6 H); mass spectrum, *m/e* 168 (M + 1, 1), 139 (2), 124 (1), 108 (1), 99 (23), 74 (15), 68 (5), 57 (100), 43 (24); exact mass calculated for $C_{20}H_{17}NO$ 167.13101, found 167.13128.

Kinetic Studies. The 6-dram borosilicate vials to be used as reaction vessels were capped with rubber septa and purged with dry nitrogen. While continuing the nitrogen purge, the vials were flamed to remove adsorbed water and then allowed to cool. The remaining glassware for each set of reactions was dried overnight in an oven at 130 °C and cooled in a desiccator before use. The solvent acetonitrile: HPLC grade was further purified as noted and stored over 3-Å molecular sieves. In control runs, the HPLC-grade acetonitrile was used directly (over sieves) without distillation from phosphorus pentoxide. The sensitizer (DCB) was recrystallized from benzene prior to use. MVA was used as received for some reactions and was vacuum-distilled in other cases. Similarly, runs were made with dienes directly as received; in other cases the distilled dienes were used. A fortuitous effect of impurities on the kinetic results is thereby excluded.

The photosensitizer for each set of reactions (each run) was weighed into a nitrogen-purged round-bottomed flask which was septum sealed except during the addition of the photosensitizer and a magnetic stirring bar. Solvent (5 mL) was then measured and added by syringe. The mixture was then stirred magnetically under a slow nitrogen purge. The dienes and MVA were then added to the reaction vials by syringe, with the exact amounts used determined by weighing the vials before and after each addition. The photosensitizer and solvent were then added by syringe, after which the vials were shaken to mix the reagents. The septa were then wired down and covered with aluminum foil to prevent their deterioration during irradiation. The vials were hung in a room-temperature water bath which surrounded the housing of a 450-W Hanovia medium-pressure mercury-vapor lamp. The lamp was kept cool during operation by circulating chilled water through the lamp's borosilicate housing.

After completion of the allowed reaction time (never more than 20% conversion; 1–20% conversions), the gas chromatography (GC) internal standard (phenanthrene; Eastman, recrystallized from 95% ethanol) was added to each vial at a level of 30 mol % of the weighed amount of alkene used. The samples were then analyzed by GC performed on a Varian 3700 flame ionization detector chromatograph with either a 1 m × 0.125 in. or a 0.5 m × 0.125 in. column, both packed with 5% OV-101 on Chromosorb G-HP 100/120. The GC was programmed to rise from 100 to 250 °C at 25 °C C/min. GC yields were calculated with the aid of a Varian SP 4270 integrator. Six separate runs were made using DCB as the photosensitizer. The results were qualitatively and quantitatively very similar in all runs; averaged reactivities are presented in Table II.

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Registry No. 1, 5194-51-4; **2**, 592-57-4; **3**, 77142-27-9; **4**, 1000-86-8; **5**, 2004-70-8; (±)-*anti*-**6**, 116809-24-6; (±)-*syn*-**6**, 116907-41-6; (±)-*anti*-**7**, 116809-25-7; (±)-*syn*-**7**, 116907-42-7; (±)-*endo*-**8**, 116809-26-8; (±)-*exo*-**8**, 116809-37-1; (±)-*anti*-**9**, 116809-27-9; (±)-*syn*-**9**, 116907-43-8; (±)-*anti*-**10**, 116809-28-0; (±)-*syn*-**10**, 116907-44-9; *endo*-**11**, 116907-40-5; *exo*-**11**, 116907-45-0; **12**, 116809-29-1; **13**, 116809-30-4; **14**, 116809-31-5; (±)-*anti*-**15**, 116809-32-6; (±)-*syn*-**15**, 116809-38-2; (±)-*anti*-**16**, 116809-33-7; (±)-*syn*-**16**, 116809-39-3; (±)-**17**, 116809-34-8; *N*-methyl-*N*-vinylacetamide, 3195-78-6; *N*-acetyl-*N*-methyl-2-(1-propenyl)cyclobutanamine, 116809-35-9; *N*-acetyl-*N*,3-dimethyl-2-ethenylcyclobutanamine, 116809-36-0; (*E*)-anethole, 4180-23-8; 4-methyl-1,3-pentadiene, 926-56-7; 1,1′-dicyclopentenyl, 934-02-1; 2,3-dimethyl-1,3-butadiene, 513-81-5; 2-methyl-1,3-butadiene, 78-79-5; phenyl vinyl sulfide, 1822-73-7; ethyl vinyl ether, 109-92-2; phenyl vinyl ether, 766-94-9; (*E*)-2-methyl-1,3-pentadiene, 1118-58-7; α-methylstyrene, 98-83-9.

Biosynthetic Studies of Marine Lipids. 17.[1] The Course of Chain Elongation and Desaturation in Long-Chain Fatty Acids of Marine Sponges

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Abstract: Through appropriate labeling and incorporation experiments, the biosynthesis of the most characteristic sponge fatty acids, (5*Z*,9*Z*)-5,9-hexacosadienoic acid ($\Delta^{5,9}$-26:2) and (5*Z*,9*Z*,19*Z*)-5,9,19-hexacosatrienoic acid ($\Delta^{5,9,19}$-26:3), was completely elucidated. The dienoic acid is produced by the sequence 14:0 → → 26:0 → 26:1 → 26:2. Contrary to precedent in animal fatty acid biosynthesis, the first desaturation occurs both at Δ^5 and Δ^9. Either of these 26:1 acids can undergo further desaturation to the $\Delta^{5,9}$-26:2 acid. As far as 26:3 trienoic acid biosynthesis is concerned, no Δ^9-desaturase appears to act at the usual 16:0 stage. Instead, the sponge secures palmitoleic acid (Δ^9-16:1) from an exogeneous source (bacteria?) and undergoes chain elongation to Δ^{19}-26:1 before undergoing Δ^9- and Δ^5-desaturation.

