Hz, 3 H, C-26), 1.019 (s, 3 H, C-19), 0.950 (d, J = 6.4 Hz, 3 H, C-21), 0.727 (s, 3 H, C-18); mass spectrum similar to **26**.

Assignment of the Absolute Stereochemistry of the Alcohols 26–29 by Conversion to Known Dichloromethylene (30 and 32)[6b] and Cyclopropyl (34 and 36)[6b] i-Methyl Ethers. Each alcohol (**26–29**) (8 mg) was stirred with pyridinium chlorochromate (excess) and dichloromethane (1 mL) for 1 h in a test tube sealed with a rubber septum. The reaction mixture was filtered through Florisil and concentrated to yield the respective aldehydes. Each was stirred with the methyl Wittig reagent (90 mg of methyltriphenylphosphonium bromide, 5 mL of THF, and 0.25 mM *n*-butyllithium) for 30 min. The reaction mixture was diluted with water, extracted with ether, dried over sodium carbonate, concentrated, and purified by preparative TLC to afford the corresponding 23-vinyl compound. Each of the 23-vinyl compounds (5 mg) was stirred in 2-propanol with 5% rhodium on carbon (3 mg) under an atmosphere of hydrogen to afford the known 23-ethyl-24,25-(dichloromethylene)-27-norcholesterol i-methyl ethers (**30** and **32**).[6b] The other two compounds were only known as their i-methyl ethers; hence, the dichloromethylene compounds were dechlorinated to yield the respective i-methyl ethers (**34** and **36**).[6b] The ^{1}H NMR spectrum of each compound was compared with the known sample, enabling us to assign the absolute stereochemistry of the starting dichlorocyclopropyl alcohols (**26–29**).

Synthesis of Four 29-Norhebesterol Isomers (31, 33, 35, 15). A mixture of each alcohol (8 mg), *p*-toluenesulfonyl chloride (16 mg), and pyridine (1 mL) was stirred overnight, then diluted with water, extracted with ether, concentrated, and chromatographed by preparative thin-layer chromatography to afford the respective tosylates. Each tosylate, dimethyl sulfoxide (3 mL), and excess sodium borohydride was stirred for 3 h at 90 °C, then diluted with water, extracted with ether, dried over sodium carbonate, concentrated, and chromatographed to yield the four i-methyl ethers. The chlorine atoms were removed and the i-methyl ethers deprotected by methods previously described to yield the four 29-norhebesterol isomers (**31, 33, 35, 15**).[6b] The natural compound was found, after comparison of ^{1}H NMR spectra, to be the (23*R*,24*R*,25*S*)-29-norhebesterol (**15**).

(23*S*,24*S*,25*R*)-24,25-Methylene-23-methyl-27-norcholesterol (31): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.38–5.34 (bm, 1 H, C-6), 3.60–3.45 (bm, 1 H, C-3), 1.013 (s, 3 H, C-19), 1.010 (d, J = 5.8 Hz, 3 H, C-26), 0.885 (d, J = 6.0 Hz, 3 H, C-28), 0.895 (d, J = 6.4 Hz, 3 H, C-21), 0.720 (s, 3 H, C-18), 0.55–0.45 (bm, 1 H, cyclopropyl), 0.25–0.0 (bm, 3 H, cyclopropyl); mass spectrum, m/z (relative intensity) 398 $C_{28}H_{46}O$ (36.7), 365 (11), 301 (11), 300 (42), 299 (15), 285 (11), 272 (32), 271 (100), 270 (20), 253 (11), 213 (11), 55 (19).

(23*S*,24*R*,25*S*)-24,25-Methylene-23-methyl-27-norcholesterol (33): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.38–5.34 (bm, 1 H, C-6), 3.60–3.45 (bm, 1 H, C-3), 1.011 (s, 3 H, C-19), 1.002 (d, J = 6.4 Hz, 3 H, C-26), 0.888 (d, J = 5.5 Hz, 3 H, C-28), 0.876 (d, J = 6.4 Hz, 3 H, C-21), 0.694 (s, 3 H, C-18), 0.40–0.27 (bm, 2 H, cyclopropyl), 0.20–0.05 (bm, 2 H, cyclopropyl); mass spectrum similar to that of **31**.

