Biosynthetic Studies of Marine Lipids. 34.[1] Stereochemical Features of the Enzymatic C-Methylation on the Path to Isofucosterol and Fucosterol

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Abstract: Incorporation studies in the sponge *Xestospongia testudinaria* using (*E*)- and (*Z*)-[28-^{3}H]-24-methylenecholesterol establish that proton removal relative to *S*-adenosylmethionine attack occurs in the anti sense in the biosynthesis of the 24-ethylidene grouping in isofucosterol and fucosterol. A primary isotope effect is observed. Fucosterol is not isomerized in vivo to isofucosterol.

The stereochemistry at C-24 of a phytosterol is phylogenetically significant: vascular plants have the α-stereochemistry, while algae and fungi possess the opposite configuration.[2-7] The course of methylation leading to formation of the 24β-methylsterol ergosterol (**4b**), studied in Arigoni's laboratory[8,9] in the fungus *Claviceps paspali* and in an algal *Trebouxia* species, led to the following conclusions.[8-10]

(a) Methylation by attack of *S*-adenosylmethionine (SAM) on the *si*-face of the desmosterol side chain double bond (**1**) occurs with inversion of configuration at the methyl center.

(b) The resulting carbenium ion undergoes a 1,2-hydride (reversible) shift on the opposite face, followed by loss of a proton forming either the methylene intermediate **2** or the (epi)codisterol side chain (**3a** and **3b**).

The generality of these observations to other double bond systems in the sterol side chain remains to be established. Earlier incorporation experiments in our laboratory[11] related to the biosynthesis of the triply alkylated side chain of the sponge sterol strongylosterol (**10**) demonstrated two separate *si*-face SAM attacks on sequentially formed double bonds—a result which concurs with (but is reported differently from) Arigoni's assessment.

The formation of the triply alkylated 24(*S*)-24-isopropenylcholesterol (**9**) in the sponge *Pseudaxinyssa* can also be viewed as a *si*-SAM attack on the 24-ethylidene intermediates (**6** and **7**). An apparent exception to *si*-SAM attack has, however, been noted.[14-16] Finally, the detection of Δ^{23}-sterols[17,18] in trace amounts in plants suggests that in both single and double transmethylations some stabilization is received by the consequent formation of carbenium ions at C-23, implying that its C-24 carbenium ion precursor is significantly long-lived and that the usual proton transfer from C-25 to C-24 is not necessarily concerted.

The present study focuses on the methylation of 24-methylenecholesterol (**2**) going to the widely distributed iso-

Chart I

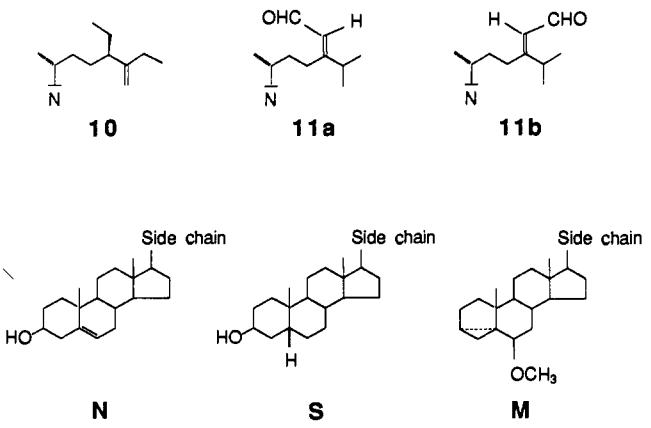
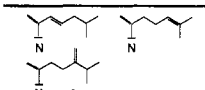
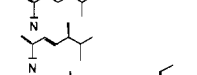
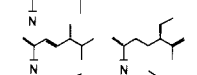
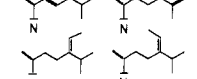
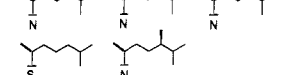
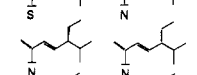
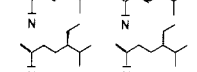
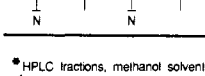