One of the most interesting chemical features of marine sponges is the presence of unusual fatty acids in their phospholipids. They are rich sources of C_{24}–C_{30} fatty acids,[2-13] in contrast to the C_{14}–C_{22} fatty acids typical of higher animals. Furthermore, contrary to most phospholipids which contain two different acyl groups attached to the glycerol backbone, sponges possess the same long-chain acyl moiety at both locations.[3] The polyunsaturated

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Table I. Composition (%) of Principal Phospholipid Fatty Acids in *Microciona Prolifera*[a,b]

fatty acid	July[c]	December
Δ^9-16:1	1.6 (0.2)	3.2
n-16:0	5.1 (0.4)	4.8
Δ^{11}-18:1	4.1 (0.4)	6.4
n-18:0	2.7 (0.3)	1.9
$\Delta^{4,7,10,13}$-20:4	4.7 (0.4)	6.2
$\Delta^{4,7,10,13,16,19}$-22:6	25.4 (1.4)	18.9
22:0	2.8 (1.3)	1.8
$\Delta^{5,9,19}$-26:3	8.2 (0.4)	11.3
$\Delta^{5,9}$-26:2	16.4 (0.5)	11.0

[a] Sponges were collected in the San Francisco Bay near Redwood City at a depth of 30 cm. [b] For head group distribution and molecular species isolation, see ref 3. [c] Averages of five replicates; standard deviations are in parentheses.

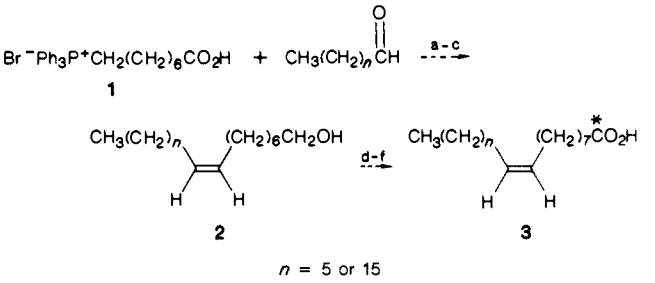
fatty acids of sponges often exhibit a $\Delta^{5,9}$ double bond pattern, whereas higher animals contain $\Delta^{6,9}$-acids (methylene-interrupted diene system). In addition, many sponge phospholipids contain methyl-branched fatty acids which have been isolated from *Petrosia ficiformis*,[5] *Calyx nicaeensis*,[7] *Petrosia hebes*,[8] *Aplysina fistularis*,[9] and *Strongylophora durissima*.[10] Unusual substituents of the fatty acid chains are also known to exist in some sponges: cyclopropyl from *C. nicaeensis*,[7] bromo from *P. hebes*,[8] methoxy from *Higginsia tethyoides*,[11,12] and acetoxy from *Polymastia gleneni*.[13] In spite of the numerous interesting structural features of sponge fatty acids, their biosynthesis has been explored only sporadically.

De Rosa et al.[14] studied the biosynthesis of fatty acids in the sponge *Verongia aerophoba* through administration of [1-^{14}C]-acetate, [2-^{14}C]mevalonate, and [CH_3-^{14}C]methionine. Some incorporation of radioactivity into total fatty acids was observed, notably with acetate, but since the individual fatty acids were neither separated nor identified, information about fatty acid composition and location of radioactivity is not available. Litchfield and Morales[15] proposed, on the basis of feeding experiments of sodium [1-^{14}C]acetate in *Microciona prolifera*, that the unusual long-chain fatty acids (e.g. $\Delta^{5,9}$-26:2) were formed by a chain elongation and desaturation mechanism from short-chain fatty acids such as palmitic (16:0) and palmitoleic (Δ^9-16:1) acids. The proposed pathway, 16:0 → 26:0 → Δ^9-26:1 → $\Delta^{5,9}$-26:2, unfortunately, does not shed any light on specific precursors, since acetate is the basic 2-carbon unit used in all fatty acid chain elongations, and no other labeled precursors were examined.

Several studies[16-18] in our laboratory on fatty acid biosynthesis in marine sponges have solved certain technical problems as well as advanced our knowledge of more advanced biosynthetic intermediates. For example, in the earlier *Verongia aerophoba* experiment,[14] the Italian investigators found the sponges dead after 48 h of incubation. By contrast, we showed[16] that the Australian sponge *Jaspis stellifera* biosynthesizes $\Delta^{5,9}$-iso-27:2, $\Delta^{5,9}$-anteiso-27:2, and $\Delta^{5,9}$-26:2 acids from iso-15:0, anteiso-15:0, and 16:0 precursors, respectively, and the sponges remained healthy even after 30 days of incubation. A subsequent biosynthetic study on *Aplysina fistularis*[18] revealed that both *R* and *S* enantiomers of 10-Me-16:0 were transformed into 22-Me-$\Delta^{5,9}$-28:2, even though the naturally occurring acid possesses the 22*R* configuration as demonstrated[18] by total synthesis from chiral precursors.

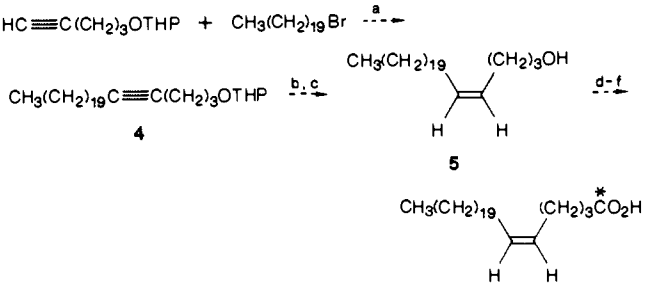
Microciona prolifera is an ideal sponge with which to study fatty acid biosynthesis since it contains[2] the long-chain fatty acids $\Delta^{5,9}$-26:2 and $\Delta^{5,9,19}$-26:3 as major components. Another advantage is the sponge's abundance in the San Francisco Bay. We were thus able to study the fate of short-chain fatty acid precursors

Scheme I[a]



[a] (a) *n*-BuLi, THF, DMSO; (b) MeOH, H$^+$; (c) $LiAlH_4$, THF; (d) MsCl, Et_3N, DMAP, CH_2Cl_2; (e) K^{14}CN, THF, DMSO; (f) KOH, EtOH.