(23*R*,24*S*,25*R*)-24,25-Methylene-23-methyl-27-norcholesterol (35): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.38–5.34 (bm, 1 H, C-6), 3.60–3.45 (bm, 1 H, C-3), 1.008 (s, 3 H, C-19), 0.993 (d, J = 6.3 Hz, 3 H, C-26), 0.856 (d, J = 6.5 Hz, 3 H, C-28), 0.827 (d, J = 6.4 Hz, 3 H, C-21), 0.687 (s, 3 H, C-18), 0.45–0.35 (bm, 1 H, cyclopropyl), 0.25–0.05 (bm, 3 H, cyclopropyl); mass spectrum similar to that of **31**.

(23*R*,24*R*,25*S*)-24,25-Methylene-23-methyl-27-norcholesterol (15): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.38–5.34 (bm, 1 H, C-6), 3.60–3.45 (bm, 1 H, C-3), 1.006 (s, 3 H, C-19), 1.002 (d, J = 5.8 Hz, 3 H, C-26), 0.898 (d, J = 6.4 Hz, 3 H, C-28), 0.811 (d, J = 6.4 Hz, 3 H, C-21), 0.683 (s, 3 H, C-18), 0.50–0.40 (bm, 1 H, cyclopropyl), 0.20–0.05 (bm, 3 H, cyclopropyl); mass spectrum similar to that of **31**.

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Biosynthetic Studies of Marine Lipids. 38.[1] Mechanism and Scope of Sterol Side Chain Dealkylation in Sponges: Evidence for Concurrent Alkylation and Dealkylation

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Abstract: A biosynthetic study has been performed to elucidate the precise mechanism of sterol side chain dealkylation in marine sponges. This process resembles that of insects by involving the oxidation of common dietary sterols with unsaturation at C-24(28) [24-methylenecholesterol (**8**), fucosterol (**3**), and isofucosterol (**4**)] to their 24,28-epoxides (**9** and **5**). Loss of formaldehyde (or acetaldehyde) then produces desmosterol (**6**), which is subsequently reduced to cholesterol (**7**). The existence of this pathway in sponges is particularly surprising as these organisms are also capable of the reverse process: *S*-adenosylmethionine-mediated alkylation. The simultaneous operation of these competing processes was demonstrated using doubly labeled sterol precursors.

Introduction

The occurrence of novel sterols with unprecedented structures in sponges is now well-documented.[2] An understanding of the biosynthetic origin of these structures is also emerging.[3a,b] In spite of the tremendous diversity of structures there are certain unifying features in the biosynthesis of these sterols. The use of *S*-adenosylmethionine (SAM) is universal in the construction of the highly alkylated side chains of sponge sterols[3a,b] as well as all phytosterols.[3c] It is also apparent that in sponges of the order Haplosclerida, which characteristically produce unusual sterols

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(1) Part 37: Silva, C. J.; Giner, J.-L.; Djerassi, C. *J. Am. Chem. Soc.*, preceding paper in this issue.

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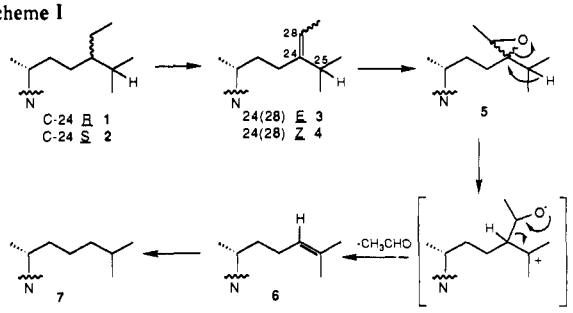
0002-7863/92/1514-299$03.00/0

Scheme I



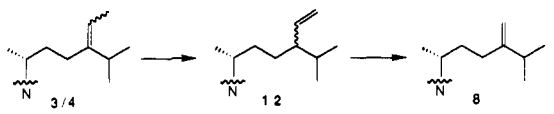
(such as cyclopropanes and cyclopropenes), there exists a single, common intermediate.[4] In two recent preliminary communications,[5a,b] we reported evidence suggesting the operation of an unexpected pathway in sterol metabolism: the dealkylation of 24-alkylated sterols to cholesterol.[5] We now describe the mechanism and scope of this process and discuss the relationship between SAM-mediated alkylation and dealkylation.