Table I. Major Sterols in *Xestospongia testudinara*

Sterol fraction*	percent†	HPLC RRT	name
	trace trace	0.80	22-dehydrocholesterol desmosterol
	5	0.86	24-methylenecholesterol
	11	0.90	crinosterol
	5 trace	0.93	brassicasterol epicleresterol
	36 3 11	1.0	isofucosterol fucosterol cholesterol
	<6>	1.08	cholestanol campesterol
	<11>	1.12	stigmasterol poriferasterol
	<11>	1.22	sitosterol clionasterol

*HPLC fractions, methanol solvent, as described in experimental.
†Bracketed values are for the whole HPLC fraction.

(1) For Part 33 in this series, see: Giner, J.-L.; Djerassi, C. *J. Org. Chem.*, **1991**, *56*, 2357-2363.
(2) Harrison, D. M. *Nat. Prod. Rep.* **1985**, 526-560.
(3) Goad, L. J.; Goodwin, T. W. In *Progress in Phytochemistry*; Rhinehold, L., Liwschitz, V., Eds.; Interscience: London, 1972; Vol. 3, pp 113-98.
(4) Nes, W. R.; McKean, M. C. *Biochemistry of Steroids and Other Isopentenoids*; University Park Press: Baltimore, MD, 1977.
(5) Goodwin, T. W. In *The Biochemistry of Plants*; Tevini, M., Lichtenthal, H. K., Eds.; Springer: Berlin, 1980, pp 485-505.
(6) Lederer, E. *Quart. Rev. London* **1969**, *23*, 453-481.
(7) Caspi, E. *Tetrahedron* **1986**, *42*, 3-50.
(8) Arigoni, D. *Ciba Foundation Symposium* **1978**, *60*, 243-261.
(9) Mihailovic, M. M. Dissertation ETH No. 7535, **1984**, 1-81.
(10) Floss, H. G.; Tsai, M. O.; Woodard, R. B. *Top. Stereochem.* **1984**, *15*, 253-321.
(11) Stoilov, I. L.; Thompson, J. E.; Cho, J.-H.; Djerassi, C. *J. Am. Chem. Soc.* **1986**, *108*, 8235-8241.
(12) Stoilov, I. L.; Thompson, J. E.; Djerassi, C. *Tetrahedron* **1986**, *42*, 4147-4155.
(13) Catalan, C. A. N.; Thompson, J. E.; Kokke, W. C. M. C.; Djerassi, C. *Tetrahedron* **1985**,, *41*, 1073-1084.
(14) Nicotra, F.; Ronchetti, F.; Russo, G.; Toma, L. *J. Chem. Soc., Perkin Trans. I* **1985**, 521-524.
(15) Seo, S.; Uomori, A.; Yashimura, Y.; Takeda, K. *J. Am. Chem. Soc.* **1983**, *105*, 6343-6344.
(16) Seo, S.; Uomori, A.; Yashimura, Y.; Seto, H.; Ebizuka, Y.; Noguchi, H.; Samkawa, U.; Takeda, K. *J. Chem. Soc., Perkin Trans. I* **1990**, 105-109.
(17) Itoh, T.; Shimizu, N.; Tamura, T.; Matsumoto, T. *Phytochemistry* **1981**, *20*, 1353-1356.
(18) Scheid, F.; Rohmer, M.; Benveniste, P. *Phytochemistry* **1982**, *21*, 1959-1967.

