ISOLATION, STEREOCHEMISTRY, AND BIOSYNTHESIS OF ŠORMOSTEROL, A NOVEL CYCLOPROPANE-CONTAINING SPONGE STEROL*

Christopher J. SILVA and Carl DJERASSI

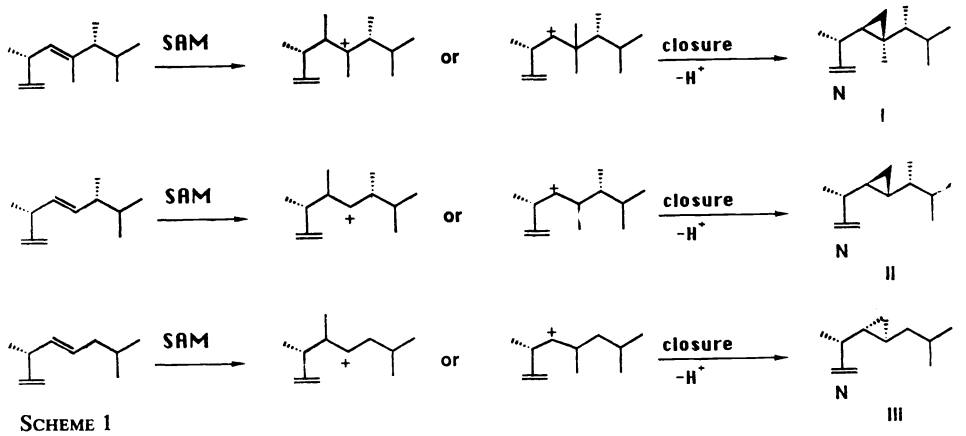
Department of Chemistry, Stanford University, Stanford, California 94305

Received July 4, 1990
Accepted July 30, 1990

Dedicated to the memory of Professor František Šorm.

Šormosterol ((24*R*)-24,25-methylenecholesterol, *XVIII*), a product of de novo sterol biosynthesis in the marine sponge *Lissodendoryx topsenti*, was shown, through the use of suitably labeled precursors, to be a new alternative product of the initial S-adenosylmethionine (SAM) methylation of the 24,25 double bond in the sterol side chain. Adidtional labeling experiments demonstrated that the cyclopropane ring is not further modified in vivo by the sponge.

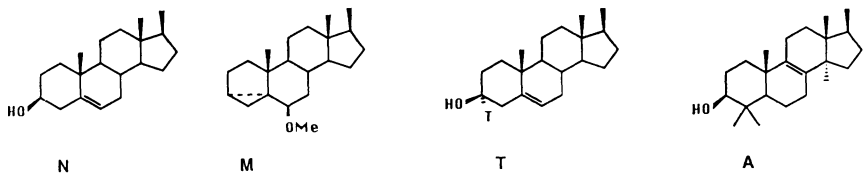
Sterols with cyclopropane rings in their side chains have so far only been found in marine organisms. They can be arranged into four groups, based on their presumed mechanism of formation. The best known of these groups is composed of sterols with a 22,23-methylene cyclopropane *I*–*III*, which presumably arises from the methylation of the 22,23 double bond (Scheme 1)[2]. Members of this class include



SCHEME 1
Gorgosterol-like sterols

* Part 64 in the series Sterols in Marine Invertebrates; Part 63: J. Org. Chem., submitted (see ref.[1]).

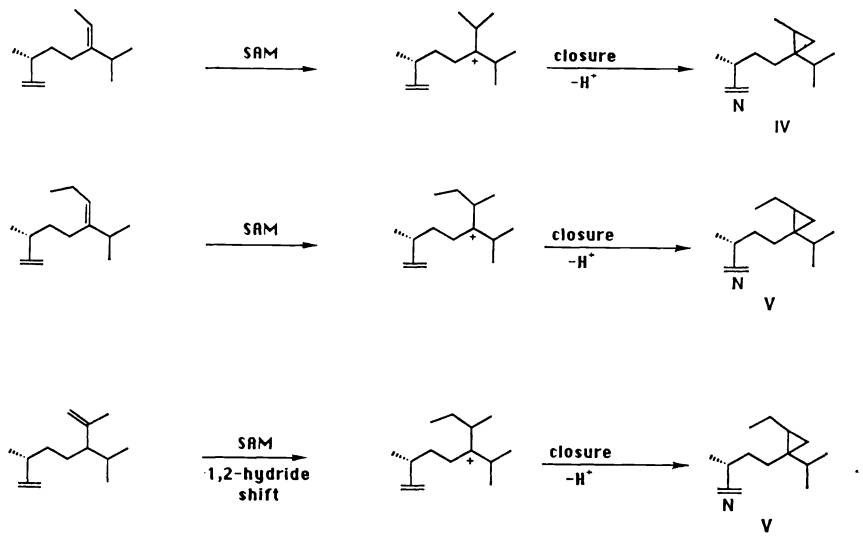
gorgosterol *I* (refs[3-5]), 23-demethylgorgosterol *II* (ref.[6]) and 22,23-methylenecholesterol *III* (refs[7,8]). The second group consists of 24,28-methylene cyclopropanes *IV* (ref.[9]) and *V* (ref.[10]) (Scheme 2). These are thought to arise from the methylation