Dealkylation of sterol side chains was first rigorously demonstrated among insects by Clark and Bloch in 1959.[6] In this pioneering work, the conversion of ergosterol to 22-dehydrocholesterol was observed in the cockroach *Blattella germanica*. In most insects, cholesterol is the principal sterol and is a required component of the lipid bilayer in cell membranes.[7] Unlike most vertebrates and plants, insects are generally incapable of de novo sterol biosynthesis, and thus those insects which do not obtain cholesterol in their diet must satisfy their sterol requirements by the modification of higher alkylated dietary sterols.

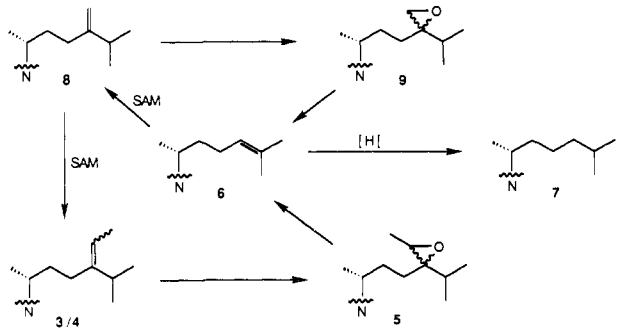
The mechanism of this dealkylation in insects has been examined in the laboratories of Svoboda,[8] Ikekawa,[9] and Goodwin.[10] Through the feeding of radiolabeled sterols and the isolation of several intermediates, the mechanism was demonstrated to be as described in Scheme I. Sitosterol (**1**) and its C-24 epimer clionasterol (**2**) are dehydrogenated to (iso)fucosterol (**3**/**4**), which are then transformed to their 24,28-epoxides (**5**). Loss of acetaldehyde generates desmosterol (**6**), which is then reduced to cholesterol (**7**). The hydrogen at C-25 of sitosterol (**1**) and clionasterol (**2**) has been shown to migrate to C-24 of desmosterol.[10b,11] Dealkylation of sterol side chains is not common in the marine environment, but it has been documented in certain molluscs,[12] arthropods,[13] and coelenterates.[14] The mechanism of dealkylation in these organisms has not been investigated.

We have recently demonstrated that sponges are capable of de novo sterol biosynthesis,[15] and from our earlier work[5] and the work described herein, it is evident that sponges can also assimilate dietary cholesterol (**7**), dealkylate dietary phytosterols to cholesterol, and alkylate sterol side chains. Goad has earlier commented[2c] that "it would certainly be an extraordinary situation if animals of the same phylum have evolved the enzyme systems required for both C-24 alkylation and C-24 dealkylation reactions." We now show such a combination is indeed possible. This unique set of capabilities in a single organism offers sponges unparalleled metabolic plasticity.

Scheme II



Scheme III



Results

In our preliminary communication,[5a] we reported that 24-methylenecholesterol (**8**) and its 24,28-epoxide (**9**) were converted to cholesterol (**7**) using live sponges. This suggested that the mechanism of dealkylation in sponges may be analogous to that in insects (Scheme I). In a subsequent communication,[5b] the conversion of 24-methylenecholesterol (**8**) to its epoxide (**9**) was confirmed using cell-free extracts of several sponges. Numerous experiments have now been performed with *Tethya aurantium* (*T. aurantia*, order Hadromerida, family Tethyidae), an abundant sponge in Monterey Bay, in order to examine the precise mechanism and scope of this dealkylation. Table I summarizes the metabolic fate of six ^{3}H-labeled sterols that were efficiently metabolized, while Figure 1 lists sterols (24-methyl-, 24-ethyl-, and 24-vinylcholesterol (**10**, **11**, and **12**)) that were not transformed. It should be stressed that for all of the sterols in Figure 1, a significant amount of radioactivity was recovered with each precursor, indicating that all sterols were incorporated into the sponge cells but were not metabolized. For the experiments in Table I in which the precursor was metabolized, the individual sterols of the sponge were rigorously purified by HPLC to constant specific activity. Unless stated otherwise, sterols were labeled with ^{3}H at C-3 by a previously described procedure.[16]