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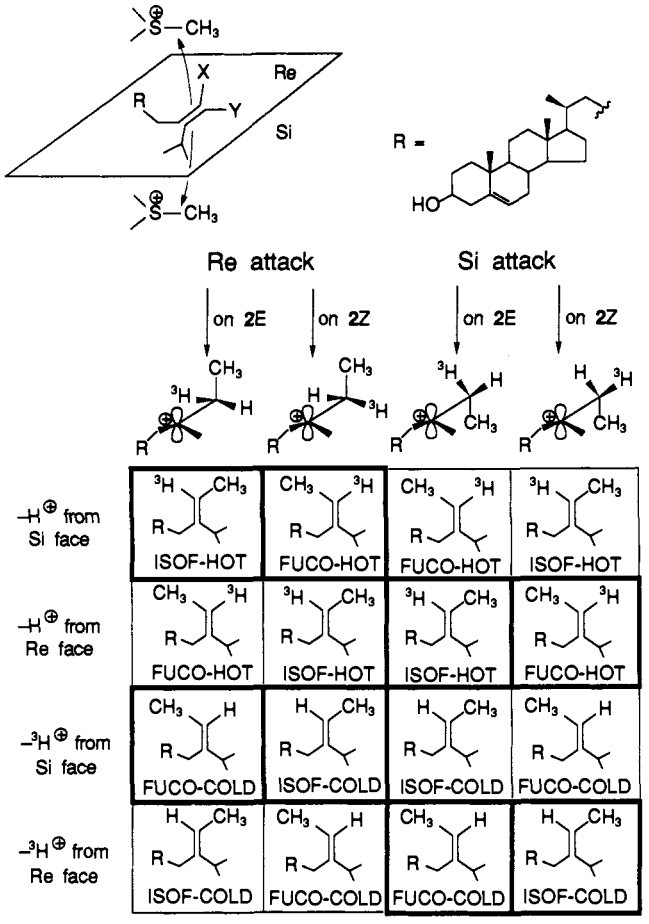
Scheme I



fucosterol (**6**) and fucosterol (**7**). It has been suggested[19] that the same enzyme (or complex) is responsible for these successive transmethylations, implying that the same stereochemical restrictions apply as those established for the trisubstituted double bond in **1**. We chose C-28 tritium-labeled 24-methylenecholesterol (**2*E*** and **2*Z***) as substrates to test for an antimechanism in the methylation at C-28 of the sterol side chain by examining the stereochemical product distributions which are anticipated as diagrammed in Scheme II.

Preparation of the stereospecifically labeled precursors (*E*- and *Z*-)-[28-^{3}H]-24-methylenecholesterol (**2*E*** and **2*Z***) was accomplished as follows: The mixture of isomeric aldedhydes **11a** and **11b**, prepared by the method of Anastasia et al.[20] from the 24-ketone, was separated into its pure *E* (**11a**) and *Z* (**11b**) components. Each isomer was separately reduced with sodium borohydride, oxidized with pyridinium chlorochromate, and decarbonylated in the presence of Wilkinson's catalyst which is known to proceed with retention of configuration.[20] Incorporations, according to previously described techniques,[21] were conducted by using 12.3 μCi of the *E*-isomer (**2*E***) and 17 μCi of the *Z*-isomer (**2*Z***) on 14-g fragments of one specimen of *Xestospongia testudinaria*, the sterol composition of which is shown in Table I. Isofucosterol (**6**) is a major (ca. 36%) and fucosterol (**7**) is a trace (ca. 3%) component of this sponge's sterol mixture.

To rule out the possibility that isofucosterol (**6**) and fucosterol (**7**) might be related through an isomerase, [6-^{3}H]-fucosterol[22] was incorporated into the sponge. Under these conditions, less than 0.03% of the recovered radioactivity[23] in the isolated fucosterol–isofucosterol mixture was located in the isofucosterol component, thus demonstrating the absence of interconversion between these two naturally occurring sterols.

Table II. Recovered Radioactivity[a] ($\times 10^{-3}$) in Isolated Isofucosterol and Fucosterol

precursor	isofucosterol[b]	fucosterol[c]
(*E*)-[28-^{3}H]-24-methylene-cholesterol (**2*E***)	38.3 (expt 1) 69.0 (expt 2)	4.0 5.6
(*Z*)-[28-^{3}H]-methylene-cholesterol (**2*Z***)	169.0 (expt 1) 16.7 (expt 2)	143.0 12.6
[6-^{3}H]-fucosterol	0	285.0
[6-^{3}H]-24-methylene-cholesterol	787.0	73.0

[a] Activity is reported in dpm. [b] Recovered 20.2 mg/14 g sponge. [c] Recovered 1.8 mg/14 g sponge.

