were made at 30 ± 0.1 °C and at a total ionic strength of 0.3 M (NaCl). The majority of kinetic runs were made in the absence of buffer by use of a pH stat–spectrophotometer combination.[34] Rates of lactonization were followed by the increase in absorption at 240–260 nm or the decrease at 270–285 nm, and the reverse for lactone hydrolysis. Phenolic acids **3a–c** were sufficiently stable for isolation; in the case of **3d**, however, the lactone was opened in 0.08 M KOH (20% dioxane), and aliquots of this stock solution were added to appropriate buffer media or to media of the desired pH value. For the majority of runs, initial and final pH values differed by less than 0.05 unit. For calculations, final pH values were taken routinely. In kinetic runs, correlation coefficients generally exceeded 0.998, and values of k_{obsd} were usually reproducible to ±2% or better, reproducibility decreasing somewhat for the very fast reactions.

Registry No. **3a**, 614-75-5; **3b**, 86549-97-5; **3c**, 86549-98-6; **3d**, 86549-99-7; **4a**, 553-86-6; **4b**, 33901-25-6; **4c**, 13524-76-0; **4d**, 86562-99-4; **6**, 86550-00-7; **7a**, 26172-13-4; **7b**, 86563-00-0.

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Isolation, Partial Synthesis, and Structure Determination of Sterols with the Four Possible 23,24-Dimethyl-Substituted Side Chains[1]

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Received March 15, 1983

(23*R*,24*R*)- and (23*S*,24*R*)-4α,23,24-trimethyl-5α-cholestan-3β-ol, (23*R*,24*R*)- and (23*S*,24*R*)-23,24-dimethyl-5α-cholestan-3β-ol, and (23*S*,24*R*)-23,24-dimethylcholest-5-en-3β-ol were isolated from various marine organisms. Methyl substitution at C-23 and C-24 was proved by comparison of their 360-MHz ^{1}H NMR spectra with those of the hydrogenation products of dinosterol ((*E*,24*R*)-4α,23,24-trimethyl-5α-cholest-23-en-3β-ol) and synthetic 24-epidinosterol and 4-demethyl-5-dehydro-24-epidinosterol. The configuration at C-23 in the natural products was determined by chemical correlation of one of them with peridinosterol ((*E*,23*R*,24*R*)-4α,23,24-trimethyl-5α-cholest-17(20)-en-3β-ol). The configuration at C-23 of sterols with a 23,24(*S*)-dimethyl-substituted side chain was solved by X-ray analysis of the *p*-bromobenzoate of (23*R*,24*S*)-23,24-dimethyl-5α-cholestan-3β-ol.

Research on cultured unicellular marine algae has solved the problem of the origin of two cyclopropyl sterols, viz., gorgosterol (**3m**) and 23-demethylgorgosterol (**3l**), as it has been shown[2] that dinoflagellates (unicellular algae belonging to the phylum Pyrrhophyta) are primary sources of sterols with such side chains (**m,l**). Dinoflagellates are also sources of sterols with other uncommon side chains such as dinosterol[3] (**1i**) and its Δ^5,[4] $\Delta^{8(14)}$,[5,6] Δ^{14},[5] 4-demethyl[2,7] (**2i**), and 4-demethyl-5-dehydro[2] (**3i**) analogues, 24-demethyldinosterol[8,9] (**1h**) and its 4-demethyl[2] (**2h**) and 4-demethyl-5-dehydro[2,8] (**3h**) analogues, and 24-epioccelasterol[10] (**3g**). Sterols with the corresponding saturated side chains have not yet been unequivocally found in marine organisms with the exception of one claim[11] whose validity is discussed below.

We now report the partial synthesis and structure determination of sterols with the four possible 23,24-dimethyl-substituted side chains (**a–d**) and the isolation of five sterols having two of these side chains and three different nuclei.

Results and Discussion

The 360-MHz ^{1}H NMR and chromatographic mobility data of the natural products (**1a,b**, **2a,b**, and **3b**, Figure 1) discussed in this paper are listed in Table I together with their sources. The NMR data clearly show that the five naturally occurring sterols have two different side chains, **a** and **b**. In other papers[5,6] we have described how the structure of the skeleton and the site of nuclear unsaturation are deduced by NMR and mass spectra, including application of Zürcher's rules.[12] To avoid repetition, we will discuss here only the determination of the structure of the side chains by a combination of chemical and spectroscopic methods.

The NMR spectra of the 4α-methyl sterols of M_r = 430 (Table I) include six methyl doublets (one at δ 0.946 due

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Table I. Chromatographic Behavior, Sources, and 360-MHz ^{1}H NMR Data[a] of Five Natural Sterols (1a,b, 2a,b, 3b) and of 15 Structurally Related, Synthetic Steroids