Scheme II[a]



[a] (a) *n*-BuLi, THF, hexane, HMPA; (b) Lindlar catalyst, *n*-hexane; (c) H$^+$, MeOH; (d) MsCl, Et_3N, CH_2Cl_2; (e) K^{14}CN, THF, DMSO; (f) KOH, EtOH.

such as lauric (12:0), myristic (14:0), palmitic (16:0), palmitoleic (Δ^9-16:1), and stearic (18:0) acids in addition to the long-chain hexacosanoic (26:0), hexacosenoic (Δ^5-, Δ^9-, and Δ^{19}-26:1), and hexacosadienoic ($\Delta^{5,9}$-26:2) acids. To find the optimum time of biosynthesis of such long-chain fatty acids, time-dependent radioactivity analyses were also performed for all of these precursors.

Results and Discussion

The major fatty acids in the total phospholipids of *Microciona prolifera* are listed in Table I. Two long-chain fatty acids ($\Delta^{5,9}$-26:2 and $\Delta^{5,9,19}$-26:3) are major components[2,3] in the phospholipids of *Microciona prolifera*. They are different from the common fatty acids in two respects: very long chain length and non-methylene interrupted polyunsaturation. The $\Delta^{5,9}$-diene system is known in a few plant tissues[19-22] and in the slime mold *Dictyostelium discoideum*,[23] but only in C_{16}–C_{18} chain length acids.

The composition of the two long-chain sponge fatty acids varies depending on the season (cf. Table I). In the winter, the content of the $\Delta^{5,9,19}$-26:3 acid increases at the expense of the $\Delta^{5,9}$-26-dienoic acid; the reverse happens in the summer. This phenomenon has been explained[2b] as an adjustment by the sponge to maintain constant membrane flexibility with variations in environmental temperature. The seasonal variation of these two components is more pronounced in *Microciona prolifera* collected in New Jersey (26:2/26:3 = 18.1%/12.2% in June;[2a] 14%/31% in March[2b]) than in the San Francisco Bay (26:2/26:3 = 16.4%/8.2% in July; 11.0%/11.3% in December) and is presumably attributable to the larger temperature differences in New Jersey as compared to those in San Francisco. One major component (22:6, docosahexaenoic acid) most likely is derived from the diet.

For our biosynthetic experiments, [1-^{14}C]palmitoleic (Δ^9-16:1), [1-^{14}C]hexacosanoic, [1-^{14}C]hexacosenoic (Δ^5, Δ^9, Δ^{19}-26:1), [1-^{14}C]hexacosadienoic ($\Delta^{5,9}$-26:2), and [5,6-^{3}H]-5-hexacosenoic

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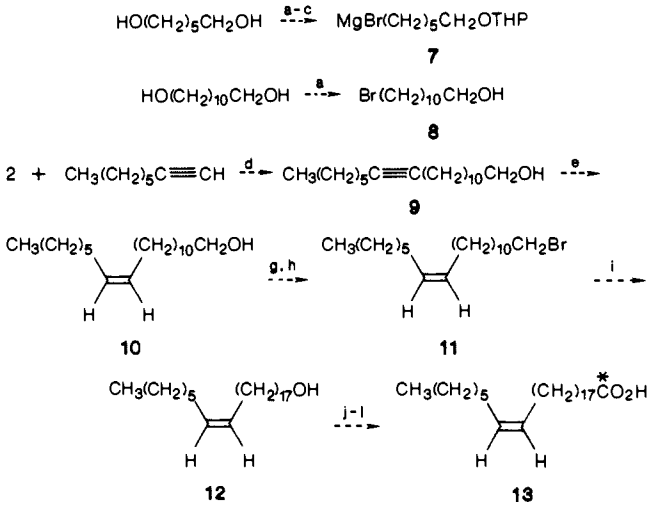
Scheme III[a]



[a](a) 48% HBr, *n*-heptane; (b) *p*-TsOH, DHP; (c) Mg, THF; (d) *n*-BuLi, THF, HMPA; (e) Lindlar catalyst, H_2, *n*-hexane; (g) MsCl, NEt_3, CH_2Cl_2; (h) LiBr, THF; (i) **7**, THF, Li_2CuCl_4; (j) MeOH, H^+; (k) $K^{14}CN$, DMF; (l) KOH, EtOH

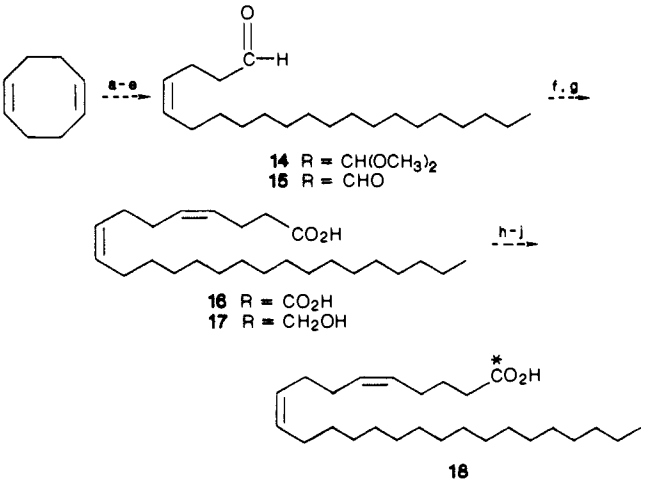
(Δ^5-26:1) acids were synthesized as depicted in Schemes I–V. The key step for the synthesis of the labeled Δ^9-16:1 and Δ^9-26:1 acids (Scheme I) was Wittig reaction of heptanal or heptadecanal with (7-carboxyheptyl)triphenylphosphonium bromide (**1**), affording the *Z*-configured C_{15}- and C_{25}-Δ^8 acids. Reduction of these acids to the corresponding alcohols **2**, mesylation, and treatment with 1 mCi (57.6 mCi/mmol) of $K^{14}CN$ in a 1:1 mixture of dimethyl sulfoxide and tetrahydrofuran afforded the corresponding nitriles, which were then converted to the desired acids **3** by basic hydrolysis. The 26:0 acid was synthesized in the same manner as the Δ^9-26:1 acid except that the olefinic intermediate after Wittig reaction was first subjected to catalytic hydrogenation.