N M T A

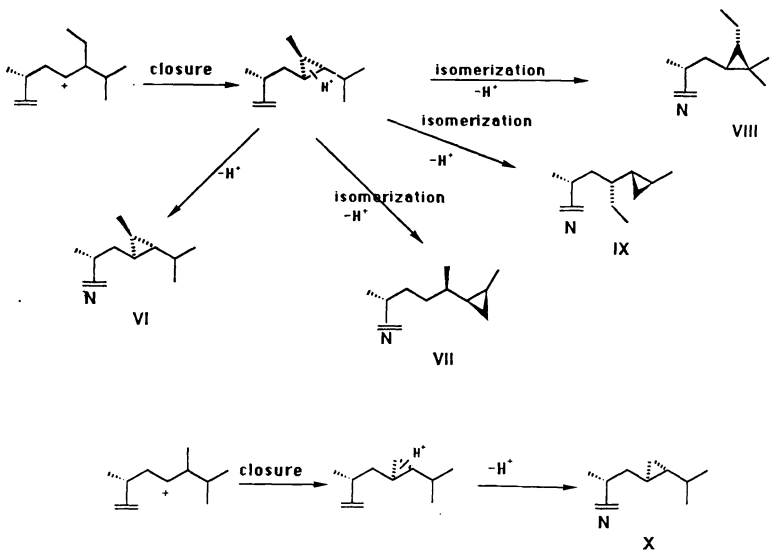
Sterol nuclei: N (Δ^5), M (i-methyl ether), T (Δ^5 3-T), and A (lanosterol)

of the 24,28 double bond[9,10]. The third is the collection of rearrangement products of the C-23 carbonium ion and the C-24 substituent (Scheme 3) (ref.[11]). Members of this group include dihydrocalysterol *VI* (ref.[12]), petrosterol *VII* (refs[13-15]),



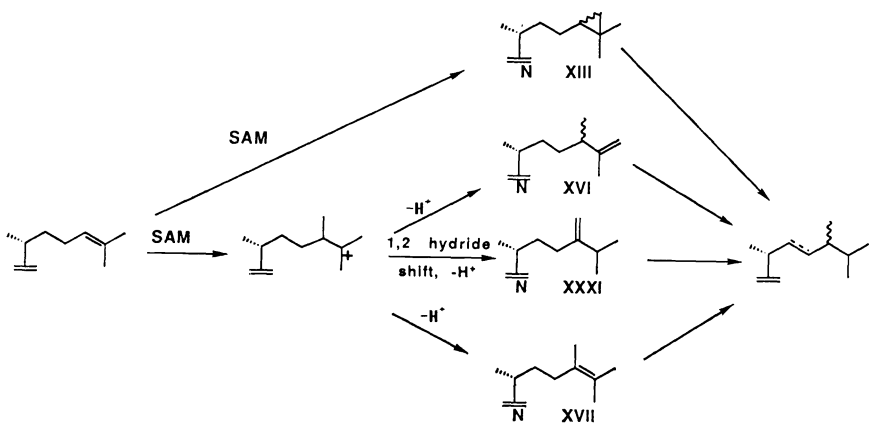
SCHEME 2
24,28-Methylene cyclopropanes

nicasterol *VIII* (ref.[16]), hebestrol *IX* (ref.[17]), and 23,24-methylenecholesterol *X* (refs[18,19]). Such a rearrangement process has been demonstrated experimentally[20] for petrosterol *VII* and dihydrocalysterol *VI*. Papakusterol (glaucostereol) *XI* (refs[21,22]) and (24*S*, 25*S*)-24,26-cyclocholesterol *XII* (refs[23,24]) represent the fourth class. It is not known how they are biosynthesized. We now wish to report the isola-



SCHEME 3

Cyclopropanes related to dihydrocalysterol

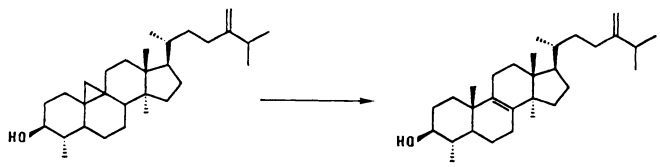


SCHEME 4

Lederer's scheme for the biosynthesis of 24-methyl sterols

tion of (24*R*)-24,25-methylenecholesterol *XVIII*, representing a fifth class that arises from the initial biomethylation of the desmosterol *XXX* side chain (Scheme 8). In honor of the occasion of this journal volume, we have named it šormosterol.*

Lederer proposed the intermediacy of the 24,25-methylenecholesterol *XIII* side chain (of undetermined C-24 stereochemistry) in the biosynthesis of 24-methyl sterols (Scheme 4)[25]. This side chain was synthesized in radiolabeled form[26] with the lanosterol nucleus (A) and fed to yeast, but no transformation to ergosterol was observed[27]. Later, an enzyme was found that isomerized the cyclopropane ring of cycloeucalenol *XIV* to obtusifoliol *XV* (Scheme 5)[28-30]. Tarchini and Rohmer, in