structure	M_r	GC RRT[b]	HPLC RRT[c]	^{1}H NMR, δ								
				C3 H	C4 Me	C18 H	C19 H	unassigned methyl doublets				
1a[d,e]	430	1.91	1.77	3.1 (m)	0.946 (d, 6.3)	0.661 (s)	0.823 (s)	0.880 (6.8)	0.872 (6.0)	0.813 (7)	0.724 (6.9)	0.700 (6.7)
1b[f]	430	1.80	1.65	3.1 (m)	0.946 (d, 6.3)	0.648 (s)	0.822 (s)	0.918 (6.6)	0.887 (6.8)	0.872 (6.7)	0.789 (6.8)	0.759 (7.0)
1c	430	1.95	1.75	3.1 (m)	0.945 (d, 6.4)	0.660 (s)	0.822 (s)	0.876 (6.7)	0.864 (6.3)	0.812 (7)	0.806 (6.7)	0.723 (6.9)
1d	430	1.85	1.59	3.1 (m)	0.947 (d, 6.3)	0.658 (s)	0.825 (s)	0.891 (6.4)	0.876 (6.5)	0.871 (6)	0.691 (6.9)	0.685 (6.8)
1f	430	1.64	1.51	3.1 (m)	0.944 (d, 6.4)	0.645 (s)	0.818 (s)	0.887 (6.6)	0.875 (6.5)	0.784 (6.5)	0.718 (6.8)	0.684 (6.8)
1x[g]	430			3.1 (m)	0.945 (d, 6.7)	0.643 (s)	0.822 (s)	0.915 (6.5)	0.905 (6.9)	0.855 (6.0)	0.781 (6.5)	0.772 (6.9)
2a[e,h]	416	1.63	1.54	3.6 (m)		0.661 (s)	0.801 (s)	0.878 (6.7)	0.871 (6.4)	0.811 (7)	0.721 (6.9)	0.698 (6.7)
2b[h,i]	416	1.62	1.44	3.6 (m)		0.647 (s)	0.798 (s)	0.916 (6.5)	0.887 (6.7)	0.873 (6.5)	0.788 (7)	0.757 (7.0)
2c	416	1.67	1.52	3.6 (m)		0.660 (s)	0.800 (s)	0.874 (6.7)	0.863 (6.3)	0.809 (6.8)	0.805 (6.4)	0.721 (6.9)
2d	416	1.55	1.39	3.6 (m)		0.656 (s)	0.800 (s)	0.889 (6.3)	0.871 (6.1)	0.866 (6.6)	0.684 (6.9)	0.677 (6.9)
3b[j]	414			3.5 (m)		0.679 (s)	1.006 (s)	0.933 (6.5)	0.887 (6.7)	0.875 (6.7)	0.790 (6.8)	0.761 (7.0)
4f	430	1.64	1.35	3.73 (m)	0.908 (d, 6.3)	0.645 (s)	0.800 (s)	0.887 (6.7)	0.875 (6.5)	0.783 (6.5)	0.717 (6.8)	0.683 (6.8)
5a	416	1.61	1.24	4.06 (m)		0.663 (s)	0.775 (s)	0.879 (6.8)	0.875 (6.3)	0.813 (6.7)	0.724 (6.9)	0.699 (6.7)
5b	416	1.56	1.13	4.06 (m)		0.647 (s)	0.772 (s)	0.917 (6.5)	0.883 (7)	0.869 (6.4)	0.785 (7)	0.756 (7.0)
5c	416	1.64	1.22	4.06 (m)		0.664 (s)	0.775 (s)	0.877 (6.6)	0.868 (6.5)	0.812 (6.7)	0.807 (6.6)	0.724 (6.9)
5d	416	1.52	1.09	4.06 (m)		0.659 (s)	0.776 (s)	0.889 (6.6)	0.874 (6.3)	0.873 (6.1)	0.689 (6.9)	0.682 (6.8)
6a	428	2.05	2.02		0.971 (d, 6.5)	0.689 (s)	1.026 (s)	0.880 (7)	0.877 (6)	0.814 (6.7)	0.725 (7.1)	0.702 (7)
6b	428	1.99	1.89		0.669 (d, 6.5)	0.673 (s)	1.063 (s)	0.919 (6.5)	0.886 (6.7)	0.871 (6.6)	0.788 (6.7)	0.758 (7.0)
6c	428	2.08	2.00		0.971 (d, 6.5)	0.689 (s)	1.026 (s)	0.877 (6.6)	0.869 (6.6)	0.815 (6.6)	0.807 (6.7)	0.725 (6.9)
6d	428	1.93	1.83		0.971 (d, 6.5)	0.684 (s)	1.067 (s)	0.893 (6.3)	0.876 (6)	0.873 (7)	0.692 (6)	0.683 (6.8)
6f	428	1.70	1.84		0.970 (d, 6.5)	0.676 (s)	1.062 (s)	0.891 (6.5)	0.879 (6.5)	0.791 (6.4)	0.723 (6.8)	0.688 (7)

[a] Recorded in $CDCl_3$; internal standard $CHCl_3$. Shifts are δ values. Multiplicities and splitting constants (in hertz) are given in parentheses. Rounded values for J indicate that no accurate values could be given because of overlapping peaks. [b] Capillary SE54 column, 260 °C, standard cholesterol. [c] Altex Ultrasphere ODS columns, absolute MeOH, standard cholesterol. [d] From *Protoceratium reticulatum*[14] and *Ascidia nigra*.[15] [e] From *Orbulina universa* zooxanthellae. [f] From *Siphonogorgia* sp. [g] 220-MHz NMR data.[11] [h] This is a known synthetic compound.[7] [i] From *Bugula neritina*.[16] [j] From *Stelleta conulosa*.[17]

to the 4-methyl group[4-6]); the other spectra show five methyl doublets, three of which are caused by C-21, C-26, and C-27 protons, leaving two doublets for methyl substituents in the side chain. Although there are three possible sets of side chains with two additional methyl groups that would give five methyl NMR doublets (22,23-, 22,24-, and 23,24-dimethyl), only the last alkylation pattern was consistent with the mass spectra which, irrespective of the nuclei (**1**–**3**), showed strong peaks at m/z 98 (usually 100%) and weak ($M^+ - 97$) peaks. These peaks are best explained by cleavage of the C-22,C-23 bond with generation of a $C_7H_{14}^+$ or a ($M^+ - C_7H_{13}$) fragment.

Reference sterols with the four epimeric 23,24-dimethyl-substituted side chains (**a**–**d**) are available by hydrogenation of naturally occurring dinosterol (**1i**) and of synthetic 24-epidinosterol[13] (**1j**) or its 4-demethyl-5-dehydro analogue (**3j**) as well as of synthetic 23-methylene-24-methyl-5α-cholestan-3β-ol[7] (**2k** or epimer at C-24).

Hydrogenation of dinosterol (**1i**), 24-epidinosterol (**1j**), and 4-demethyl-5-dehydro-24-epidinosterol (**3j**) in hexane–HOAc or EtOAc–HOAc, using Adam's catalyst, appeared to be completely stereoselective (vide infra) as the products isolated by reverse-phase HPLC (ODS-2 column) were pure by 360-MHz ^{1}H NMR. This hydrogenation product of dinosterol and sterols of M_r = 430 isolated from the dinoflagellate *Protoceratium reticulatum*,[14] the tunicate *Ascidia nigra*,[15] and cultured zooxanthellae (algal symbionts) of the foram *Orbulina universa* were identical by 360-MHz ^{1}H NMR. In this manner the stereochemistry (24*R*) at C-24 in side chain **a** was determined. A trace sterol of M_r = 416 (**2a**) of the zooxanthellae from *Orbulina* had the same side chain (see Table I). The last sterol (**2a**) is a known synthetic compound[7] obtained as the major product in the hydrogenation of 23-methylene-24(*R*)-methyl-5α-cholestan-3β-ol (**2k**). The minor product of this