The synthesis of the Δ^5-26:1 acid (Scheme II) entailed two key steps. The first was the acetylenic coupling of the tetrahydropyranyl derivative of 4-pentyn-1-ol with 1-bromoeicosane with *n*-butyllithium as a base. The second was the hydrogenation of the acetylenic compound with Lindlar catalyst to afford the desired *cis*-olefinic product. Methods similar to those used for the previously mentioned acids were then followed to obtain the ^{14}C-labeled Δ^5-26:1 acid **6**.

A different route (Scheme III) was followed for the synthesis of the Δ^{19}-26:1 acid **13**. The acetylenic coupling of 1-octyne with 11-bromo-1-undecanol (**8**) afforded the C_{19} acetylenic alcohol **9**. Subsequent hydrogenation of **9** with Lindlar catalyst, mesylation, and bromine substitution of the mesylate product gave 1-bromo-12-nonadecene (**11**). The Grignard coupling of **11** with the tetrahydropyranyl derivative of 6-bromo-1-hexanol (**7**) was performed in the presence of lithium tetrachloric cuprate as a catalyst. The remaining steps from the resulting C_{25} olefinic alcohol (**12**) to the final Δ^{19}-26:1 acid (**13**) were essentially identical with those in Schemes I and II. The only exception was the solvent for the cyanation reaction of the mesylate. The reaction was faster and no elimination product was observed when dry DMF instead of a 1:1 mixture of DMSO and THF was employed.

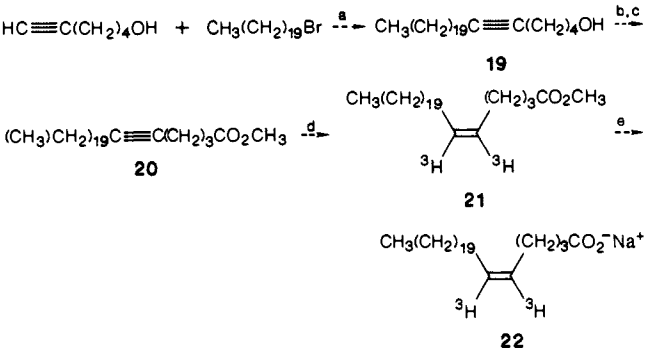
The [1-^{14}C]-5,9-hexacosadienoic acid (**18**) was synthesized by the method shown in Scheme IV. The starting material was 1,1-dimethoxy-4-heneicosene (**14**) which could be obtained from 1.5-cyclooctadiene as reported previously.[24] After removal of the acetal protective group of **14**, the resulting aldehyde (**15**) was treated with (3-carboxypropyl)triphenylphosphonium bromide to provide (4*Z*,8*Z*)-4,8-pentacosadienoic acid (**16**). HPLC analysis of its methyl ester showed the presence of about 8% of the 4*E*,8*Z* isomer. Reduction to the alcohol (**17**) followed by cyanide displacement and hydrolysis provided the required ^{14}C-labeled dienoic

Scheme IV[a]



[a](a) 1 equiv of O_3, MeOH, −78 °C; (b) TsOH, 1 h; (c) $NaBH_4$, −10 °C; (d) *n*-$C_{13}H_{27}MgBr$, THF, Li_2CuCl_4; (e) H^+, acetone; (f) $Br^-Ph_3{}^+PCH_2CH_2CH_2CO_2H$; (g) $LiAlH_4$; (h) MsCl, NEt; (i) $K^{14}CN$, DMF; (j) KOH, EtOH.

Scheme V[a]



[a](a) *n*-BuLi (2 equiv), HMPA; (b) Jones reagent, acetone; (c) TsOH, CH_3OH; (d) $^3H_2$, Lindlar catalyst; (e) NaOH, H_2O, EtOH.

acid **18**. For the double-label experiments (^3H and ^{14}C) of Δ^5-26:1 and Δ^9-26:1, tritium-labeled Δ^5-26:1 was also synthesized (Scheme V). The acetylenic alcohol (**19**) was obtained by acetylenic coupling between 5-hexyn-1-ol and 1-bromoeicosane. Jones oxidation of the alcohol (**19**) followed by esterification gave the acetylenic ester (**20**). A catalytic tritiation of acetylenic ester **20** using Lindlar catalyst afforded the tritium-labeled ester (**21**), which was hydrolyzed to the desired acid (**22**).

The sodium salts of these ^{14}C-labeled precursors and commercially available, labeled lauric, myristic, palmitic, and stearic acids were incorporated with aeration for 9–14 h into live, intact sponges by aquarium incubation in a precursor–seawater solution. The above sponges were then transferred into the natural marine environment for a given period after which time the sponge was processed. The fatty acid methyl esters (FAME) of the total phospholipids, obtained by the method described in the Experimental Section, were fractionated by reverse-phase HPLC on an Altex column. The observed incorporation of radioactivity into the 26:1, 26:2, and 26:3 acid fractions are listed in Table II.

The purity of the C_{26} fatty acids after HPLC fractionation was checked by gas chromatography on a capillary column, which disclosed the following facts: (1) The 26:1 acid is accompanied by the 24:0 acid (<31%). (2) No appreciable impurity exists in the 26:2 fraction. (3) About 15% of the $\Delta^{5,9,19}$-26:3 fraction was contaminated by the $\Delta^{5,9}$-24:2 and $\Delta^{5,9,17}$-26:3 acids.