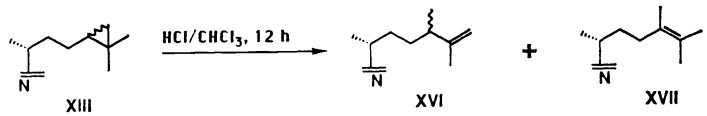


XIV XV

SCHEME 5

Isomerization of cycloeucalenol *XIV* to obtusifoliol *XV*

our laboratory, synthesized a number of sterols with cyclopropanes in the side chain to see if they would undergo acid-catalyzed ring opening, in a biomimetic fashion, to yield olefins known to be intermediates in sterol side chain biosynthesis[31]. Included in these biomimetic precursors was 24,25-methylenecholesterol *XIII* (as a mixture of C-24 epimers), which yielded upon acid-catalyzed ring opening (Scheme 6)



XIII XVI XVII

SCHEME 6

Acid-catalyzed ring opening of (24*R*/*S*)-24,25-methylenecholesterol *XIII*

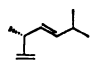
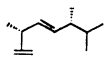
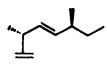
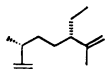
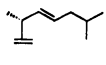
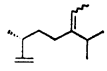
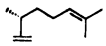
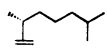
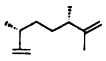
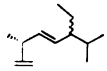
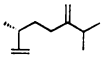
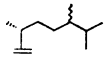
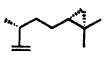
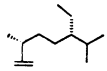
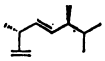
24-methylcholesta-5,25-dien-3β-ol *XVI* and 24-methylcholesta-5,24-dien-3β-ol (*XVII*), both of which are known to be converted biosynthetically to other 24-methyl sterols[32,33]. Now that we have isolated šormosterol *XVIII*, we decided to test its role in vivo (vide infra).

* Named in memory of Academician František Šorm — a superb steroid chemist and an esteemed friend.

The sterol mixture of the marine sponge *Lissodendoryx topsenti* was sequentially fractionated by reverse phase HPLC (Altex Ultrasphere ODS-5, mobile phase methanol, then acetonitrile–methanol–ethyl acetate 11 : 4 : 4) to yield šormosterol *XVIII* as a component of the mixture (Table I). The 300 MHz ^{1}H NMR spectrum showed specific resonances at 1·005δ (s, 3 H), 0·676δ (s, 3 H) and 5·35δ (bd, 1 H) as well as other general characteristics consistent with the Δ^5 (N) nucleus. The high-resolution mass spectrum had a strong molecular ion peak at m/z 398·3522 ($C_{28}H_{46}O$), indicating that the side chain contained nine carbons with one degree of unsaturation. Additional resonances in the 300-MHz ^{1}H NMR spectrum at 0·3 to 0·5δ (bm, 2 H), −0·15δ to −0·10 (bt, 1 H), 1·006δ (s, 3 H), 1·022δ (s, 3 H) and 0·908δ (d, J = 6·5 Hz) suggest a trisubstituted cyclopropane, two methyl groups attached to a quaternary carbon and a typical C-21 configuration, respectively. These data led to the formulation of šormosterol *XVIII* as 24,25-methylenecholesterol *XIII* of undetermined stereochemistry at C-24. The 300 MHz ^{1}H NMR spectrum

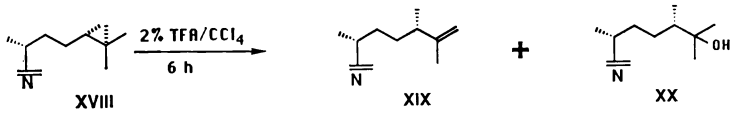
TABLE I

Sterol composition of *Lissodendoryx topsenti* (all sterols have the Δ^5 nucleus (N))

	2 %	 +	< 2 %
	< 1 %		
	5 %		3 %
	6 %		22 %
	< 1 %		< 2 %
	39 %		< 2 %
	5 %		2 %
	8 %		

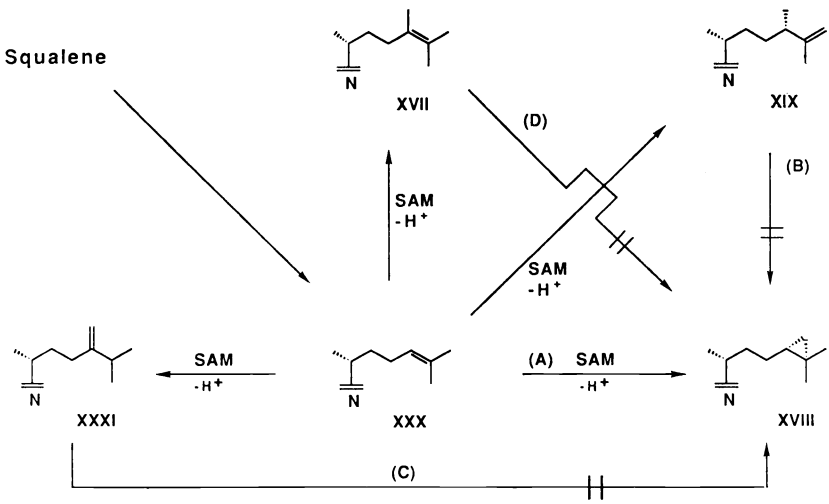
matched that of synthetic 24,25-methylene cholesterol *XIII* (ref.[31]). We chose to determine the stereochemistry at C-24 by subjecting šormosterol *XVIII* to acid-catalyzed ring opening.