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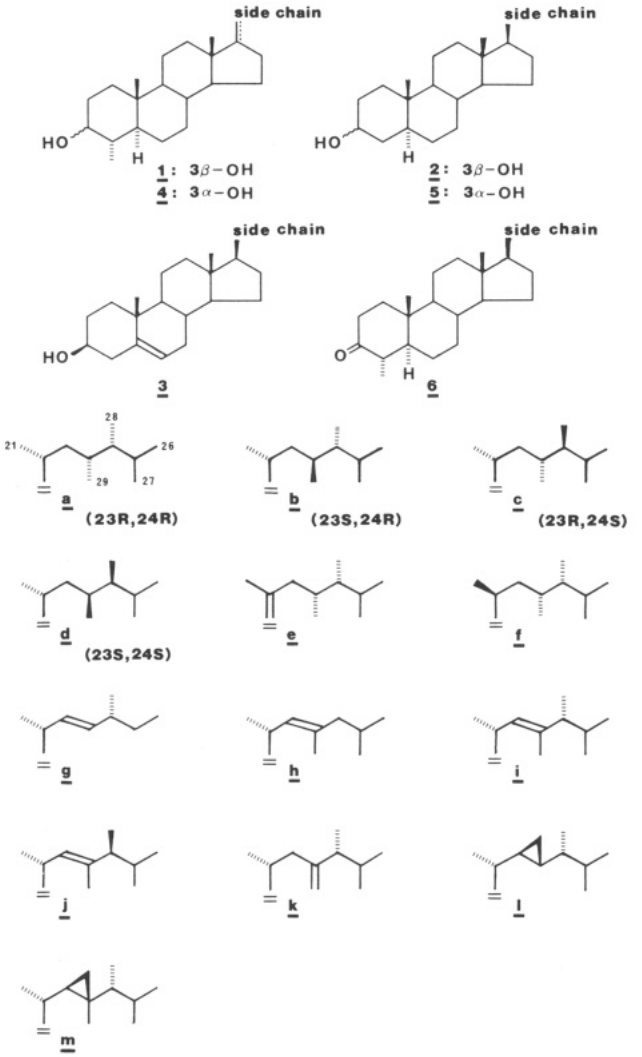

Figure 1. Structures of selected steroids.

reaction and a sterol isolated from the bryozoan *Bugula neritina*[16] were identical by NMR; thus the *Bugula* sterol also has the 24*R* configuration (side chain **b**). A 4α-methyl sterol of M_r = 430 and a Δ^5 sterol of M_r = 414 had the same side chain (**b**). They were isolated from the soft coral *Siphonogorgia* sp. and for the sponge *Stelleta conulosa*,[17] respectively.

An argument in favor of the 23*R*,24*R* configuration of side chain **a** was the co-occurrence of two sterols with side chain **a** (M_r = 430 and 416; see Table I) and peridinosterol (**1e**) in the sterol fraction of the zooxanthellae from *Orbulina universa* (for abundances see Experimental Section). X-ray analysis has shown[18] that the methyl groups in the side chain of peridinosterol (**1e**) both have the *R* configuration, and it seemed very unlikely that the above two saturated sterols of M_r = 430 (**1a**) and 416 (**2a**), isolated from the same alga, should have a different stereochemistry at C-23 and C-24.

This deduction could be confirmed by chemical correlation. Four products were obtained on hydrogenation (PtO_2) of peridinosterol (**1e**) in HOAc–EtOAc (6:1); one of the minor hydrogenation products and the M_r = 430 sterol isolated from the alga *Protoceratium reticulatum* were identical by 360-MHz ^{1}H NMR. This means that side

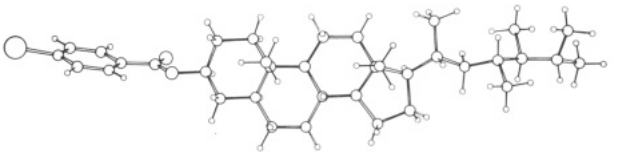

Figure 2. Observed absolute configuration and conformation of (23*R*,24*S*)-23,24-dimethyl-5α-cholestan-3β-ol *p*-bromobenzoate.

chain **a** is 23*R*,24*R* and side chain **b** is 23*S*,24*R*. The expected main product (**1f**) of the hydrogenation of peridinosterol (**1e**) has the normal configuration at C-17 (17β) but the "unnatural" configuration at C-20 (*S*); the two other minor products were a 3-ketone (**6f**) and a 3α-alcohol (**4f**) with the same side chain as the main product as is proved by chemical interconversion (see Experimental Section).

We decided to solve the problem of the stereochemistry of the side chain (**c**) of sterols, prepared by hydrogenation of 24-epidinosterol (**1j**) and 4-demethyl-5-dehydro-24-epidinosterol (**3j**) with Adam's catalyst, by X-ray analysis as this could not be achieved readily by chemical methods. In order to obtain single crystals of the quality required for structure determination, it was necessary to prepare a bromobenzoate derivative of 23,24-dimethyl-5α-cholestan-3β-ol (**2c**). The overall conformation and the observed 23*R*,24*S* (**c**) chiralities in the molecule are illustrated in Figure 2. The methyl substituents appear to further stabilize the fully extended minimum-energy conformation of the side chain. Previous studies have revealed that 3β-benzoate substituents on steroids are restricted by intramolecular constraints to two principal conformations differing primarily in the magnitudes of the C(2)–C(3)–O(3)–C(*n*) torsion angles.[19] The 3β-bromobenzoate substituent on this structure belongs to the more restricted subset [C(2)–C(3)–O(3)–C(*n*) = 91°, C(3)–O(3)–C(*n*)–O = 8°].

Alam et al.[11] reported the isolation from a cultured dinoflagellate of a new 4α-methyl sterol of M_r = 430 to which the structure 4α,23ξ,24ξ-trimethyl-5α-cholestan-3β-ol was assigned. They claimed that this sterol could be synthesized by hydrogenation of dinosterol by using 10% Pd/C in ethyl acetate. Because the published 220-MHz ^{1}H NMR data (compound **1x**, Table I) did not agree with our NMR data of the two natural products of M_r = 430 and of the PtO_2 hydrogenation product (**1c**) of 24-epidinosterol (**1j**), we decided to try to reproduce Alam's experiment.

In our hands, hydrogenation of sterols with the dinosterol (**1j**) or epidinosterol (**1j**) side chain using Pd/C in ethyl acetate gave rise to mixtures of products. Epimerization and catalystic oxidation (apparently caused by oxygen adsorbed by the catalyst) were important side reactions; 3α-alcohols **4** and 3-ketones **6** were obtained in addition to 3β-alcohols **1**. The workup of reaction mixtures by reverse-phase HPLC afforded sets of four compounds with the same skeleton but with different side chains. We already determined the stereochemistry at C-23 amd C-24 of three of the side chains (**a**–**c**); this left 23*S*,24*S* as the only possibility for the configuration of the fourth 23,24-dimethyl-substituted side chain (**d**).

Relative retention times, both in reverse-phase HPLC and capillary GC, of all isolated hydrogenation products of dinosterol (**1i**), 24-epidinosterol (**1j**), and 4-demethyl-5-dehydro-24-epidinosterol (**3j**) are included in Table I. The data show that the sets of four epimeric 4-methyl

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(19) Duax, W. L.; Weeks, C. M.; Rohrer, D. C. *Top. Stereochem.* **1976**, *9*, 271 (Figure 77).