The extent of these contaminations in each radioactive fraction was checked by epoxidation of the relevant fatty acids with *m*-chloroperoxybenzoic acid, since in this manner the epoxides were readily separated. Taking experiment 3 (Table II) as an example, the monoepoxide of the 26:1 acid was separated by preparative

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Table II. Observed Radioactivities of C_{26} Fatty Acids from Various Precursors

expt no.	precursor	month	incubation period, days	radioactivity, dpm		
				24:0 and 26:1	26:2	24:2 and 26:3
1	12:0	November	8	100	100	100
2	14:0	November	7	22 500	129 000	15 400
			30	5 000	50 000	6 300
3	16:0	July	3	13 800	62 800	4 100
			8	92 800[a]	564 000[b]	30 100[c]
			15	38 400	499 000	21 500
			30	3 900	139 000	6 700
4	18:0	July	7	132 000	331 000	23 300
			15	65 000	444 000	17 800
			30	41 300	225 000	15 500
5	Δ^9-16:1	July	7	124 000	31 300	355 000
			15	134 000	32 500	418 000[d]
			30	41 300	5 800	135 000
6	26:0	July	10 (h)	32 000	60 300	940
			4	46 300	215 000	810
			15	5 900	166 000	2 100
			30	2 000	76 800	1 200
7	Δ^9-26:1	July	14 (h)	4.65×10^6	168 000	1 900
			4	65 500	308 000	2 500
			15	44 400	250 000	2 700
			30	29 900	70 600	600
8	Δ^5-26:1	October	9 (h)	1.31×10^6	155 000	7 500
			4	218 000	248 000	6 000
			15	90 100	178 000	8 600
			30	40 500	36 900	3 500
9	Δ^{19}-26:1	July	2	230 000	32 500	486 000
			4	200 000	42 500	513 000
			15	138 000	38 800	400 000
			30	55 000	26 300	211 000
10	$\Delta^{5,6}$-26:2	May	3		1.26×10^6	8 800

[a] The composition of this fraction was shown to consist of one-third 24:0 and two-thirds 26:1 by silica gel chromatography of the mixture after epoxidation. [b] Approximately 94% of this fraction was the 26:2 acid as shown by purification via its diepoxide. [c] Virtually the entire radioactivity resided in the 24:2 fraction as shown by separation of the derived epoxides. [d] Virtually the entire radioactivity resided in the 26:3 fraction after purification of the derived triepoxide.

TLC from the saturated 24:0 acid contaminant. Subsequent counting showed that approximately 30 000 dpm of the 92 800 dpm (Table II) is due to simple chain elongation of palmitic acid after 8 days to the 24:0 acid, whereas the remaining 66% of radioactivity is associated with the 26:1 acid. The location of the double bond of the 26:1 acid was not established in this experiment.

The diepoxide of the 26:2 acid corresponding to 564 000 dpm in experiment 3 (Table II) was separated by silica gel chromatography from a small unknown impurity. Most (94%) of the radioactivity was derived from the diepoxide (i.e. 26:2 acid); about 2% was found in other fractions, and 4% was lost during the process.

The 30 100 dpm count of the 24:2 + 26:3 fraction in experiment 3 was checked by triepoxidation and subsequent separation by preparative TLC. The triepoxide from the 26:3 acid was not radioactive, most of the radioactivity being associated with the diepoxide from the $\Delta^{5,9}$-24:2 acid. It is extremely likely that the same situation applies to the 24:2 + 26:3 fraction in experiments 2 and 4 (14:0 and 18:0 precursors).

In experiment 5, the 24:2 + 26:3 fraction from the 15-day sample was epoxidized and the diepoxide of the 24:2 acid and the triepoxide of the 26:3 acid were separated by TLC. The diepoxide was not radioactive with most of the radioactivity retained in the triepoxide fraction. For the other precursors, contamination of radioactivity due to impurities is not likely. For example, a 24:2 contaminant in the 26:3 fraction is not likely to be contributing to the radioactivity count of the 26:3 acid from a Δ^{19}-26:1 precursor (experiment 9).

The possibility of randomization of radioactivity via two-carbon degradation followed by chain elongation was checked especially for the 26:2 fraction (499 000 dpm) of the 15-day palmitic acid experiment (experiment 3 in Table II). The $\Delta^{5,9}$-26:2 acid methyl ester was subjected to ozonolysis and the degraded C_{17} monocarboxylic ester was fractionated by HPLC. The radioactivity yield, after degradation, was about 80% for all fractions. Of this recovered radioactivity, 90% was associated with the C_{17} ester as expected. Considering that some of the radioactivity must have been lost during the workup, significant randomization of the radioactive label has thus been shown to be absent.

Scheme VI

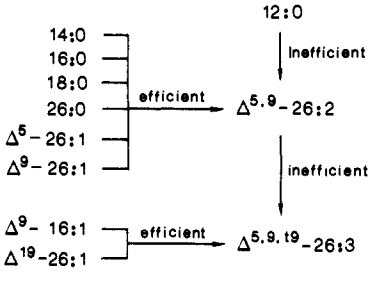

Table III. Radioactivities[26] of C_{26} Fatty Acids from Various Precursors (Winter)

expt no.	precursor	days	obsd radioactivities, dpm		
			24:0 + 26:1	26:2	24:2 + 26:3
11	16:0	7 (Feb)	31 300	71 300	5 000
		14	54 300	126 000	12 200
12	18:0	7 (March)	58 100	86 600	29 500
		15	27 300	151 000	23 300
		30	23 800	159 000	28 100