It is apparent from Table I that 24-methylenecholesterol (**8**), fucosterol (**3**), isofucosterol (**4**), and their 24,28-epoxides (**9** and **5**) are degraded to desmosterol and cholesterol in *Tethya aurantium*. It is noteworthy that there is no significant difference in the degree of metabolism of fucosterol (**3**) and its stereoisomer, isofucosterol (**4**). In contrast to insects, only sterols with C-24(28) unsaturation were degraded by *T. aurantium*. (24*R*/*S*)-24-Methylcholesterol (**10**) and (24*R*/*S*)-24-ethylcholesterol (**11**) were not transformed. Sponges evidently lack the ability to effect a dehydrogenation at C-24(28) of saturated sterol side chains. This difference in insect and sponge metabolism may reflect a difference in the sterol composition of the diets of these organisms.

A number of entries in Table I were puzzling to us and required

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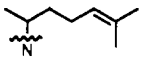
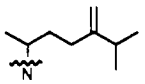
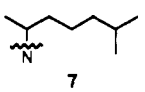
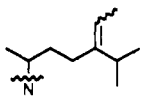
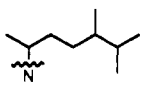
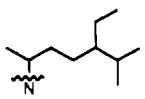
Table I. Incorporation Experiments in *Tethya aurantium*[a]

sponge sterol		precursor[b] [label position]					
		6 [3-^{3}H]	**8** [3-^{3}H]	**3** [3-^{3}H]	**4** [6-^{3}H]	**9**[c] [3-^{3}H]	**5**[d] [3-^{3}H]
 **6**	dpm[e]	2850	14250	203000	295220	14400	12000
	dpm/mg[f]	950	7130	67670	73800	4800	6000
 **8**	dpm	195200	249750	281300	634430	96600	166000
	dpm/mg	8490	8920	11720	21150	4200	5930
 **7**	dpm	116000	13950	536500	441380	74200	110700
	dpm/mg	2070	240	8650	6310	1325	1910
 **3/4**	dpm	41400	6220	1255700	3002660	cold	cold
	dpm/mg	82800	6220	1255700	3002600		
 **10**	dpm	10000	7950	19900	22040	cold	cold
	dpm/mg	2500	1140	2490	2760		
 **11**	dpm	3000	cold	cold	cold	cold	cold
	dpm/mg	500					

[a] Details of incorporation experiments are outlined in the experimental section. [b] 20 μCi (4.4 × 10^7 dpm) of each precursor was fed to each sponge, and each experiment was performed in duplicate. [c] Recovered 7260 dpm in precursor. [d] Recovered 2800 dpm in precursor. [e] Total radioactivity. [f] Specific activity.

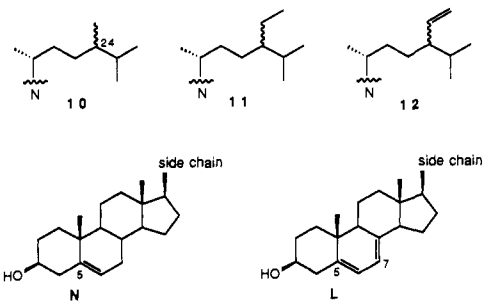


Figure 1.

further experimental scrutiny. Fucosterol (**3**) and isofucosterol (**4**) were both transformed to desmosterol (**6**) and cholesterol (**7**) as one might expect on the basis of the literature of insect sterol metabolism; however, 24-methylenecholesterol (**8**) was also radioactive. There are two plausible explanations for this observation, both amenable to experimental testing.