Table II. Selected Hydrogenation Experiments of Dinosterol (1i), 24-Epidinosterol (1j), and 4-Demethyl-5-dehydro-24-epidinosterol (3j)[e]

run	starting matl	catalyst	recovd/isolated epimerized starting matl	epimeric 3β-alcohols formed	byproducts
1	1i	PtO_2[a]	1i (17)	1a (73), 1b (5), 1c (3), 1d (2)	
2	1i	PtO_2[b]		1a (84), 1b (9), 1c (4), 1d (3)	
3	1i	Pd/C[c]	1i (12), 1j (9)	1a (39), 1b (15), 1c (8), 1d(7)	6a (6), 6b (2), 6d (1), 6d (1)
4	1i	Pd/C[c]		1a (38), 1b (19), 1c (14), 1d (15)	6a (6), 6b (3), 6c (2), 6d (3)
5	1j	PtO_2[a]		1a (2), 1c (98)	
6	3j	PtO_2[a]		2a (9), 2b (4), 2c (83), 2d (4)	
7	3j	PtO_2[d]		2a (7), 2b (4), 2c (70), 2d (19)	
8	3j	Pd/C[c]		2a (14), 2b (8), 2c (36), 2d (14)	5a (6), 5b (5), 5c (12), 5d (8)

[a] In 4:1 hexane-HOAc. [b] In 14:1 EtOAc-HOAc. [c] In EtOAc. [d] In 9:1 HOAc-EtOAc. [e] Abundances are given in parentheses. See the Experimental Section for details.

sterols **1a–d**, 3α-hydroxysteranes **5a–d**, and 4-methyl-3-oxosteranes **6a–d**, but not the 3β-hydroxysteranes **2a–d**, are completely separable by capillary GC.

Once we had determined the chromatographic behavior of sets of four epimers (**a–d**), we reexamined the PtO_2 hydrogenation of dinosterol (**1i**) and 24-epidinosterol (**1j**) to determine whether it was as stereoselective as had previously been assumed. Control experiments indicated that reduction products with the 23*S* configuration (**b, d**) had been overlooked because they were eluted faster than the main product with the 23*R* configuration (**a** or **c**), and their peaks had not been resolved from a noisy base line (because initial experiments were done on a 1-2 mg scale), or because these byproducts had been mistaken for unreacted starting material. A minor product with the 23*R* configuration (**a** or **c**) and the opposite configuration at C-24 as the main product (**c** or **a**) has about the same retention time in reverse-phase HPLC as the main product. This minor component had not been detected because capillary GC had not been used, and 360-MHz ^1H NMR is not a good tool for the quantitation of a minor component which has an NMR spectrum not much different from that of the main component of a mixture.

A more careful analysis of mixtures of PtO_2 hydrogenation products of dinosterol (**1i**) and 4-demethyl-5-dehydro-24-epidinosterol (**3j**) now showed that minor products with side chains **b**, **c**, **d** and **a**, **b**, **d**, respectively, were present.

The composition of some typical mixtures of PtO_2 and Pd/C hydrogenation products is listed in Table II; the NMR data of all identified compounds are included in Table I. It should be emphasized that NMR data of the four epimeric dinostanols **1a–d**) are different from those reported by Alam et al.[11] for the natural product which they isolated (compound **1x**, Table I).

The data in Table II show that epimerization is a very important side reaction if hydrogenation is carried out with Pd/C. One then must ask the question whether only epimerization at C-24 takes place or whether also the stereochemistry at C-20 is affected. If epimerization at C-20 had taken place, a mixture of sterols with the eight possible epimeric side chains (at C-20, C-23, and C-24) would have been obtained. We found no evidence for the formation of 20-iso sterols, such as **1f**, during hydrogenation. It has been reported that 20-iso sterols consistently have a shorter retention times (RTs) in GC than normal sterols,[20] which is in agreement with our observation (cf. the relative retention time (RRT) of **1a** and **1f**, Table I). We noted (Table I) that **1f** also has a shorter RT in HPLC than any of the four dinostanols **1a–d**; thus if more than a trace of **1f** had been formed in the hydrogenation of dinosterol (**1i**) or epidinosterol (**1j**) we would have detected it using HPLC.

Published NMR data[20,21] for 20-epi sterols suggest that inversion of the normal configuration at C-20 results in a significant upfield shift ($\Delta\delta \approx 0.1$) for the C-21 methyl doublet. We find no such pronounced difference between the NMR data for the normal steroids **1a** and **6a** and the corresponding 20-iso steroids **1f** and **6f** (Table I).

Experimental Section

X-ray Analysis. 23(*R*),24(*S*)-Dimethyl-5α-cholestan-3β-ol *p*-bromobenzoate crystallized in a monoclinic space group ($P2_1$) with cell dimensions of a = 19.568 (18) Å, b = 5.998 (1) Å, c = 14.115 (3) Å, and β = 98.05 (2)°. The cell constants were determined by least-squares analysis of the θ values of 25 reflections centered on a diffractometer. The integrated intensities for 4014 reflections having $\theta < 75°$ were measured on an Enraf-Nonius CAD-4 diffractometer by using Cu Kα radiation. The structure was solved by direct methods[22] and refined by full-matrix least-squares methods. Hydrogen atoms were introduced at theoretically expected positions and not refined. The crystal size and shape produced data suitable for unambiguous determination of the configurations at C-23 and C-24. However, the rapid falloff in intensities as a function of θ from the best crystals obtainable precluded H-atom refinement and the degree of geometric accuracy routinely achieved in X-ray crystal structure determinations. The final conventional R indices were 12.2% for the 3711 independent reflections and 7.8% for the 2467 reflections having intensities greater than 3 times their standard deviation. Positional parameters for the nonhydrogen atoms are given in Table III. The C–C bond lengths in the B–D rings of the steroid ranged from 1.496 to 1.544 Å, and those in the benzene ring ranged from 1.36 to 1.44 Å. Coordinates for the H-atom positions, anisotropic thermal parameters for nonhydrogen Atoms, and other crystallographic details are available from the Buffalo author upon request.