The C-24 epimeric mixture of 24,25-methylenecholesterol *XIII*, protected as the i-methyl ether (M), was synthesized by the literature procedure[31] and then subjected to reverse phase HPLC separation (Altex Ultrasphere ODS-5, mobile phase methanol) as the protected i-methyl ethers (M), followed by deprotection to yield the two pure sterol epimers. The epimer displaying all of the spectral characteristics of šormosterol *XVIII* was subjected to acid-catalyzed ring opening (2% TFA in CCl_4, 6 h) to furnish, upon workup, two products (Scheme 7); codisterol *XIX* (refs[34,35]), (24*S*)-25-



SCHEME 7
Acid-catalyzed ring opening of šormosterol *XVIII*

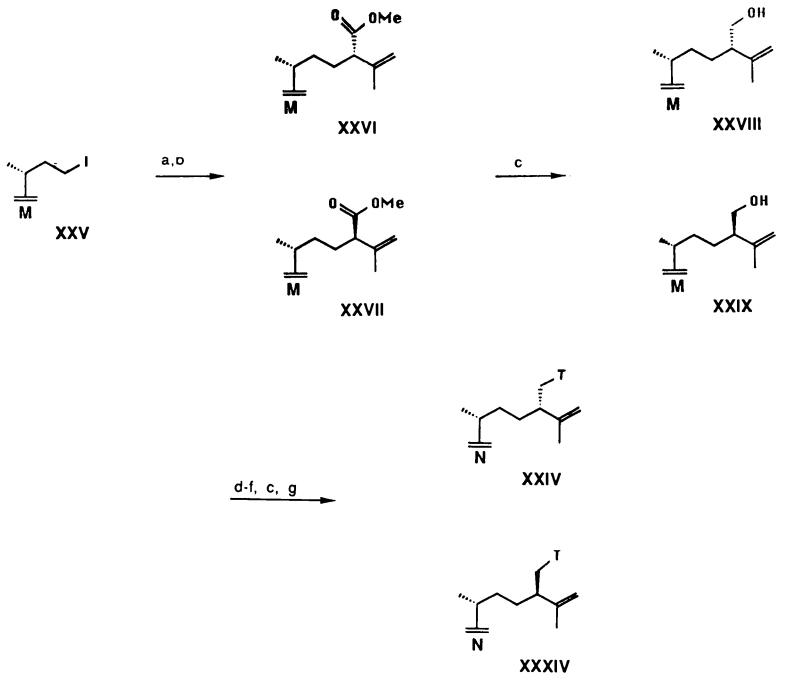
-hydroxy-24-methylcholesterol *XX* (ref.[36]), and unchanged šormosterol *XVIII*. The stereochemistry at C-24 is, therefore, *R*. For purpose of comparison, the other C-24 epimer was subjected to acid-catalyzed ring opening to yield the same products, but of opposite C-24 stereochemistry.



SCHEME 8
Four possible biosynthetic routes to šormosterol *XVIII*

We felt that there are four plausible biosynthetic routes to šormosterol *XVIII* which are outlined in Scheme 8. The first (Scheme 8A) is the direct biomethylation of the desmosterol *XXX* side chain to yield šormosterol *XVIII*. The remaining three (Scheme 8 B, C, and D) involve the isomerization to šormosterol *XVIII* of three alternate initial biomethylation products: codisterol *XIX*, 24-methylenecholesterol *XXXI*, or 24-methyldesmosterol *XVII*, respectively. In order to examine the de novo biosynthesis of šormosterol *XVIII*, we chose to synthesize radiolabeled squalene and desmosterol *XXX*, whereas labeled codisterol *XIX* and 24-methylenecholesterol *XXXI* were prepared to enable differentiation among the four possibilities outlined in Scheme 8. The radiolabeled form of šormosterol *XVIII* permitted us to test Lederer's hypothesis in vivo.

[3-^{3}H]-Squalene was prepared by a modification[37] of a previously described procedure[38]. [3-^{3}H]-24-Methylenecholesterol *XXI* and [3-^{3}H]-šormosterol *XXII* (Table II) were prepared from their respective sterols in a manner previously des-

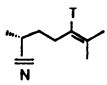
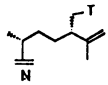
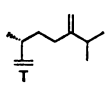
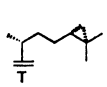
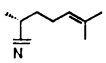
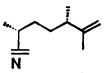
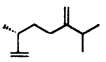
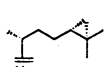
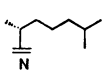
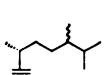


SCHEME 9

Synthesis of [28-^{3}H]-codisterol *XXIV* and [28-^{3}H]-epicodisterol *XXXIV*. a $(CH_3)_2C{=}CHCO.OMe$, LDA, THF/HMPA −78° → 0°; b HPLC; c LAH, THF; d PCC, CH_2Cl_2; e [^{3}H]-$NaBH_4$, EtOH; f MsCl, CH_2Cl_2; g *p*-TosOH, aq. dioxane

cribed[39]. [24-^{3}H]-Desmosterol *XXIII* was obtained from the known 24-oxo-cholesterol i-methyl ether[40] in the manner described by Sato and Sonoda[41]. [28-^{3}H]--Codisterol *XXIV* was prepared by the following sequence (Scheme 9); condensation of the 23-iodide *XXV* (ref.[42]) with the lithium dienoate of methyl 3-methylbut-2-en--oate afforded a mixture of the epimeric esters *XXVI* and *XXVII*, which were sepa-