One possibility is the initial isomerization of (iso)fucosterol (**3**/**4**) to 24-vinylcholesterol (**12**) (Scheme II). Dealkylation of **12**, presumably via its epoxide, would result in the formation of 24-methylenecholesterol (**8**) and suggests the possibility that desmosterol and cholesterol are not produced directly from a dealkylation of (iso)fucosterol but through the intermediacy of 24-methylenecholesterol. A C-24 epimeric mixture of ^{3}H-labeled 24-vinylcholesterols (**12**) was, however, not metabolized by *T. aurantium*, thus implying that **12** is not the source of 24-methylenecholesterol in this sponge.

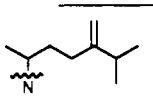
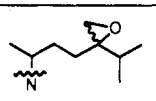
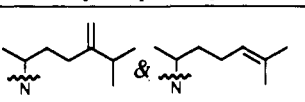
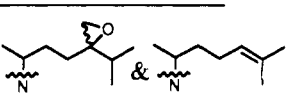
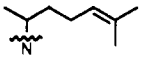
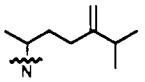
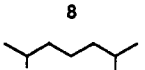
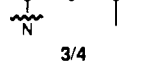
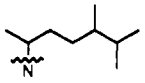
Another possible explanation for the production of "hot" 24-methylenecholesterol from labeled (iso)fucosterol (**3**/**4**) is that **3** and **4** are degraded to desmosterol (**6**), which is not only reduced to cholesterol but is also realkylated to 24-methylenecholesterol (**8**) (Scheme III). As shown in Table I, desmosterol (**6**) is converted, inter alia, to cholesterol (**7**) and 24-methylenecholesterol (**8**). The alkylation of desmosterol (**6**) to 24-methylenecholesterol (**8**) was further demonstrated by the feeding of ^{3}H-labeled *S*-adenosylmethionine. There was poor uptake of this precursor, relative to sterols,[17] yet 24-methylenecholesterol was clearly radioactive (2450 dpm) after the usual incubation period with 20 μCi of [^{3}H]SAM. If there is indeed a dealkylation of (iso)-fucosterol (**3**/**4**) followed by a realkylation, then one would expect that the same might occur with 24-methylenecholesterol (**8**).

To examine the possibility of such a sequence of events, a double labeling experiment with [3-^{3}H,28-^{14}C]-24-methylenecholesterol was performed (first column, Table II). Desmosterol (**6**) and cholesterol (**7**) were found to contain ^{3}H but not ^{14}C as expected. The 24-methylenecholesterol (**8**) contained both ^{14}C and ^{3}H, but the ratio of ^{14}C:^{3}H after the usual incubation period was 1:11.8, while in the precursor it was 1:1. This indicates that over 90% of the radioactivity associated with 24-methylenecholesterol is produced by a dealkylation and subsequent realkylation (Scheme III) and that the remainder (<10%) is recovered precursor. In this and other double labeling experiments, the location of the ^{3}H label was confirmed by oxidation of the sterols with pyridinium chlorochromate to 3-keto steroids. In all cases, such treatment resulted in the loss of radioactivity, confirming that the tritium label had remained at C-3. Conversion of the recovered 24-methylenecholesterol (**8**) to its i-methyl ether followed by ozonolysis resulted in the loss of radioactivity, confirming that the ^{14}C label was at C-28.

From Table I it is apparent that the epoxide of 24-methylenecholesterol (**9**) is converted to desmosterol (**6**), chole-

(17) Sponges are more efficient at removing insoluble material from sea water than dissolved substances. *S*-Adenosylmethionine (SAM) is very soluble in water, whereas sterols are not; it is this solubility difference that most likely explains the varying uptake of SAM and sterols.

Table II. Double Labeling Experiments with *Tethya aurantium*

	precursor[a] [label position]							
								