General Methods. We used Waters HPLC equipment (M6000, M6000A, and M45 pumps, R401 and R403 refractometers, U6K injector) and also Valco CV-6-UHPa-N60 and Rheodyne 7125 injectors, two Waters semipreparative μ-Porasil columns in series for normal phase separations (eluent toluene–EtOAc, 95:5) and Whatman Partisil M9 10/50 ODS-2 columns and two Altex Ultrasphere-ODS columns (5 μm, 10 mm i.d. × 25 cm) in series for reverse-phase separations (eluent absolute MeOH unless stated otherwise). A Hewlett-Parkard Model 402 gas chromatograph with FID (3% SP2250 column, 2 mm I.D. × 1.80 m, 260°) and a Carlo Erba Fractovap Model 4160 gas chromatograph with FID (fused silica capillary SE54 column, 30 m, 260 °C) were used for analytical GC. Cholesterol was used as a standard, both in GC and HPLC. The beginning of a solvent peak, and not the point of injection, was used to calculate relative retention times (RRT) in GC and HPLC. Reverse-phase HPLC fractions of sterol

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Table III. Non Hydrogen Atom Coordinates (×10⁴) and Isotropic Thermal Parameters (×10) for (23*R*,24*S*)-23,24-Dimethyl-5α-cholestan-3β-ol *p*-Bromobenzoate

atom	*x/a* (σ)	*y/b* (σ)	*z/c* (σ)	B_{ISO} (σ)
C(1)	3278 (4)	7503 (23)	9967 (5)	54 (3)
C(2)	2610 (5)	7259 (25)	10395 (6)	59 (3)
C(3)	2017 (5)	8186 (21)	9763 (7)	58 (3)
C(4)	1942 (4)	7190 (25)	8790 (6)	57 (3)
C(5)	2596 (4)	7508 (21)	8359 (6)	50 (3)
C(6)	2524 (4)	6848 (36)	7299 (6)	73 (3)
C(7)	3148 (4)	7673 (24)	6868 (5)	62 (3)
C(8)	3822 (4)	6809	7420 (5)	48 (2)
C(9)	3878 (4)	7308 (18)	8502 (5)	42 (2)
C(10)	3240 (4)	6562 (20)	8943 (5)	44 (2)
C(11)	4571 (4)	6579 (24)	9024 (5)	58 (3)
C(12)	5191 (4)	7445 (25)	8581 (5)	57 (3)
C(13)	5146 (4)	6974 (22)	7516 (5)	41 (2)
C(14)	4447 (4)	7812 (20)	7041 (5)	46 (2)
C(15)	4486 (4)	7752 (29)	5990 (6)	79 (4)
C(16)	5234 (5)	8359 (28)	5924 (6)	69 (3)
C(17)	5638 (4)	8246 (20)	6944 (5)	44 (2)
C(18)	5226 (5)	4388 (21)	7356 (8)	71 (4)
C(19)	3218 (5)	3985 (21)	8997 (7)	62 (3)
C(20)	6379 (4)	7551 (21)	6976 (6)	49 (2)
C(21)	6771 (4)	7250 (31)	7958 (7)	78 (4)
C(22)	6758 (4)	9337 (21)	6411 (6)	54 (3)
C(23)	7433 (4)	8563 (21)	6098 (6)	51 (2)
C(23M)	7261 (5)	7101 (29)	5193 (7)	74 (3)
C(24)	7883 (4)	10587 (21)	5915 (6)	50 (2)
C(24M)	8119 (5)	11804 (31)	6844 (7)	73 (3)
C(25)	8494 (5)	9998 (22)	5385 (6)	56 (3)
C(26)	8857 (5)	12147 (29)	5080 (6)	74 (3)
C(27)	9038 (5)	8520 (26)	5946 (9)	82 (4)
C(28)	1197 (5)	9505 (24)	10724 (6)	58 (3)
C(29)	601 (4)	8898 (21)	11195 (6)	48 (2)
C(30)	290 (4)	6717 (25)	11071 (6)	55 (3)
C(31)	−238 (5)	6256 (22)	11583 (7)	62 (3)
C(32)	−447 (4)	7727 (23)	12218 (6)	56 (3)
C(33)	−137 (6)	9791 (23)	12348 (7)	67 (3)
C(34)	376 (5)	10362 (23)	11836 (7)	62 (3)
BR(32)	−1127 (1)	6911 (15)	12967 (1)	91 (1)
O(38)	1387 (3)	7890 (18)	10171 (4)	62 (2)
O(28)	1482 (4)	11299 (20)	10823 (6)	86 (3)

mixtures were examined by GC/MS using a Varian MAT-44 spectrometer (3% OV-17 column or fused silica capillary SE54 column, 9 M, 260 °C). High-resolution mass spectra were obtained by using a MS-50 spectrometer at the Midwest Center for Mass Spectrometry, University of Nebraska–Lincoln. The 360-MHz ^{1}H NMR spectra were recorded with a Brucker HXS-360 spectrometer. Angles of rotation and melting points were determined witn an Autopol III automatic polarimeter (Rudolph Research, Fairfield, NJ) and with a Hoover capillary melting point apparatus (A. H. Thomas Co., Philadelphia, PA), respectively. Grace grade 62 silica gel (60–200 mesh) and Florisil (100–200 mesh) were used for column chromatography, and TLC was done with precoated TLC sheets (E. Merck, No. 5539).

***Orbulina universa* Zooxanthellae.** An inocculum of the zooxanthellae of *O. universa* was obtained from the culture collection of Dr. R. K. Trench, University of California at Santa Barbara. The algae were mass cultured[23a] during 15 days at 18 °C in two batches of 200 L each in GPM medium[23b] in polyethylene drums with constant aeration. The drums were illuminated with two banks of four "cool white" (General Electric) fluorescent lights (no dark period). Cells were harvested by continuous centrifugation (Sharpless centrifuge); yield 36.2 g of wet cells. They were homogenized in $CHCl_3$–MeOH (1:1, Waring blender), and the homogenate was filtered. The material on the filter was resuspended in $CHCl_3$–MeOH, and the mixture was filtered again. This was repeated once; the weight of the extracted residue on the filter (air dried) was 6.8 g. The filtrates were combined and evaporated, and the residue was partitioned between $HCCl_3$ and water. The residue of the $CHCl_3$ layer was dissolved in $CHCl_3$ (40 mL), and a much larger volume of acetone (500 mL) was added to precipitate phospholipids. After storage overnight in a freezer the acetone solution was decanted and evaporated to give 1.22 g of residue. This was applied to a silica gel column (40 g), and sterol esters and free sterols were isolated by using hexane–toluene (3:2) and hexane–ether mixtures. The residue of the hexane–toluene eluate (containing the sterol esters) was saponified (NaOH, 2 pellets; 95% EtOH, 50 mL; reflux, 2 h); a workup of the neutral unsaponifiables (silica gel, 5 g; hexane–ether, 2:1) afforded 14.2 mg of sterols from sterol esters. Purification of the residue of the free sterol fractions of the silica gel column (138 mg) over Florisil (15 g) with hexane–ether (2:1) gave 98.5 g of free sterols. Both sterol samples were mixtures of 4α-methyl sterols (1) and regular (4-demethyl) sterols (2, 3) which were separated by HPLC (semipreparative μ-Porasil columns). 4-Methyl sterols were separated by using first an ODS-2 column and then Altex columns; the more complicated mixtures of demethyl sterols were separated over Altex columns. A list of abundances of all identified components of the complex sterol mixture will be given elsewhere;[24] the abundances of a few components mentioned earlier in the text follow: (23*R*,24*R*)-4α,23,24-trimethyl-5α-cholestan-3β-ol (**1a**), 26.9% of free sterols, 14.6% of sterols from esters; peridinosterol (**1e**), 0.3% of free strols, 4.8% of sterols from esters; (23*R*,24*R*)-23,24-dimethyl-5α-cholestan-3β-ol (**2a**), not detected in free sterols, 0.1% of sterols from esters. It should be noted that the *Orbulina* zooxanthellae are the second example of algae that contain an anomalous side-chain sterol, 24-epioccelasterol (**3g**) as a major sterol (26.0% of free sterols, 24.0% of sterols from esters) (the first example was *Gymnodinium simplex*)[10] and its partially saturated analogue, 24-epipatinosterol (**2g**), as a minor sterol. The last sterol is a known synthetic compound[25] but has never before been isolated from nature.