TABLE II
Summary of the feeding experiments

Recovered radioactivity[b] (specific activity)[c]	Labeled precursors[a]				
	[3-^{3}H]-Squalene	 XXIII	 XXIV	 XXI	 XXII
 XXX	3.1 x 10^5 (1.0 x 10^6)	1.0 x 10^6 (1.0 x 10^7)		2.6 x 10^4 (8.7 x 10^4)	
 XIX[d]	1.5 x 10^4	8.0 x 10^4	5.2 x 10^6	2.6 x 10^3	
 XXXI	2.6 x 10^5 (1.5 x 10^5)	4.3 x 10^5 (6.1 x 10^5)		3.1 x 10^5 (1.6 x 10^5)	
 XVIII	1.1 x 10^5 (5.5 x 10^5)	1.4 x 10^5 (1.4 x 10^6)		3.3 x 10^3 (1.7 x 10^4)	2.1 x 10^6 (6.6 x 10^6)
 XXXIII		1.0 x 10^4 (2.5 x 10^4)			
 XXXII[d]		2.4 x 10^3	1.6 x 10^4	2.5 x 10^3	

[a] An amount of 20 μCi of each precursor was used per experiment, except in the case of squalene where 50 μCi was used. [b] This value is in dpm. [c] This value is in dpm/mg. [d] Due to the small amounts of these sterols present in the sponge sample, the specific activity could not be calculated accurately.

rated by normal phase HPLC Altex Ultrasil-Si. Each was transformed to its respective primary alcohol (*XXVIII* or *XXIX*), oxidized to the corresponding aldehyde, reduced with [^{3}H]-sodium borohydride to introduce the label, deoxygenated, and then deprotected. The results of the feeding experiments are summarized in Table II.

The feeding experiment, performed in the usual manner[40], with [3-^{3}H]-squalene demonstrated the de novo biosynthetic origin of desmosterol *XXX*, 24-methylenecholesterol *XXXI*, codisterol *XIX* and šormosterol *XVIII*. The latter three presumably originate from the biomethylation of the 24,25 double bond of the desmosterol *XXX* side chain. The [24-^{3}H]-desmosterol *XXIII* experiment shows that the biomethylation occurs with the conventional Δ^5-3β-hydroxy sterol nucleus (N), but this does not mean that it cannot occur with alternate nuclei (eg. A). This biosynthesis has also been verified in a cell free system[45]. As expected, it also shows that desmosterol *XXX* is reduced to cholesterol *XXXIII*. In order to demonstrate that šormosterol *XVIII* does no arise from the isomerization of codisterol *XIX* (Scheme 8 B) or 24-methylenecholesterol *XXXI* (Scheme 8 C), we fed [28-^{3}H]-codisterol *XXIV* and [3-^{3}H]-24-methylenecholesterol *XXI* to the sponge.

The results (Table II) show that codisterol *XIX* is reduced to 24-methylcholesterol *XXXII*, but is not isomerized to šormosterol *XVIII*. 24-Methylenechclesterol *XXXI* is reduced to 24-methylcholesterol *XXXII*, but radioactivity was also encountered in cholesterol *XXXIII*, codisterol *XIX*, and šormosterol *XVIII*. We have shown in other sponges that 24-methylenecholesterol *XXXI* is dealkylated to desmosterol *XXX* (refs[43,44]) which can then suffer reduction or SAM alkylation of the 24,25 double bond, thus accounting for the appearance of radioactivity in the latter three compounds. In both the [24-^{3}H]-desmosterol *XXIII* and [3-^{3}H]-squalene feeding experiments the isolated šormosterol *XVIII* is radioactive. This observation excludes the possibility (Scheme 8 D) that 24-methyldesmosterol *XVII* is isomerized to šormosterol *XVIII*, because radioactive 24-methyldesmosterol *XVII* cannot arise from [3-^{3}H]-squalene and [24-^{3}H]-desmosterol *XXIII* for the following reasons.

The C-3 and C-24 positions of squalene are equivalent. Upon cyclization of [3-^{3}H]-squalene to lanosterol one would expect to find tritium at C-3 and C-24 in a 1 : 1 ratio. The tritium at C-3 is lost, through exchange, during the removal of the two C-4 methyl groups and the formation of 24-methyldesmosterol *XVII* requires the loss of the C-24 proton (tritium in the case C-24 tritium labeled compound). Therefore, šormosterol *XVIII* is a product of the initial biomethylation of the desmosterol *XXX* side chain and not a product of the isomerization of one of the alternative initial biomethylation products codisterol *XIX*, 24-methylenecholesterol *XXXI* or 24-methyldesmosterol *XVII*. This is further substantiated in a recent cell-free system experiment which demonstrated that šormosterol *XVIII*, 24-methylenecholesterol *XXXI*, and codisterol *XIX* are products of the initial biomethylation of desmosterol *XXX* by S-adenosyl methionine[45].

In order to test Lederer's hypothesis (Scheme 4) in vivo we fed [3-^{3}H]-šormosterol *XXII* to the sponge. The results (Table II) indicate that the cyclopropane ring of šormosterol *XVIII* is not metabolized. This indicates that, at least for this sponge, there exists no enzyme analogous to the isomerase responsible for the conversion (Scheme 5) cycloeucalenol to obtusifoliol that might operate on the 24,25-methylene-cholesterol *XIII* side chain.