The time-dependent change of radioactivity in the 26:2 and 26:3 fractions is shown in Figures 1 and 2, respectively. The results clearly indicate that long-chain fatty acid biosynthesis is very active. The maximum radioactivity in the 26:2 or 26:3 acids is reached after 2–15 days, depending on the precursors studied. Figure 2 also contains the decrease of radioactivity recovered in the precursors over time. As expected, the radioactivities of the precursors decreased with time. The results also show that the short-chain precursors (16:0, 18:0 and Δ^9-16:1) take a longer time to reach the optimum radioactivity at the 26:2 (Figure 1) or 26:3

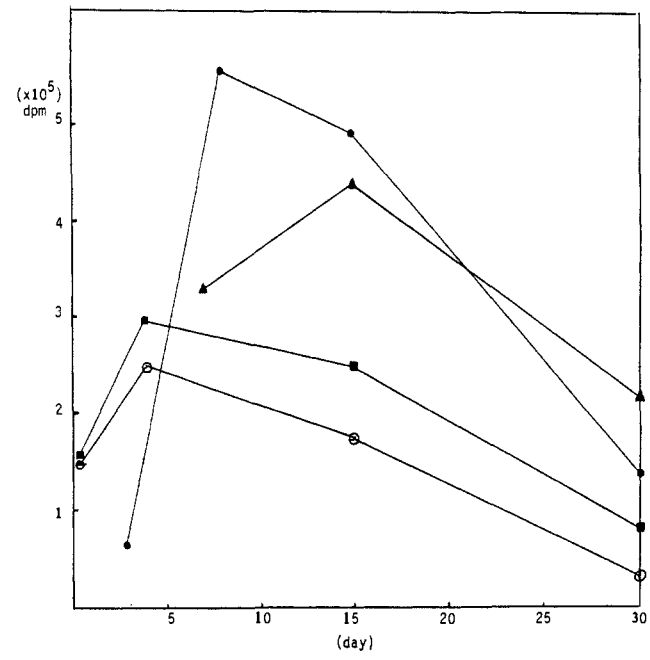

Figure 1. Plot of radioactivity of 26:2 vs time from various precursors. ● for 16:0 (July), ▲ for 18:0 (July), O for Δ^5-26: (October), and ■ for Δ^9-26:1 (July).

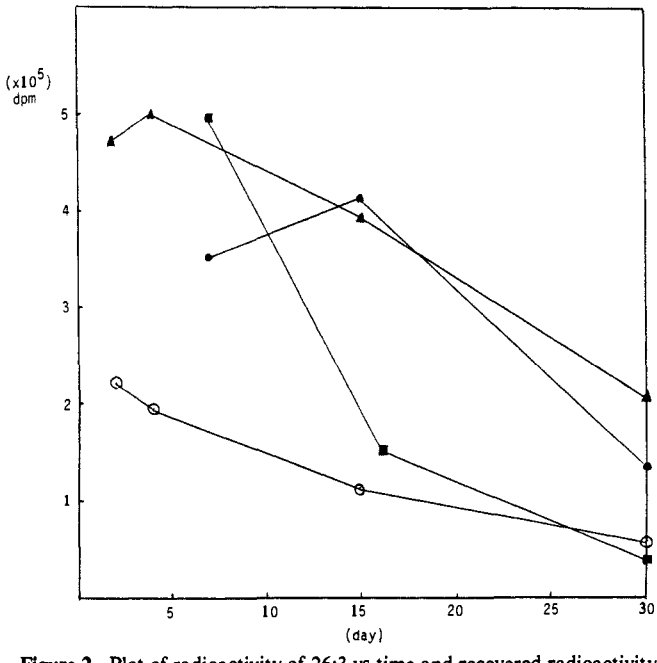

Figure 2. Plot of radioactivity of 26:3 vs time and recovered radioactivity of precursor vs time. ● for 26:3 from Δ^9-16:1, ▲ for 26:3 from Δ^{19}-26:3, ■ for recovered radioactivity of Δ^9-16:1, and O for recovered radioactivity of Δ^{19}-26:1.

(Figure 2) stages. This is reasonable since short-chain precursors require chain elongation to long-chain fatty acids before desaturation can ensue. A summary of our results is given in Scheme VI. The data in Table III, obtained by incorporations in the winter, when contrasted with the predominantly summer results of Table II, show that the biosynthetic efficiency of long-chain fatty acid biosynthesis is seasonal and possibly temperature dependent (e.g. experiments 3 vs 11 or 4 vs 12).

Morales and Litchfield[15] discussed the formation of double bonds at C-5 and C-9 of the long-chain fatty acids in *Microciona prolifera* when labeled acetate was incorporated. On the basis of a series of oxidative degradation studies (partly illustrated in Figure 3), they proposed the following biosynthetic pathway for the $\Delta^{5,9}$-26:2 acid: *n*-16:0 → *n*-26:0 → Δ^9-26:1 → $\Delta^{5,9}$-26:2. They also concluded that the $\Delta^{5,9,19}$-26:3 acid was generated by chain

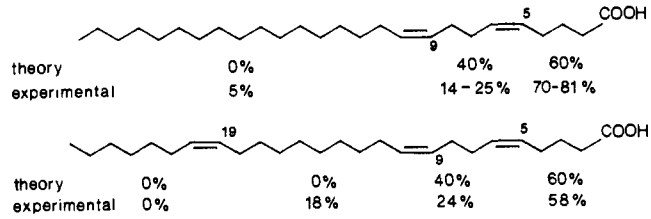

Figure 3. Summary of [1-^{14}C]acetate incorporation results of Morales and Litchfield (ref 15): Experimental = radioactivity encountered in ozonization fragments. Theorg = results based solely on chain elongation from 16:0 rather than de novo biosynthesis.

elongation and subsequent desaturation of palmitoleic acid (Δ^9-16:1), known to be present among the short-chain fatty acids of the animal, without, however, testing such labeled intermediates. The weakness of this early pioneering study was that it employed only the most basic two-carbon unit of all fatty acids and never pursued bioincorporation of more complicated, structurally unique precursors.