Protoceratium reticulatum (Clap. and Lachm.) Bütschli, 1885 (=*Gonyaulax grindleyi* Reinecke, 1967)[26] was obtained from the culture collection (No. PY-29) of Dr. F. T. Haxo, Scripps Institution of Oceanography, La Jolla. The alga was mass cultured[23a] during 29 days at 24 °C in 200 L of GPM medium[23b] in a polyethylene drum with constant aeration. The drum was illuminated with two banks of four "cool-white" (General Electric) fluorescent lights (no dark period). Cells were harvested by continuous centrifugation. The yield was 37.4 g of wet cells, giving after extraction (as above) and removal of the water-solubles 1.0 g of extract (phospholipids were not precipitated) and 7.3 g of extracted, dry residue. Free sterols were isolated by silica gel column chromatography (40 g) and further purified over a Florisil column (12 g); yield 70 mg. Cholesterol was the main sterol (84%); (23*R*,24*R*)-4α,23,24-trimethyl-5α-cholestan-3β-ol (**1a**) accounted for 5% of the mixture. Abundances of all identified components will be reported elsewhere.[24]

Isolation of (23*S*,24*R*)-23,24-Dimethyl-5α-cholestan-3β-ol (2b). Extract of the bryozoan *Bugula neritina* (collected in the Gulf of Mexico)[27] was supplied by Dr. G. R. Pettit, Arizona State University, Tempe. Free sterols were isolated by silica gel column chromatography with hexane–ether mixtures. The yield was about 0.5 g of crude sterols from 30 g of extract. **2b** (13% of the sterols) was obtained pure by fractionation of the sterol mixture with an ODS-2 column, and it has since been synthesized.[7] Cholesterol was the main sterol (50%) of *B. neritina*; **2b** was found in the next to last peak in HPLC, the last peak corresponding to dinosterol (**1i**).[16]

Isolation of (23*S*,24*R*)-23,24-Dimethylcholest-5-en-3β-ol (3b). The sponge *Stelleta conulosa* (collected on Leigh Reef, New Zealand) was supplied by Dr. J. Baker, of the former Roche

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Research Institute of Marine Pharmacology, Dee Why, NSW, Australia. The powedered, freeze-dried sponge (50.2 g) was stirred in a blender with $CHCl_3$ (200 mL), MeOH (200 mL), and water (100 mL). The mixture was filtered, and the $CHCl_3$ layer was washed with water and evaporated to give 1.35 g of extract from which free sterols were isolated by silica gel column chromatography. The sterols were fractionated by using an ODS-2 column. Main components were 24-methylenecholesterol (43.2%) and fucosterol (30.9%);[17] **3b** (1%) was contained in the second half of the last peak (RRT [HPLC, ODS-2] = 1.23). **3b** was further purified by HPLC using Altex columns (eluent MeOH–water, 98:2).

Isolation of (23*S*,24*R*)-4α,23,24-Trimethyl-5α-cholestan-3β-ol (1b). The free sterol mixture of the Indo-Pacific soft coral *Siphonogorgia* sp. (collected in 1978 by Mr. Jean Pierret at Laing Island, Papua-New Guinea) was donated by Dr. J. C. Braekman, Free University of Brussels. Separation of the mixture (600 mg) over a silica gel column with hexane–ether (3:1) gave 4-demethyl sterols (446 mg) and 16 mg of a mixture of 4α-methyl sterols and 4-demethyl sterols from which 4 mg of pure 4-methyl sterols was obtained by rechromatography over Florisil and silica gel. The demethyl sterol mixture, after further purification over Florisil, was fractionated by using an ODS-2 column, and fractions were analyzed by GC/MS. Fourteen components were identified; cholesterol was by far the main sterol (45%), and no 23-demethylgorgosterol (**3l**) or gorgosterol (**3m**) was detected, suggesting that *Siphonogorgia* sp. does not have algal symbionts.[2,28] The mixture of 4-methyl sterols was also fractionated over an ODS-2 column; **1b** (RRT [HPLC, ODS-2] = 1.54) was the only novel sterol isolated (4.3% of the 4-methyl sterols; 0.018% of the total free sterols).

Hydrogenation of Peridinosterol (1e). Peridinosterol (2.5 mg, isolated from the dinoflagellate *Crypthecodinium cohnii*[24] [UTEX 1649]), PtO_2 (14.6 mg, Aldrich) in EtOAc (0.5 mL), and HOAc (3 mL) were stirred under H_2 for 4 days. Abundances and RRTs in HPLC (Altex columns) of the four identified products: peak 1 (**4f**), 5.0%, 1.35; peak 2 (**1f**), 78.8%, 1.51; peak 3 (**1a**), 6.2%, 1.77; peak 4 (**6f**), 9.9%, 1.84. Assuming bottom-side attack of hydrogen, the main product had to have the normal configuration at C-17 and the unnatural configuration at C-20: **1f**. The NMR spectra of the main PtO_2 hydrogenation product of dinosterol (**1i**) and that of the compound of peak 3 were identical, thus establishing the latter's structure as **1a**. The compound with the longest RT in HPLC had M_r = 428 (GC/MS). Its NMR spectrum did not show any proton at the same carbon as an OH group. Because of significant differences in the shift of C-4 Me and C-19 H as compared with the NMR spectra of **1f** and **1a** it became obvious that this compound was the 3-ketone corresponding to **1f** (thus **6f**). This could be confirmed by Jones oxidation[29] of **1f**: the Jones oxidation product and the ketone isolated from the above hydrogenation mixture had identical 360-MHz NMR spectra. The peridinosterol (**1e**) hydrogenation product with the shortest RT in HPLC had M_r = 430. Notable differences between its NMR spectrum and that of the main product **1f** (C-3 H, δ 3.7 vs. 3.1 for **1f**; C-4 Me, δ 0.908 vs. 0.944 for **1f**; C-19 H, δ 0.800 vs. 0.818 for **1f**) suggested that this minor hydrogenation product might be the 3α isomer **4f** of the main product **1f**. This was confirmed by reduction of the ketone **6f** (**6f**, 2 mg; PtO_2 [Aldrich], 25.8 mg; EtOAc, 4 mL; stirred for 2 days under H_2). Two products with the same RT in GC (RRT = 1.49, 3% SP2250, 260 °C) were separated by HPLC (Altex columns). The product (42%) with the shorter RT in HPLC and the peridinosterol hydrogenation product with the shortest RT in HPLC were identical by NMR, thus establishing structure **4f**. The other hydrogenation product (58%) of the ketone **6f** was the 3β-alcohol **1f**.