Table III. Relative Radioactivities of Products

precursor	isofucosterol	fucosterol
(*E*)-[28-^{3}H]-24-methylene-cholesterol (**2*E***)	1	0.09 ± 0.01
(*Z*)-[28-^{3}H]-24-methylene-cholesterol (**2*Z***)	1	0.81 ± 0.02
[6-^{3}H]-24-methylene-cholesterol	1	0.09

The predicted outcomes for SAM methylation of the 24-methylene side chain are shown in Scheme II, where the only mechanistic restriction of the departing proton is that it must depart in the same plane as the p-orbital of the adjacent carbenium ion. As indicated by the darkened boxes, the antimechanism requires the fucosterol (**7**) to be nonradioactive ("cold") for the precursor (*E*)-24-methylenecholesterol (**2*E***) and radioactive ("hot") for the precursor (*Z*)-24-methylenecholesterol (**2*Z***); in the equivalent analysis, the isofucosterol (**6**) must be "hot" from the precursor *E*-isomer (**2*E***) and "cold" from the precursor *Z*-isomer (**2*Z***). A syn mechanism requires compliance with the opposite outcome. Finally, total lack of stereochemical control would give identical results from either progenitor.

The total radioactivities for the incorporations of the stereospecifically labeled precursors (*E*- and *Z*-)-[28-^{3}H]-24-methylenecholesterols are given in Table II. The (*E*)-[28-^{3}H]-24-methylenecholesterol (**2*E***) precursor definitively yields hot isofucosterol and nearly cold fucosterol in a ratio of 1:0.09 (Table III), which is qualitatively consistent with an antimechanism (Scheme II).

By contrast, the incorporation of the **2*Z*** precursor does not produce the expected cold isofucosterol and hot fucosterol (Scheme II) but rather hot isofucosterol and hot fucosterol (Table II) in a ratio of 1:0.81 (Table III). To explain the apparently anomalous generation of hot isofucosterol from the *Z*-isomer **2*Z***, the isotope effect $(k_H)/(k_T)$ operating in this reaction must be considered.

The deuterium isotope effect $(k_H)/(k_D)$ for sterol side chain alkylation was first observed by Goad[24] and later was reported by Mihailovic to be 3.5–4.8 for methylation of the 24-methylene side chain.[9] This value can be extended through the Swain equation[25] to predict a value of 5.8–9 for the tritium isotope effect $(k_H)/(k_T)$. The product ratio isofucosterol/fucosterol was determined from the incorporation of [6-^{3}H]-24-methylenecholesterol and found to be 11:1 (Table III). When the prevailing anti-