Our first question of the Morales and Litchfield paper[15] concerns their interpretation that the 5% of radioactivity recovered in the C_{17} unit (shown in Figure 3) after oxidative degradation of the $\Delta^{5,9}$-26:2 acid implied negligible radioactivity. Therefore, they proposed that palmitic acid was the precursor in the sponge for subsequent elongation to long-chain fatty acids. However, our myristic acid (14:0) experiment (experiment 2 in Table II) showed that the sponge converted this precursor reasonably efficiently into the $\Delta^{5,9}$-26:2 acid. The shortest fatty acid studied by us (experiment 1), lauric acid (12:0), was not taken up. Our observations, coupled with Morales and Litchfield's degradation study[15] showing some radioactivity in the C_{17} unit, indicate that myristic acid is the shortest precursor utilized by *M. prolifera*. At this time, it is not certain why the sponge is incapable of de novo biosynthesis of 14:0 or 16:0 acids from acetate.

Morales and Litchfield's assumption[15] that desaturation occurs only after completion of chain elongation is supported by our experimental work (Table II). For instance, saturated intermediates such as the 18:0, 22:0, and 24:0 acids from the 16:0 experiment (experiment 3 in Table II) were purified by HPLC and proven to be radioactive. The good incorporation into 26:2 from 14:0 (experiment 2), 16:0 (experiment 3), and 18:0 (experiment 4) precursors indicates that there is no substantial substrate preference by the chain elongation enzyme system. Presumably *M. prolifera* would use 20:0, 22:0, and 24:0 precursors for chain elongation into 26:0. This is also supported by the degradation results (Figure 3) of the 26:2 acid from the acetate incorporation,[15] which displayed more radioactivity in the first five carbons at the carboxyl end than would be expected from a simple 16:0 → 26:0 elongation process alone.

To explain the presence of 18% radioactivity in the Δ9–Δ19 segment of the $\Delta^{5,9,19}$-26:3 degration (summarized in Figure 3), Morales and Litchfield proposed[15] the following alternative pathway for this fatty acid: 14:0 → 16:0 → Δ^9-16:1 → Δ^{19}-26:1 → $\Delta^{9,19}$-26:2 → $\Delta^{5,9,19}$-26:3.

According to our results, since myristic (experiment 2, Table II) and palmitic (experiment 3) acids were not useful precursors for the 26:3 acid (most of the radioactivity in this fraction is due to the 24:2 acid component; vide supra), such a pathway is not operative. If the 16:0 → Δ^9-16:1 process were efficient in *Microciona*, there would have to be significant radioactivity in the 26:3 acid from the 14:0 or 16:0 precursors.

Still another question of the Morales and Litchfield proposal[15] is the order of double-bond introduction. By analogy to general precedent in fatty acid desaturation,[25] the Δ^9 double bond was assumed to be introduced first, followed by Δ^5 desaturation. However, experiments 7 and 8 in Table II demonstrate that either the Δ^5 or Δ^9 double bonds can be introduced into the C_{26} fatty acid chain.

(25) Cook, H. W. *Biochemistry of Lipids and Membranes*; Vance, D. E., Vance, J. E., Eds.; Benjamin: Menlo Park, CA, 1985; pp 196–197.

Table IV. Radioactivity[26] of 5,9-Hexacosadienoic Acid from Concurrent [1-^{14}C]-9-Hexacosenoic and [5,6-^{3}H]-5-Hexacosenoic Acid Incorporations

incubation period, days	month	radioactivity, dpm	
		^{3}H count	^{14}C count
2	May	117000	81000
4	May	170000	85600
15	May	100000	75000

One way to settle this problem is a double labeling study, whereby any possible idiosyncracies in sponge specimens are eliminated. Therefore, we undertook an experiment (cf. Table IV) in which the same sponge was fed with equimolar and equiradiospecific [1-^{14}C]hexacosenoic (Δ^9-26:1) and [5,6-^{3}H]-hexacosenoic acid (Δ^5-26:1) precursors. Since part of the ^{14}C counts overlap with the ^{3}H counts, appropriate adjustments of both counts were made.[26] In any event, the results clearly demonstrate the existence and operation of Δ^5- and Δ^9-desaturases in the generation of the $\Delta^{5,9}$-dienoic acid, contrary to assumptions[15] based on precedent.[25]

So far, we have not shown whether in marine sponges the desaturation of the long-chain fatty acids occurs at the CoA or phospholipid stage of these long-chain fatty acids, although in virtually all previously studied instances[27] the CoA form of the acid is the appropriate substrate. We hope to shed some light on this question through appropriate experiments with specially synthesized labeled substrates.

Another manner of examining the order of desaturation would be through direct radioactivity counts of any Δ^5-26:1 and Δ^9-26:1 acids that might be produced in experiment 6 (Table II) from the labeled 26:0 acid. The separation of these two 26:1 fatty acids was possible by reverse-phase HPLC, with methanol as a solvent and with addition of inactive carriers. A 20 μCi 26:0 experiment after 2 days (performed in late May) showed ca. 25000 dpm in the Δ^9-26:1 acid and 5000 dpm in the Δ^5-26:1 acid. This clearly indicates some Δ^5-desaturase activity on the saturated 26:0 acid. Quint and Fulco[28] reported that *Bacillus licheniformis* 9259 synthesizes two monounsaturated C_{16} fatty acids (Δ^5-16:1 and Δ^{10}-16:1) along with the $\Delta^{5,10}$-16:2 dienoic acid from added palmitate. Further desaturation of these two monounsaturated 16:1 acids to the $\Delta^{5,10}$-16:2 acid was also demonstrated. The product ratio of Δ^{10}-16:1 to Δ^5-16:1 from palmitic acid was 96:4. However, the further desaturation of the individual Δ^5-16:1 and Δ^{10}-16:1 acids to the $\Delta^{5,10}$-16:2 acid proceeded equally well. Qualitatively, the same situation seems to operate in our sponge system.