Hydrogenation of Dinosterol (1i), 24-epidinosterol (1j), and 4-demethyl-5-dehydro-24-epidinosterol (3j) was carried out at room temperature (H_2 pressure 1.0–1.5 atm). Reactions were usually followed by GC (3% SP2250 column). Products with saturated side chains (**a**–**d**) have a longer RRT than the starting material. For quantitation of the composition of an epimeric mixture of hydrogenation products we used the HPLC trace which gives the ratio **d**/**b**/(**c** + **a**) and the capillary GC trace (SE54 column) of the mixture of **c** + **a**. Compounds with side chains **c** and **a** (and the same skeleton) can be separated by reverse-phase HPLC if MeOH–water (95:5) is used: the peak has to be cut, and fractions have to be reinjected. Experimental details for the various runs (Table II) follow. Yields are listed in Table II, and RRTs by reverse-phase HPLC and capillary GC are listed in Table I.

Run 1. Dinosterol (**1i**, 2 mg) and PtO_2 (2 mg, Aldrich) in AR hexane (4 mL) and glacial HOAc (1 mL) were stirred overnight. Altex columns were used to fractionate the components. The sample corresponding to the main peak was pure by NMR (**1a**), but a low percentage of **1c** was detected by capillary GC; the two other minor products, **1d** and **1c**, were identified on the basis of their retention time in HPLC and capillary GC.

Run 2. Dinosterol (**1i**, 15 mg) and PtO_2 (50 mg, Aldrich) were stirred in glacial HOAc (14 mL) and EtOAc (1 mL) for 4 days. Components were separated by HPLC (Altex columns). The material corresponding to the first and second peak was reinjected (Altex columns) and NMR samples of **1e** and **1b**, respectively, were obtained in this manner. The ratio of **1a** and **1c** in the third peak was determined by capillary GC. (23*R*,24*R*)-4α,23,24-Trimethyl-5α-cholestan-3β-ol (**1a**) has the following: mp 196–199 °C (from MeOH); $[\alpha]_D$ +22.2° (c 5 × 10^{-3}, $CHCl_3$).

Run 3. Dinosterol (**1i**, 5 mg) and 10% Pd/C (10 mg, Alfa Inorganics) in EtOAc (5 mL) were stirred under H_2 for 24 h. After HPLC (Altex columns) NMR spectra were recorded of the main components [epidinosterol (**1j**), dinosterol (**1i**)], of the material of two of the saturated sterol fractions (**1b** and **1a** + **1c**), and of the main ketone fraction (**6a** + **6c**). The composition of the last two fractions was obtained by capillary GC.

Run 4. The above experiment was repeated on a larger scale in order to get samples of all components for NMR. Dinosterol (15 mg), 10% Pd/C (50 mg), and EtOAc (5 mL) were used with a workup after 3 days (Altex columns). The main peak (**1a** + **1c**) was cut at the top; the fraction corresponding to the first half of that peak was enriched in the minor component (**1c**). Material of peaks caused by one compound (**1b**,**d**, **6b**,**d**) was reinjected (Altex columns), and pure samples of these compounds were obtained for NMR. Reinjection of the first half of the main peak (**1a** + **1c**) and of the last peak (**6a** + **6c**) (Altex columns; MeOH–water, 95:5), cutting at the top, reinjection of fractions under the same conditions, etc. eventually produced samples of the remaining four compounds (**1a**,**c**, **6a**,**c**) for NMR.

Run 5. The scale of the experiment and the conditions were the same as for run 1. The starting material was epidinosterol (**1j**). The workup was done with an ODS-2 column; only one peak was observed (**1c** + a small amount of **1a**).

Run 6. 4-Demethyl-5-dehydro-24-epidinosterol (**3j**, 70 mg) and PtO_2 (70 mg, Aldrich) in AR hexane (8 mL) and glacial HOAc (2 mL) were stirred overnight. The mixture was worked up by using an ODS-2 column. Sterols **2d** and **2b** were identified on the basis of their RRT in HPLC, and the other minor component (**2a**) was identified on the basis of its RRT in capillary GC. The mixture of **2c** (plus some **2a**) was pure by NMR. For (23*S*,24*S*)-23,24-dimethyl-5α-cholestan-3β-ol (**2c**): mp 165.0–165.5 °C (from MeOH); $[\alpha]_D$ +43.7° (c 9 × 10^{-3}, $CHCl_3$).

Run 7. 4-Demethyl-5-dehydro-24-epidinosterol (**3j**, 5 mg) and PtO_2 (40.5 mg, Aldrich) in glacial HOAc (4.5 mL) and EtOAc (0.5 mL) were stirred under H_2 for 2 days. Components were identified on the basis of their RRT in HPLC (Altex columns) and capillary GC.

Run 8. 4-Demethyl-5-dehydro-24-epidinosterol (**3i**, 9.5 mg) and 10% Pd/C (31.1 mg, Alfa Inorganics) in (EtOAc 5 mL) were stirred under H_2 for 2 days. GC showed that reaction was not complete. An almost identical GC trace was obtained after the reaction had been continued for 1 more day. The sterols were then isolated, and the reaction was continued with fresh solvent (5 mL) and catalyst (62.4 mg) for 3 days. NMR showed the byproducts to be 3α-alcohols, which, apparently, had been formed by reduction of products of catalytic oxidation. All components were obtained pure for NMR by using Altex columns: two 3α-alcohols (**5d** and **5b**) and two 3β-alcohols (**3d** and **3b**) were purified by reinjection; to obtain the remaining four components pure, MeOH–water (95:5) had to be used, peaks had to be cut at the top, and the second half of the peak (enriched in **5a** and **3a**,

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respectively) had to be reinjected.