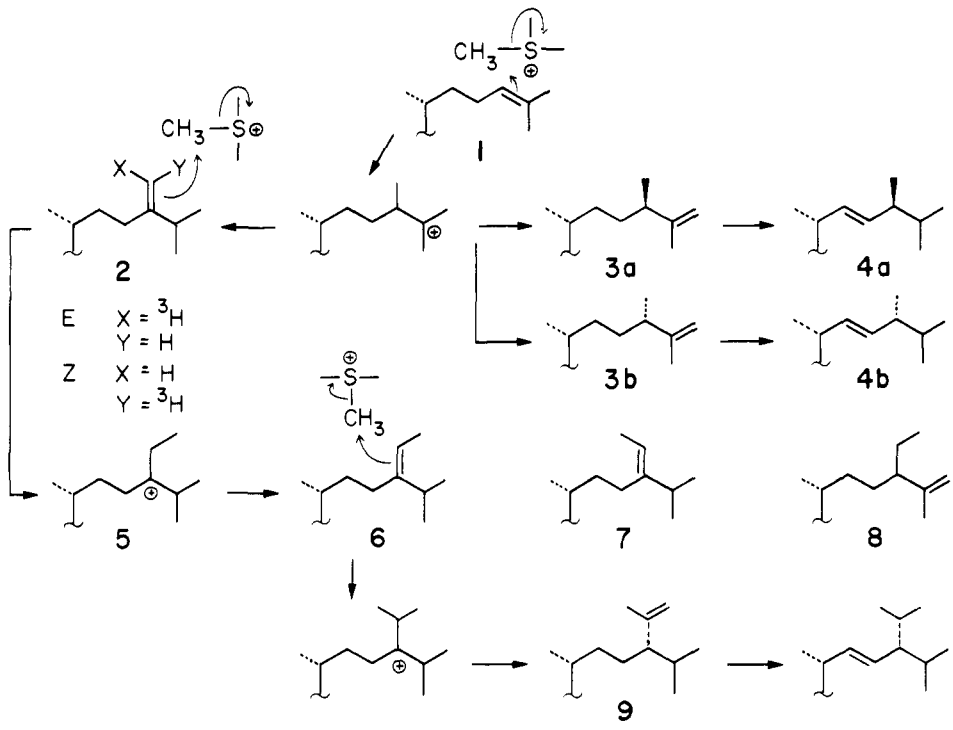
(19) Bansal, S. K.; Knoche, H. W. *Biochemistry* **1981**, *20*, 1269–1277.
(20) Anastasia, M.; Allevi, P.; Ciuffreda, P.; Fiecchi, A.; Gariboldi, P.; Scala, A. *J. Chem. Soc., Perkin Trans. 1* **1985**, 595–598.
(21) Carballeira, N.; Thompson, J. E.; Ayanoglu, E.; Djerassi, C. *J. Org. Chem.* **1986**, *51*, 2751–2756.
(22) Palmer, M. A.; Goad, L. J.; Goodwin, T. W. *Nouv. J. Chim.* **1978**, *2*, 401–404.

(23) Relative radioactivity is expressed as

100 × [dpm of isomer A/% of A in A + B] + [[dpm of isomer B/% of B in A + B] + [dpm of isomer A/% of A in A + B]]

(24) Goad, L. J.; Knapp, F. F.; Lenton, J. R.; Goodwin, T. W.; *Biochem. J.* **1972**, *129*, 219–222.
(25) Swain, C. G.; Stivers, E. C.; Reuwer, J. F., Jr.; Schaad, L. J. *J. Am. Chem. Soc.* **1958**, *80*, 5885–5893.

Scheme II



mechanism forces the production of hot isofucosterol (requiring loss of H) eleven times over the product of cold fucosterol, the isotope effect is masked by a factor on the order of 1/11, as was the case in the incorporation of (*E*)-[28-^{3}H]-24-methylenecholesterol (**2*E***) (Table III).

On the other hand, when the prevailing mechanism forces the production of cold isofucosterol as the major (11×) product, the isotope effect is enhanced, as was the case in the incorporation of (*Z*)-[28-^{3}H]-24-methylenecholesterol (**2*Z***) (Table III). This value appears somewhat low, presumably due to the fact that hydrogen instead of tritium may be lost from C-25 as well as C-28. The extent to which C-25 loses hydrogen is qualitatively known, as the trace sterol fraction epiclerosterol (**8**) is hot for both *E*- and *Z*-28-tritiated isomers.[26] We do not expect proton loss from C-25 to be dependent on the isotopic chirality at C-28, because the C-26 and C-27 methyl groups in both fucosterol and isofucosterol are free to rotate in the absence of the enzyme, as demonstrated by the degeneracy of their NMR signals. The availability of proton vs tritium loss from C-25 vs C-28 can, therefore, be predicted to work against the prevailing anti-mechanism, because, when the precursor (*Z*)-[28-^{3}H]-24-methylenecholesterol (**2*Z***) is introduced, the anti-mechanism requires fission of a stronger carbon–tritium bond in the formation of the major product (isofucosterol).