EXPERIMENTAL

High-performance liquid chromatography (HPLC) was carried out on a Waters Associates HPLC system (M 6000 pump, UK6 injector, R403 differential refractometer) using two Altex Ultrasphere ODS 5-μm columns (25 cm × 1 cm, i.d.) connected in series or one Altex Ultrasil-Si 10-μm column (25 cm × 1 cm, i.d.). ^{1}H NMR spectra were recorded on a Nicolet Magnetics Corporation NMC-300 spectrometer equipped with a 1280 data system and operating at 300 MHz in $CDCl_3$. All ^{1}H NMR spectra were referenced to the residual $CHCl_3$ signal (7·260 ppm). Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. Low-resolution mass spectra were obtained with a Hewlett–Packard Model 5995 GC/MS in the direct inlet mode. [^{3}H]-Sodium borohydride (specific activity: 1·4 Ci/mmol) was purchased from ICN Biomedicals Inc. High-resolution mass spectra were recorded on an AEI MS-30 instrument with a direct probe inlet system at the University of Minnesota mass spectrometry service laboratory or on a Krakos Analytic MS- instrument with a direct probe at the University of California, San Francisco mass spectrometry facility.

Isolation of Sterols

Lissodendoryx topsenti was collected at a depth of 13 m in Carmel Bay, California near the Hopkins Marine Station of Stanford University. The sponge was sliced into small pieces and extracted with dichloromethane–methanol (1 : 1) overnight. The extract was evaporated to dryness under reduced pressure and the resultant oil fractionated on an open silica gel gravity column. The sterol fractions were refractionated on a silica gel HPLC column (mobile phase, ethyl acetate and hexanes 6 : 94) to obtain the Δ^5-sterols. These were sequentially fractionated by reverse phase HPLC (mobile phase, methanol, then acetonitrile, ethyl acetate and methanol 11 : 4 : 4) to obtain šormosterol (*XVIII*) as 5% of the sterol mixture (see Table I).

Feeding Experiments

Samples of approximately 10 g wet weight of *L. topsenti* were placed in 300 ml jars with 200 ml of fresh sea water. The aeration of the sponges was provided by small electric air pumps. Each radiolabeled precursor (20 μCi) was dissolved in 0·5 ml ether and added to a jar containing a sponge, and left with earation to incorporate the precursor overnight[40]. Each sponge piece was washed (to prevent cross-contamination) and, then placed in a labeled porous container for protection from predators and mechanical abrasion). These containers were taken to the ocean and attached to a nylon grid (depth 10 m) that had previously been set up for the purpose of securing the sponges for an incubation period of four weeks. Each sponge piece was subjected to the analysis described above.

Labeled [3-^{3}H]-squalene[37] was prepared by a modification of a literature procedure[38]. [3-^{3}H]-24-Methylenecholesterol *XXI* and [3-^{3}H]-šormosterol *XXII* (ref.[24]) were prepared in

a manner already described[39] from the respective sterols. Labeled [24-^{3}H]-desmosterol *XXIII* was prepared from the known 24-oxocholesterol i-methyl ether[40] according to the procedure of Sato and Sonoda[41]. The synthesis of [28-^{3}H]-codisterol *XXIV* and [28-^{3}H]-epicodisterol *XXXIV* is described below.

(24*S*)-24-(Methoxycarbonyl)-6β-methoxy-3α,5-cyclo-5α-cholest-25(26)-ene (*XXVI*)
and (24*R*)-24-(Methoxycarbonyl)-6β-methoxy-3α,5-cyclo-5α-cholest-25(26)-ene (*XXVII*)

A flame-dried 25 ml flask equipped with a magnetic stirrer and sealed with a rubber septum was charged with 15 ml of a 10% solution of dry HMPA in dry THF. Into this solution was injected 0·28 ml of dry diisopropylamine followed by 1·3 ml of a 1·6M solution of n-butyllithium in hexanes. The solution was stirred at ambient temperature for 10 min before being cooled to −78°C, then 0·26 ml of methyl 3-methylbut-2-en-oate was injected into the solution and stirred for 20 min before a solution of 100 mg of the iodide *XXV* (ref.[42]) in 2 ml of dry THF was injected. The reaction mixture was stirred at −78°C for 20 min before allowing it to warm to room temperature over the course of 2 h. The reaction was quenched with 25 ml of water and extracted with ether. The ether layer was dried over sodium carbonate and concentrated to yield a colorless oil, which was purified by normal phase HPLC (ultrasil-Si, mobile phase ethyl acetate and hexanes 1 : 99) to give two compounds.

Compound XXVI: yield 37% (by HPLC). ^{1}H NMR spectrum: 3·670 (3 H, s, $COOCH_3$); 3·317 (3 H, s, OCH_3); 1·728 (3 H, s, H-27); 1·014 (3 H, s, H-19); 0·925 (3 H, d, $J = 6·5$ Hz, H-21); 0·702 (3 H, s, H-18). Low-resolution mass spectrum, *m/z* (relative intensity): 456·5 (M^+, 11), 424·4 (14), 401·4 (18), 285·3 (10), 255·2 (17), 253·2 (20), 55·1 (100).