(23*R*,24*R*)-4α,23,24-Trimethyl-5α-cholestan-3β-ol (1a): high-resolution MS (70 eV, probe), *m/z* (assignment, relative intensity) 430.4179 ($C_{30}H_{54}O$, M^+, 22), 415.3941 ($C_{29}H_{51}O$, 16), 412.4089 ($C_{30}H_{52}$, 6), 397.3844 ($C_{29}H_{49}$, 15), 341.3194 ($C_{25}H_{41}$, 3), 303.3067 ($C_{22}H_{39}$, 2), 299.2745 ($C_{22}H_{35}$, 2), 290.2988 ($C_{21}H_{38}$, 7), 271.2433 ($C_{20}H_{31}$, 8), 262.2300 ($C_{18}H_{30}$, 8), 247.2056 ($C_{17}H_{27}O$, 38), 229.1957 ($C_{17}H_{25}$, 57), 179.1432 ($C_{12}H_{19}O$, 38), 98.1094 (C_7H_{14}, 100).

(23*S*,24*R*)-23,24-Dimethyl-5α-cholestan-3β-ol (2b): high-resolution MS (70 eV, probe), *m/z* (assignment, relative intensity) 416.4019 ($C_{29}H_{52}O$, M^+, 33), 401.3761 ($C_{28}H_{49}O$, 17), 398.3945 ($C_{29}H_{50}$, 9), 383.3683 ($C_{28}H_{47}$, 13), 359.3289 ($C_{25}H_{43}O$, 4), 344.3456 ($C_{25}H_{44}$, 4), 327.3048 ($C_{24}H_{39}$, 4), 317.2857 ($C_{22}H_{37}O$, 3), 290,2978 ($C_{21}H_{38}$, 6), 285.2584 ($C_{21}H_{33}$, 5), 257.2268 ($C_{19}H_{29}$, 14), 248.2140 ($C_{17}H_{28}O$, 11), 233.1904 ($C_{16}H_{25}O$, 64), 215.1802 ($C_{16}H_{23}$, 74), 165.1275 ($C_{11}H_{17}O$, 42), 98.1091 (C_7H_{14}, 100).

(23*R*,24*R*)-4α,23,24-Trimethyl-5α-cholestan-3-one (6a): high-resolution MS (70 eV, probe), *m/z* (assignment, relative intensity) 428.4029 ($C_{30}H_{52}O$, M^+, 25), 413.3791 ($C_{29}H_{49}O$, 14), 357 (3), 331.2992 ($C_{23}H_{39}O$, 16), 315.2695 ($C_{22}H_{35}O$, 3), 287.2377 ($C_{20}H_{31}O$, 6), 269.2286 ($C_{20}H_{29}$, 2), 260.2134 ($C_{18}H_{28}O$, 13), 245.1901 ($C_{17}H_{25}O_1$, 100), 231.1743 ($C_{16}H_{23}O$, 23), 177.1641 ($C_{13}H_{21}$, 3), 177.1278 ($C_{12}H_{17}O$, 21), 98.1092 (C_7H_{14}, 77).

Acknowledgment. We thank F. Elaine DeJarnette and Phyllis Strong for technical assistance. We are indebted to our colleagues Lian Niang Li, Manfred Eggersdorfer, and Xian Li for the isolation of compounds **1a**, **2b**, and **3b**, respectively, to James R. Lance, who mass cultured the *Orbulina universa* zooxanthellae and *Protoceratium reticulatum* at Scripps Institution of Oceanography, La Jolla, CA, to Professor Robert K. Trench, University of California at Santa Barbara, for an inocculum of these zooxanthellae, to Professor Francis T. Haxo, Scripps Institution of Oceanography, for an inocculum of *P. reticulatum*, to Professor George R. Pettit, Arizona State University, Tempe, AZ, for the extract of *Bugula neritina*, to Dr. J. C. Braekman, of the Free University of Brussels, for the sterol fraction of *Siphonogorgia* sp., to Dr. J. Baker, of the former Roche Research Institute of Marine Pharmacology, Dee Why, NSW, Australia, for a gift of *Stelleta conulosa*. The 360-MHz NMR spectra were recorded under the supervision of Dr. Lois Durham. Financial support from the National Institutes of Health (Grants No. AM-26546, No. GM-09840, and No. GM-28352) and the Wendt Foundation is gratefully acknowledged. Operation and maintenance of the Stanford 360-MHz NMR Facility were supported by NIH Grant No. RR-0711 and NSF Grant No. GP-23633. High-resolution mass spectra were recorded at the Midwest Center for Mass Spectrometry, University of Nebraska–Lincoln. This center is supported by grants from the National Science Foundation.

Registry No. **1a**, 86708-32-9; **1b**, 86708-33-0; **1c**, 86708-34-1; **1d**, 86708-35-2; **1e**, 77617-71-1; **1f**, 86708-36-3; **1i**, 58670-63-6; **1j**, 81445-03-6; **2a**, 85505-68-6; **2b**, 85505-67-5; **2c**, 86708-37-4; **2d**, 86708-38-5; **2g**, 81520-53-8; **3b**, 86708-39-6; **3g**, 64783-84-2; **3j**, 81445-04-7; **4f**, 86708-40-9; **5a**, 86708-41-0; **5b**, 86708-42-1; **5c**, 86708-43-2; **5d**, 86708-44-3; **6a**, 86708-45-4; **6b**, 86709-22-0; **6c**, 86708-46-5; **6d**, 86708-47-6; **6f**, 86708-48-7; 23(*R*),24(*S*)-dimethyl-5α-cholestan-3β-ol *p*-bromobenzoate, 86668-14-6; fucosterol, 17605-67-3; cholesterol, 57-88-5; 24-methylenecholesterol, 474-63-5.

Studies of Vitamin D Oxidation. 3. Dye-Sensitized Photooxidation of Vitamin D and Chemical Behavior of Vitamin D 6,19-Epidioxides

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Received February 8, 1983

Dye-sensitized photooxidation of vitamin D and the chemical reactions of the resulting oxidation products have been studied in detail. Vitamin D undergoes 1,4-cycloaddition and ene-type reactions with singlet oxygen to yield two C(6) epimers of 6,19-epidioxyvitamin D (**3** and **4**) as the major products (55–65% total isolated yields) and two C(6) epimers of the $\Delta^{4,7,10(19)}$ 6-hydroperoxide (**5** and **6**) as the minor products (15–25% total yields). The structures of the oxidation products are determined unambiguously by spectral data in combination with X-ray analysis. The chemical behavior of the endoperoxides **3** and **4** is examined in the reactions with basic reagents, Lewis and proton acids, transition-metal complexes, and reducing agents.

As a part of our studies[1] of the chemistry of the conjugated triene group of vitamin D, which is believed to play an important role in the biological activity of the vitamin,[2] we have been investigating the oxidation of the triene group. The oxidation is of interest not only from the chemical but also from the biological point of view, because vitamin D apparently undergoes biological oxidation at the unsaturated part,[3] as unsaturated fatty acids do in the well-known biosynthesis of prostaglandins and leucotrienes.[4] Seeming to support this possibility is the recent

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