The system could avoid this induced hurdle with two responses: (1) make hot fucosterol by losing the alternate C-28 proton (which we recognize as the normal isotope effect) or (2) make hot epiclerosterol (**8**) by removing the proton at C-25. Because responses 1 and 2 are in competition, when path 2 is taken, less hot fucosterol is produced. It is fortuitous that in our study path 1 dominated enough to recognize the operation of the anti mechanism. The experimentally determined isotope effect (Table III, ratio 0.81/0.09 = 9) is at the higher end of that extrapolated from the above deuterium data.[9]

Due to the planar nature of the 24-methylene and 24-ethylidene intermediates in phytosterol biosynthesis, it has so far been hard to establish the orientation of proton removal relative to SAM attack in the formation of 24-ethylidene intermediates. Here we have demonstrated that the pioneering work performed in Arigoni's laboratory[8,9] on the formation of C_{28} phytosterols (24-methyl) extends with analogous stereochemical restrictions to C_{29} phytosterols (24-ethyl) in the formation of isofucosterol and fucosterol by a sponge. The heavy-lined boxes in Scheme II correspond to our data: once the isotope effect is taken into consideration, in each case the best fit is for the C-24 double bond to be formed by proton removal on the opposite face from the SAM attack.

(26) The production of "hot" epicholerosterol (**8**) was demonstrated in a cell-free system (Giner, J.-L.; Djerassi, C. *Tetrahedron Lett.* **1990**, *31*, 5421–5424) with [methyl-^{14}C]-*S*-adenosylmethionine and "cold" 24-methylenecholesterol.

Experimental Section

General Methods. High-performance liquid chromatography (HPLC) was carried out in reversed phase on a Waters Associates HPLC system (M 6000 pump, UK6 injector, R403 differential refractometer). Two Altex Ultrasphere ODS 5-m columns (25 cm × 10 mm i.d.) in series using as a mobile phase sequentially (as required) methanol, acetonitrile–methanol–water (11:4:4), and methanol–water (97:3) containing 40 mmol silver nitrate were used for the fractionation of sterol mixtures. The flow rate was 3 mL/min. The purity of HPLC fractions was determined by gas chromatography (GLC) using a Carlo Erba series 4160 Fractovap chromatograph equipped with a 15 × 0.32 mm fused silica column coated with SE-54 (programmed at 280–290 °C with a warming rate of 1 °C/min), a cooled column injection system, and a flame ionization detector. Cholesterol was used as the standard for relative retention times (RRT). Fourier transform ^{1}H NMR spectra were recorded on a Nicolet Magnetic Corp. NMC-300 spectrometer equipped with a 1280 data system. All NMR spectra were measured in $CDCl_3$ and were referenced to $CHCl_3$ (7.259 ppm). Low-resolution mass spectra were recorded on a Hewlett-Packard 5970 series mass spectrometer system equipped with a 5890A GC for sample introduction and a Hewlett-Packard 9133 system for data acquisition. The sponge *Xestospongia testudinaria* was collected at a depth of 14 m at John Brewer Reef (Central Australian Great Barrier Reef).

Preparation of the Stereospecifically Labeled Precursors (*E*- and *Z*-)-[28-^{3}H]-24-Methylenecholesterol. The isomethyl ether aldehyde **11** mixture[20] was separated into *E* and *Z* isomers by thin-layer chromatography (TLC) by using precoated 20 × 20 cm aluminum sheets with a 0.2-mm thickness of silica gel (EM Reagents) with hexane–ethyl acetate (10:1) as the mobile phase and developing each plate twice until UV analysis of the plate showed R_f values of 0.46 and 0.38 for the *E* and *Z* isomers, respectively. This separation was repeated three times whereupon the *E* isomer was free of the *Z* isomer as shown by loss of the aldehyde proton absorption at 10.1 ppm in the 300-MHz NMR spectrum. Likewise, the *Z* isomer was shown to be pure by the loss of such a signal at 10.0 ppm.

E Isomer: ^{1}H NMR ($CDCl_3$) 9.996 (d, J = 8 Hz, C-29), 5.83 (d, J = 8.2 Hz, C-28), 3.32 (s, OCH_3), 1.100 (d, J = 6.8 Hz, C-26, 27), 1.019 (s, C-19), 1.005 (d, J = 6.7 Hz, C-21), 0.719 (s, C-18).