Compound XXVII: yield 55% (by HPLC). ^{1}H NMR spectrum: 3·672 (3 H, s, $COOCH_3$); 3·317 (3 H, s, OCH_3); 1·733 (3 H, s, H-27); 1·014 (3 H, s, H-19); 0·903 (3 H, d, $J = 6·5$ Hz, H-21); 0·704 (3 H, s, H-18). High resolution-mass spectrum, *m/z* (relative intensity): 456·3643 (M^+, 33; for $C_{30}H_{48}O_3$ calculated 456·3591), 441·3345 ($C_{29}H_{45}O_3$, 60), 424·3323 ($C_{29}H_{44}O_2$, 64), 402·3126 ($C_{26}H_{42}O_3$, 36), 401·3070 ($C_{26}H_{41}O_3$, 100), 255·2124 ($C_{19}H_{27}$, 41).

(24*R*)-24-(Hydroxymethyl)-6β-methoxy-3α,5-cyclo-5α-cholest-25(26)-ene (*XXIX*)

To a solution of 20 mg of the ester *XXVII* in dry THF was added excess LAH. The reaction was allowed to stir for 1 h before being quenched sequentially with ethyl acetate, water, and then a 10% solution of aqueous acetic acid. The mixture was extracted with ether, dried over sodium carbonate, and the ether removed in vacuo. Alcohol *XXIX* was obtained in 95% yield as a colourless oil. ^{1}H NMR spectrum: 3·321 (3 H, s, OCH_3); 1·666 (3 H, s, H-27); 1·017 (3 H, s, H-19); 0·908 (3 H, d, $J = 6·5$ Hz, H-21); 0·706 (3 H, s, H-18). High-resolution mass spectrum, *m/z* (relative intensity); 428·3657 (M^+, 28; calculated for $C_{29}H_{48}O_2$ 428·3642), 413·3448 ($C_{28}H_{45}O_2$, 47), 396·3390 ($C_{28}H_{44}O$, 40), 373·3083 ($C_{25}H_{41}O_2$, 100), 255·2129 ($C_{19}H_{27}$, 27).

(24*S*)-24-(Hydroxymethyl)-6β-methoxy-3α,5-cyclo-5α-cholest-25(26)-ene (*XXVIII*)

Alcohol *XXVIII* was obtained from 20 mg of ester *XXVI* in a 95% yield by the procedure described above. ^{1}H NMR spectrum: 3·319 (3 H, s, OCH_3); 1·663 (3 H, s, H-27); 1·017 (3 H, s, H-19); 0·908 (3 H, d, $J = 6·5$ Hz, H-21); 0·706 (3 H, s, H-18). Low-resolution mass spectrum, *m/z* (relative intensity); 428·5 (M^+, 7), 396·4 (12), 373·3 (17), 255·2 (15), 253·2 (26), 229·2 (12), 55·1 (100).

General Procedure for the Conversion of the Alcohols *XXVIII* and *XXIX* to [28-^{3}H]-Codisterol *XXIV* and [28-^{3}H]-Epicodisterol *XXXIV*

Pyridinium chlorochromate (10 mg, 0·05 mmol) was added to a stirred solution of the alcohol *XXVIII* or *XXIX* (10·5 mg, 0·02 mmol) in dichloromethane. After 1 h, the reaction mixture was chromatographed to isolate the corresponding aldehyde. The purified aldehyde was immediately dissolved in a solution of [^{3}H]-sodium borohydride (10 mCi) in ethanol (0·2 ml) and allowed to stir for 2 h. The ethanol was removed in a stream of nitrogen, the reaction mixture filtered (silica gel, eluant ether), and ether was removed in a stream of nitrogen. Dichloromethane (0·5 ml), triethylamine (0·1 ml), and mesyl chloride (50 µl) were added to the residue and stirred for 3 h. The solvents were removed in a stream of nitrogen, the residue dissolved in ether (2 ml) and then stirred with a large excess of LAH for 2 h. The reaction mixture was sequentially quenched with ethyl acetate, water, a dilute acetic acid solution and then extracted with ether. The residue, after evaporation of the ether, was stirred for four hours at 100°C in 1 ml of a 10% aqueous dioxane solution with a catalytic amount of *p*-toluenesulfonic acid. The free sterol was purified by HPLC to give an overall yield of 32%.

The authors wish to acknowledged the financial support of the National Institute of Health through grant No. GM-*06840. The use of the* 300-MHz NMR *spectrometer was made possible by NSF Grant CHE-8109064. We wish to thank the Monterey Bay Aquarium and Hopkins Marine Station for generously providing materials and facilities for this work; Dr Christopher Harrold, Dr James Watanabe, Dr Mary Garson, and Toby Cole for their assistance in collecting the sponges; and Prof. Patricia Bergquist (University of Auckland) for kindly identifying the sponge. We also wish to thank the University of California, San Francisco mass spectrometry facility (A. L. Burlingame dir.; National Institute of Health Frant # NIH P-41 RR01614) for obtaining some of the high-resolution mass spectra.*