	8 [3-^{3}H, 28-^{14}C]		**9** [3-^{3}H, 28-^{4}C]		**8** [3-^{3}H]	**6** [24-^{24}C]	**9** [3-^{3}H]	**6** [24-^{14}C]
sponge sterol	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
 **6** dpm[b]	5000	cold	4100	cold	3100	3450	2510	9600
dpm/mg[c]	3330		2800		3100	2760	2090	9600
 **8** dpm	450100	38000	29200	cold	116100	35300	16600	68300
dpm/mg	40920	2920	2750		8930	2940	1510	5250
 **7** dpm	9900	cold	48000	cold	3700	37800	73200	66200
dpm/mg	370		1850		130	1510	2710	2550
 **3/4** dpm	3040	1950	540	cold	1140	700	600	980
dpm/mg	15200	7800	2700		4560	2690	2400	6530
 **10** dpm	2600	220	1100	cold	2100	1700	1700	4300
dpm/mg	1300	90	550		1050	740	680	2150
 **11** dpm	2500	300	cold	cold	1500	cold	cold	cold
dpm/mg	830	80			500			
total activity dpm in Δ^5 sterols	473140	40470	82940	cold	127640	78950	96610	149380

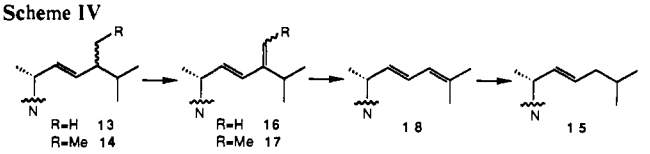
[a] 5 μCi (1.1 × 10^7 dpm) ^{3}H and 5 μCi (1.1 × 10^7 dpm) ^{14}C of each precursor or mixture of precursors were fed to each sponge. All experiments were performed in duplicate. [b] Total radioactivity. [c] Specific activity.

sterol (**7**), and 24-methylenecholesterol (**8**). To examine the mechanism of these conversions, a double labeling experiment with [3-^{3}H,28-^{14}C]-24,28-epoxy-24-methylcholesterol (**9**) was performed (second column, Table II). If the 24-methylenecholesterol (**8**) was generated by the opening of the epoxide followed by dehydration, the ^{14}C label at C-28 should be retained. Alternatively, if **8** is generated from dealkylation to desmosterol (**6**) and realkylation, the ^{14}C label should be absent. The presence of only ^{3}H in the 24-methylenecholesterol indicates that the latter mechanism is the correct one and corroborates the results from the first double labeling experiment.

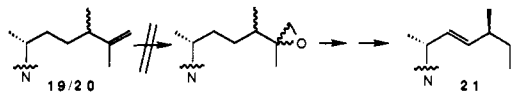
To further confirm the co-occurrence of alkylation and dealkylation, two additional double labeling experiments were performed. One involved the feeding of [24-^{14}C]desmosterol and [3-^{3}H]-24-methylenecholesterol (third column, Table II), while in the other [24-^{14}C]desmosterol and [3-^{3}H]-24,28-epoxy-24-methylcholesterol (fourth column, Table II) were administered to *Tethya aurantium*. Labels were introduced in positions such that they are retained after all biosynthetic steps. In both experiments, ^{3}H and ^{14}C activity were recovered, inter alia, in desmosterol (**6**), cholesterol (**7**), and 24-methylenecholesterol (**8**), thus demonstrating the concurrent alkylation and dealkylation in a single organism.

(Epi)brassicasterol (**13**) and (epi)stigmasterol (**14**) were examined as potential precursors for 22-dehydrocholesterol (**15**) biosynthesis. Sterol **15** has been found in many marine invertebrates,[2a] including *T. aurantium*, yet its biogenetic origin remains unknown. We reasoned that dealkylation of the C-24 alkyl group of **13** and/or **14** could result in the formation of 22-dehydrocholesterol (**15**), presumably via dienes **16**, **17**, and **18** as shown in Scheme IV. This is indeed the process that has been demonstrated in insects.[6] (Epi)brassicasterol (**13**) and (epi)stigmasterol (**14**) were not degraded; the only observed transformation was

Scheme IV



Scheme V



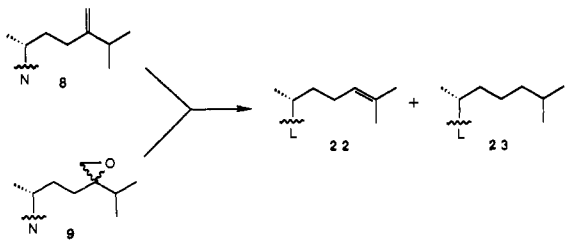
the conversion to their side chain saturated analogues. Similarly, dienes **16** and **18** were not transformed. Labeled 22-dehydrocholesterol (**15**) and cholesterol (**7**) were not metabolized by *T. aurantium*, indicating that, in this organism, they are metabolic end products and/or obtained from the diet.