Z Isomer: 10.100 (d, J = 8 Hz, C-29), 5.800 (d, J = 8 Hz, C-28), 3.330 (s, OCH_3), 1.152 (d, J = 6.9 Hz, C-26, 27), 1.019 (s, C-19), 0.965 (d, J = 6.5 Hz, C-21), 0.720 (s, C-18).

The following sequence of reactions was separately carried out on each isomer: The aldehyde (**11E** or **11Z**) (2 mg) in methanol (0.5 mL) was added to 10 μmol (6 mCi) of sodium borotritide (ICN) and stirred at room temperature for 5 min, when TLC analysis showed the reaction to be complete. The solvent was evaporated (N_2) and then passed through a short column (0.5 g) of silica gel in CH_2Cl_2. The resulting alcohol in 2 mL of CH_2Cl_2 was treated dropwise (over 30 min) with 15 mg of pyridinium chlorochromate in 1.5 mL of CH_2Cl_2. When a TLC monitor showed the reaction to be complete (40 min), the mixture was run through a short silica gel column (5 g, CH_2Cl_2), the aldehyde was divided into two portions, and each aliquot was subjected to the decarbonylation reaction.

When the preceding sequence was carried out with sodium borodeuteride, more than 99% of the recovered aldehyde retained the deuterium, which was shown to be the stereotopically pure E isomer by a clean NMR doublet centered at 5.35.

Deuterated E alcohol: ^{1}H NMR 5.35 (d, J = 7 Hz, C-28), 4.11–4.20 (m, C-29), 3.32 (s, OCH_3), 1.019 (s, C-19), 1.017 (d, J = 7 Hz, C-26, 27), 0.969 (d, J = 7 Hz, C-21), 0.714 (s, C-18). **Deuterated E aldehyde**: ^{1}H NMR 5.83 (s, C-28), 3.33 (s, OCH_3), 1.11 (d, J = 7 Hz, C-26, 27), 1.03 (s, C-19), 1.015 (d, J = 6 Hz, C-21), 0.730 (s, C-18). **Undeuterated alcohol (E:Z, 8:1)**: ^{1}H NMR 5.34 (t, C-28), 3.32 (s, OCH_3), 1.019 (s, C-19), 1.017 (d, J = 6.5 Hz, C-26, 27), 0.969 (d, J = 6.6 Hz, C-21).

The decarbonylation was carried out under argon in a sealed heavy-walled Wheaton glass reaction vial (2 mL) containing degassed dry (Na) toluene (0.3 mL), one portion of the above tritiated aldehyde, and 5 mg of tris(triphenylphosphine)rhodium(I) chloride (Aldrich). After heating to 120 °C for 1 h, the reaction was then cooled, extracted with methylene chloride, and passed twice through a short silica gel column with methylene chloride to remove any colored material. The isomethyl ether protecting group was removed in dioxane–water (1:1, 0.5 mL) by heating at 110 °C for 10 min with one crystal p-toluenesulfonic acid. Triethylamine (2 drops) was added, the solvents were evaporated, and the [28-^{3}H]-24-methylenecholesterol was purified by column chromatography (silica gel, CH_2Cl_2) to give (Z)-[28-^{3}H]-24-methylenecholesterol, 1.6 × 10^2 μCi/mg, and (E)-[28-^{3}H]-24-methylenecholesterol, 28 μCi/mg.

Incorporation of (E)- and (Z)-[28-^{3}H]-24-Methylenecholesterol into Sponge. Incorporations according to previously described[13,21] methods were performed by using 12.3 μCi of (E)-[28-^{3}H]-24-methylenecholesterol (**2E**) and 14 μCi of (Z)-[28-^{3}H]-24-methylenecholesterol (**2Z**) in duplicate on 14-g fragments of one specimen of *Xestospongia testudinaria*, living at a depth of 14 m at John Brewer Reef, Australia.

Incorporation of [6-^{3}H]-Fucosterol. The incorporation procedure was identical except that 20 μCi of [6-^{3}H]fucosterol was used on another specimen of *Xestospongia testudinaria* which was shown to have the same sterol content as the first specimen.