REFERENCES

1. Kerr R., Kerr S., Pettit G. R., Herald D. L., Groy t. L , Djerassi C.: J. Org. Chem., in press.
2. Djerassi C.: Pure Appl. Chem. *53*, 873 (1981).
3. Bergmann W., McMean M. J., Lester D.: J. Org. Chem. *8*, 271 (1943).
4. Hale R. L., Leclercq J., Tursch B., Djerassi C., Gross R. A. J., Weinheimer A. J., Gupta K. C., Scheuer P. J.: J. Am. Chem. Soc. *92*, 2179 (1970).
5. Ling N. C., Hale R. L., Djerassi C.: J. Am. Chem. Soc. *92*, 5281 (1970).
6. Schmitz F. J., Pattabhiraman T.: J. Am. Chem. Soc. *92*, 6073 (1970).
7. Blanc P. A., Djerassi C.: J. Am. Chem. Soc. *102*, 7113 (1980).
8. Blanc P. A., Djerassi C.: J. Am. Chem. Soc. *103*, 7036 (1981).
9. Kokke W. C. M. C., Shoolery J. N., Fenical W., Djerassi C.: J. Org. Chem. *49*, 3742 (1984).
10. Tam Ha T. B., Djerassi C.: Tetrahedron Lett. *26*, 4031 (1985).
11. Proudfoot J. R., Djerassi C.: J. Chem. Soc., Perkin Trans. 1, *1987*, 1283.
12. Li L. N., Li H. T., Lang R. W., Itoh T., Sica D., Djerassi C.: J. Am. Chem. Soc. *104*, 6726 (1982).
13. Mattia C. A., Mazzarella L., Puliti R., Sica D., Zolla F.: Tetrahedron Lett. *19*, 3953 (1978).
14. Ravi B. N., Kokke W. C. M. C., Delseth C., Djerassi C.: Tetrahedron Lett. *19*, 4379 (1978).
15. Sica D., Zollo F.: Tetrahedron Lett. *19*, 837 (1978).
16. Proudfoot J. R., Li X., Djerassi C.: J. Org. Chem. *50*, 2026 (1985).
17. Cho J. H., Djerassi C.: J. Chem. Soc., Perkin Trans. 1, *1987*, 1307.
18. Proudfoot J. R., Djerassi C.: Tetrahedron Lett. *25*, 5493 (1984).

19. Tam Ha T. B., Djerassi C.: Steroids *53*, 487 (1989).
20. Doss G. A., Proudfoot J. R., Silva C. J., Djerassi C.: J. Am. Chem. Soc. *112*, 305 (1990).
21. Bonini C., Kinnel R. B., Li M., Scheuer P. J., Djerassi C.: Tetrahedron Lett. *24*, 277 (1983).
22. Kobayashi M., Mitsuhashi H.: Steroids *40*, 665 (1982).
23. Catalan C. A. N., Lakshmi V., Schmitz F. J., Djerassi C.: Steroids *40*, 455 (1982).
24. Catalan C. A. N., Djerassi C.: Tetrahedron Lett. *24*, 3461 (1983).
25. Lederer E.: Q. Rev., Chem. Soc. *23*, 453 (1969).
26. Toubiana R., Lederer E.: Bull. Soc. Chim. Fr. *1965*, 2536.
27. Toubiana R.: Unpublished results as quoted in ref.[25].
28. Rahier A., Cattel L., Benveniste P.: J. Chem. Soc., Chem. Commun. *1976*, 287.
29. Rahier A., Cattel L., Benveniste P.: Phytochemistry *16*, 1187 (1977).
30. Heintz R., Benveniste P.: J. Biol. Chem. *249*, 4267 (1974).
31. Tarchini C., Rohmer M., Djerassi C.: Helv. Chim. Acta *62*, 1210 (1979).
32. Goad L. J., Knapp F. F., Lenton J. R., Goodwin T. W.: Biochem. J. *129*, 219 (1972).
33. Hall J., Smith A. R. H., Goad L. J., Goodwin T. W.: Biochem. J. *112*, 129 (1969).
34. Rubinstein I., Goad L. J.: Phytochemistry *13*, 481 (1974).
35. Kokke W. C. M. C., Pak C. S., Fenical W. H., Djerassi C.: Helv. Chim. Acta *62*, 1310 (1979).
36. Giner J.-L., Margot C., Djerassi C.: J. Org. Chem. *54*, 369 (1989).
37. Wünsche L., Silva C. J., Djerassi C.: Comp. Biochem. Physiol., submitted.
38. Colovich S. P., Kaplan N. O.: Methods Enzymol. *15*, 346 (1969).
39. Dayal B., Salen G., Tint G. S., Biswas C.: Steroids *42*, 635 (1983).
40. Catalan C. A. N., Thompson J. E., Kokke W. C. M. C., Djerassi C.: Tetrahedron *41*, 1073 (1985).
41. Sato Y., Sonoda Y.: Chem. Pharm. Bull. *29*, 2604 (1981).
42. Theobald N., Wells R. J., Djerassi C.: J. Am. Chem. Soc. *100*, 7677 (1978).
43. Malik S., Kerr R. G., Djerassi C.: J. Am. Chem. Soc. *110*, 6895 (1988).
44. Kerr R. G., Baker B., Kerr S., Djerassi C.: Tetrahedron Lett. *31*, 5425 (1990).
45. Giner J.-L., Djerassi C.: Tetrahedron Lett. *31*, 5421 (1990).