We also felt that, if 24-methylenecholesterol (**8**) can be degraded to the cholesterol side chain by *T. aurantium*, then codisterol (**19**) or its C-24 epimer (**20**) might conceivably be degraded in a similar fashion to the occelasterol side chain (**21**)[18] or its C-24 epimer (Scheme V). Sterol **21** is a trace sterol in *T. aurantium* and is freqently found in various marine invertebrates, yet its biosynthesis is unknown. However, neither codisterol (**19**) nor epicodisterol (**20**) was degraded by *T. aurantium*. It is possible that this is the correct metabolic route but that it operates in a microalga that is ingested by sponges and other suspension feeders.

Leucilla nuttingi is a calcareous sponge with predominantly $\Delta^{5,7}$-unsaturated sterols. Experiments with labeled 24-methylenecholesterol (**8**) and its 24,28-epoxide (**9**) demonstrated

(18) Kobayashi, M.; Mitsuhashi, H. *Steroids* **1974**, *24*, 399.

Scheme VI



that *L. nuttingi* is capable of degrading sterol side chains and simultaneously modifying the nucleus to 7-dehydrocholesterol (**23**)[19] (Scheme VI).

The generality of sterol side chain dealkylation has recently been demonstrated[5b] by the preparation of cell-free extracts of eight sponges. As we reported earlier, this metabolic process operates in all sponges studied to date, including those with unconventional sterols. It now appears that the capacity for simultaneous side chain alkylation and dealkylation is a common property of sponges and that the mechanism of the degradation largely parallels that found in insects.

Experimental Section

General. Normal-phase columns (Altex, Ultrasil-Si, 10 mm i.d. × 25 cm) with 6% ethyl acetate in hexanes as the mobile phase were used to obtain a clean sample of the Δ^5 sterols and, in the experiments with *Leucilla nuttingi*, separate the Δ^5 from $\Delta^{5,7}$ sterols. Reversed-phase columns (Altex, Ultrasphere ODS, 10 mm i.d. × 25 cm) with methanol as the mobile phase provided an initial purification of the sterols. Some of these fractions were impure and required a second reversed-phase HPLC injection with acetonitrile/methanol/ethyl acetate (11:4:3). The purity of the HPLC fractions was determined by capillary GC using an FID and an HP Ultra capillary column (0.32 mm i.d. × 25 m with 0.52-μm film thickness). The temperature program was 280 °C (1 min) and then 1 °C/min to 290 °C (40 min).

Sponge Collection and Introduction of Sterol Precursor. *Tethya aurantium* and *Leucilla nuttingi* were collected at a depth of 8–12 m in Monterey and Carmel Bays in central California. The sponges were maintained on an underwater grid at the same depth prior to use in biosynthetic experiments to enable scar tissue to heal. The in vivo experiments were performed in a similar manner to a previously described procedure.[19] An ethanolic suspension of the precursor was added to the sponge in noncirculating sea water maintained at the appropriate temperature. As a general rule, a small amount of sea water was used (one sponge volume) and the sponge allowed to filter this sea water for 24 h. The sponges were then returned to an underwater grid for a period of 4 weeks prior to being sacrificed for analysis.

Workup of Biosynthetic Experiments. The sponges were cut into ca. 0.5-cm^3 pieces and extracted with chloroform/methanol (twice) and then chloroform (twice). Where appropriate, sterol "cold carriers" were added to this crude extract prior to flash chromatography over silica and HPLC purification as described above. In all cases, individual sterols were purified to constant specific activity by repeated injections on reversed-phase HPLC.

Determination of Position of Radiolabel. The location of the radiolabel was confirmed by one of two methods for each sterol that was found to be radioactive after a feeding experiment.