Isolation and Purification of Incorporated Sterols: Isofucosterol and Fucosterol. The sterol fraction of the sponge samples from the incubation experiments was obtained according to our standard procedures.[13] Reversed-phase HPLC chromatography in methanol isolated the isofucosterol–fucosterol fraction from most of the other sterols (Table I). Further HPLC using acetonitrile–methanol–ethyl acetate removed the contaminating cholesterol. Finally methanol–water (97:3), containing silver nitrate, separated the fucosterol from the isofucosterol. The RRTs of the isofucosterol and fucosterol (+cold carrier) were 85 and 95 min, respectively. The sterols were separated from the silver nitrate by concentration of the solvents, extraction with methylene chloride, and followed by drying through sodium chloride–silica gel (0.5 g).

The radioactivity for each peak remained unchanged after repeated chromatography. Purity of each peak (>99.5%) was established on cold material by capillary GLC (RRT fucosterol, 1.5; RRT isofucosterol, 1.6). Futhermore, when [6-^{3}H]fucosterol was incorporated into the sponge, our separation technique was shown to be >99.7% efficient in terms of the purification of isofucosterol. The NMR spectra (on cold material) showed each isomer to be totally free of the other by the different shifts of the C-28 protons: fucosterol ^{1}H NMR 5.19 (q, J = 7.5 Hz, 1 H), (C-28), 1.010 (s, C-19), 0.988 (d, J = 6.6 Hz, C-21), 0.979 (d, J = 6.9 Hz, C-26, 27), 0.687 (s, C-18); isofucosterol ^{1}H NMR 5.11 (q, C-28), 1.009 (s, C-19), 0.975 (d, J = 6.9 Hz, C-26, 27), 0.946 (d, J = 6.6 Hz, C-21), 0.683 (s, C-18).

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Registry No. 11a, 81256-55-5; **11b**, 81256-56-6; isofucosterol, 481-14-1; fucosterol, 17605-67-3; (E)-[28-^{3}H]-24-methylenecholesterol, 132564-87-5; (Z)-[28-^{3}H]-24-methylenecholesterol, 132564-88-6; 22-dehydrocholesterol, 34347-28-9; desmosterol, 313-04-2; 24-methylenecholesterol, 474-63-5; crinosterol, 17472-78-5; brassicasterol, 474-67-9; epiclerosterol, 105226-41-3; cholesterol, 57-88-5; cholestanol, 80-97-7; campesterol, 474-62-4; stigmasterol, 83-48-7; poriferasterol, 481-16-3; sitosterol, 83-46-5; clionasterol, 83-47-6.

Quadrone Structural and Synthetic Studies. Total Synthesis of Natural (−)-Quadrone, the (+)-Enantiomer, and the Racemate. Conformational Analysis, Circular Dichroism, and Determination of Absolute Stereochemistry

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Abstract: A concise (17 steps, 1.8% overall yield) and highly stereoselective synthesis of the architecturally novel, cytotoxic sesquiterpene (−)-quadrone (**1**) has been achieved, together with parallel constructions of the unnatural (+)-enantiomer and the racemate. The cornerstone of the strategy involved generation of the tricarbocyclic quadrone skeleton via an efficient, acid-promoted rearrangement of [4.3.2]propellane derivative **11**. Further elaboration of rearrangement product **14** furnished enone acid **3**, an advanced intermediate which had previously been converted to terrecyclic acid A (**2**) and thence to **1**. Detailed conformational analysis of quadrone, encompassing molecular mechanics calculations, 2D NMR studies, and X-ray crystallography, converged upon two energetically significant conformers (**D** and **E**; predicted ratio 97.5:2.5 at 25 °C). An empirical method for estimation of the sign and magnitude of $\Delta\epsilon$ indicated that strain effects and octant-dissignate contributions of the pseudoaxial α-hydrogens dominate the circular dichroism spectrum of (−)-**1**.

In 1978 Ranieri and Calton reported the structure of (−)-quadrone (**1**), an architecturally novel sesquiterpene isolated from fermentation broths of the fungus *Aspergillus terreus*.[1] The intriguing structure and reported cytotoxicity of **1** at once inspired

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