(i) Experiments in which the precursor was labeled with ^{3}H at C-3. The sterol was treated with an excess of pyridinium chlorochromate in dichloromethane for 3 h at room temperature. The resulting ketone was purified by filtration through a column of silica, and the radioactivity was measured and compared with the starting sterol.

(ii) Experiments in which the precursor was labeled with ^{14}C at C-28. The sterol was converted to its i-methyl ether by treatment with tosyl chloride in pyridine followed by refluxing in methanol. The protected sterol was ozonized in a standard manner to generate the corresponding 24-ketone, whose radioactivity was then measured.

***S*-Adenosylmethionine Incorporation.** Commercially available ^{3}H-labeled *S*-adenosylmethionine (20 μCi) was added to a sponge in noncirculating sea water (one sponge volume), maintained in a bath of circulating sea water to control the temperature. The sponge was allowed to filter the sea water for 2.5 days to maximize incorporation of this water-soluble precursor. After the usual incubation period of 4 weeks, the sterols were purified as above. Very low levels of radioactivity were obtained, probably due to the inability of a filter-feeding sponge to extract a water-soluble compound from the sea water;[17] still, 2450 dpm was recovered in 24-methylenecholesterol.

Recovered Radioactivities of Precursors Not Metabolized: Cholest-5-en-3β-ol (**7**) 2 106 200 dpm; (24*R*/*S*)-24-methylcholest-5-en-3β-ol (**10**) 2 590 300 dpm; (24*R*/*S*)-24-ethylcholest-5-en-3β-ol (**11**) 438 800 dpm; (24*R*/*S*)-24-ethylcholesta-5,28-dien-3β-ol (**12**) 405 100 dpm; (24*R*/*S*)-24-methylcholesta-5,22-dien-3β-ol (**13**) 4 585 800 dpm; (24*R*/*S*)-24-ethylcholesta-5,22-dien-3β-ol (**14**) 2 003 000 dpm; cholesta-5,22-dien-3β-ol (**15**) 1 308 900 dpm; 24-methylcholesta-5,22,24(28)-trien-3β-ol (**18**) 307 600 dpm; cholesta-5,22,24-trien-3β-ol (**18**) 354 300 dpm; (24*R*/*S*)-24-methylcholesta-5,25-dien-3β-ol (**19**/**20**) 786 200 dpm.

Synthesis of Sterol Precursors. The following sterols were synthesized as described in the literature, and the ^{3}H label was introduced at C-3:[16] desmosterol (**6**), 24-methylenecholesterol (**8**), fucosterol (**3**), isofucosterol (**4**), and 24-vinylcholesterol (**12**).[20] An epimeric mixture of 24-methylcholesterols (**10**) and 24-ethylcholesterols (**11**) was prepared by the hydrogenation and subsequent deprotection of the *i*-methyl ethers of 24-methylenecholesterol and (iso)fucosterol (**3**/**4**), respectively. [24-^{14}C]Desmosterol[21] and [28-^{14}C]-24-methylenecholesterol[22] were also prepared according to literature precedents.

24,28-Epoxy-24-methylcholesterol (9) and 24,28-Epoxy-24-ethylcholesterol (5). Epoxide **9** was synthesized (in 60% yield) from 24-oxocholesterol acetate by treatment with a 10% excess of the sulfur ylide generated from trimethylsulfonyl iodide. [28-^{14}C]-24,28-Epoxy-24-methylcholesterol was prepared in a similar manner, but using [^{14}C]-methyl iodide and dimethyl sulfide to generate the sulfonium salt. Epoxide **5** was prepared by an analogous route, with the sulfonium salt being generated from diphenyl sulfide and ethyl iodide. The ^{1}H NMR spectra of these epoxides were identical to those reported previously.[23]

Cholesta-5,22,24-trien-3β-ol (**18**) and 24-methylcholesta-5,22,24-(28)-trien-3β-ol (**16**) were prepared by treating the i-methyl ether of (20*S*)-pregnanecarboxaldehyde with the Wittig reagents generated from (3-methyl-2-butenyl)triphenylphosphonium bromide and (2-isopropyl-2-propenyl)triphenylphosphonium bromide respectively. Their spectral properties corresponded with those reported earlier.[8b]

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