Polyunsaturated fatty acid generation in eucaryotes can be summarized as follows:[25] 1. The first double bond to be introduced into a saturated acyl chain is generally in the Δ^9 position. Therefore, substrates for further desaturation contain either a Δ^9 double bond or one derived from the Δ^9 position after chain elongation. 2. Animal systems cannot introduce double bonds beyond the Δ^9 locus. Thus, second and subsequent double bonds are always inserted between an existing double bond and the carboxyl end of the acyl chain.

Our experiment 6 (Table II) with the 26:0 acid, showing parallel Δ^5- and Δ^9-desaturase activities, clearly represents an exception to these rules. The minute amount of Δ^5-26:1 acid present in the sponge was not detected by Morales and Litchfield;[2] we succeeded in demonstrating its production only through the above described radioactive experiment, coupled with the utilization of "cold" carrier. However, large quantities (ca. 30% of the total fatty acid mixture) of very long-chain Δ^5-monounsaturated acids have been found in the sponge *Strongylophora durissima*.[10] This indicates the operation of a unique biosynthetic desaturation mechanism in marine sponges as compared to other eucaryotes. As summarized in Scheme VI, *M. prolifera* does not contain an active Δ^{19}-desaturase (see experiments 6, 7, 8, and 10). In this regard the marine sponge follows the general behavior of eucaryotes. As we shall demonstrate elsewhere, fresh water sponges behave differently.

Experimental Section

Microciona prolifera specimens were collected near Redwood City in San Francisco Bay at depths of 30 cm. The total lipids were extracted by the method of Bligh and Dyer.[29] The phospholipids were separated from neutral lipids and glycolipids by column chromatography on silicic acid (100–200 mesh). The neutral lipids and the glycolipids were eluted by acetone and the phospholipids finally by methanol. The fatty acyl components of the phospholipids were obtained as their methyl esters by reaction with 1.2 N methanolic hydrogen chloride followed by purification via column chromatography on a 1:1 mixture of silica gel and Florisil with hexane/ether (15:1) as eluent. The resulting methyl esters were analyzed by gas chromatography or Hewlett-Packard Model 5790 chromatograph. Both gas chromatographs were equipped with a 30 m × 0.32 mm fused silica column coated with SE-54 and a flame-ionization detector.

^{1}H NMR spectra were run in $CDCl_3$ on either a Varian Associates XL-400 (400 MHz) instrument or a Nicolet NT300WB (300 MHz) instrument. ^{13}C NMR spectra were obtained on a Varian instrument (100 MHz). High-performance liquid chromatography (HPLC) was performed on a Waters Associates HPLC system (M600 pump, R403 differential refractometer). For reverse-phase chromatography, Altex Ultrasphere ODS2 columns (25 cm × mm i.d., two columns in series) were used. The capillary gas chromatography mass spectrometry (GC/MS) analyses were performed with a HP 5890A gas chromatograph with a 5970 mass selective detector. The high-resolution mass spectra were obtained on an AEI MS-30 instrument at the University of Minnesota mass spectrometry facility.

For ozonolysis, the methyl esters (about 2 mg) were dissolved in 2 mL of BF_3/MeOH, and ozone was bubbled through briskly for about 2 min. The mixture was transferred to a capped vial and heated at 100 °C for 1 h and the cooled mixture was extracted with hexane, and the dried crude products were fractionated by HPLC. For epoxidation, the samples were dissolved in 5 mL of methylene chloride and an excess of *m*-chloroperoxybenzoic acid was added. The reaction products were directly separated by thin-layer chromatography (TLC). For the radioactivity measurements, small aliquots (usually $^{1}/_{5}$–$^{1}/_{10}$) of the ^{14}C-labeled material were dissolved in 10 mL of "Ready Organic" (Beckman Instruments) liquid scintillation cocktail. All results are calculated to correspond to the total sample. [1-^{14}C]Palmitic, -stearic, -lauric, and -myristic acids were obtained from ICN Radiochemicals (Irvine, CA). All other radioactive precursors were synthesized as shown below.

Synthesis of Precursors. General Procedure for Wittig Reaction and Reduction of Acid.[30] The phosphonium salt (**1**) of 8-bromooctanoic acid (847 mg, 1.78 mmol) was dissolved in 10 mL of tetrahydrofuran/dimethyl sulfoxide (1:1, v/v) under nitrogen. After cooling to 0 °C, *n*-BuLi (1.6 N, 2 equiv) in hexane was added dropwise, resulting in the development of a deep orange-red color. The solution was stirred at room temperature for 15 min, heptanal (1.5 mmol) was added, and the reaction mixture was then stirred at room temperature for 8 h before being poured into ice–water and acidified with 1 N HCl. After ether extraction, the crude mixture was purified by silica gel column chromatography with hexane/ether (4:1, v/v) as eluent. The acid (165 mg) was then converted to its methyl ester with 10 mL of methanolic hydrogen chloride (1.2 N) and finally reduced to the olefinic alcohol with $LiAlH_4$ (400 mg) and purified by HPLC reverse-phase chromatography (yield 37%).

General Procedure for the Preparation of Bromoalkane.[31] To a mixture of 1-eicosanol (0.896 g, 3 mmol) in 10 mL of dichloromethane containing triethylamine (0.61 g, 6 mmol) was added 0.4 mL of methanesulfonyl chloride (5 mmol) over a period of 10 min. After further stirring for an additional 10 min, the reaction mixture was washed with ice/water followed by 2 N hydrochloric acid solution, water, saturated aqueous sodium bicarbonate solution, and water. Evaporation of the solvent gave the crude mesylate (1.1 g, 80.3% yield), which was used without purification. A solution of the mesylate (1.09 g, 3 mmol) and lithium bromide (0.6 g, 7 mmol) in THF was stirred for 16 h. After removal of the solvent in vacuo, the residue was extracted with *n*-hexane, the solvent evaporated, and the product purified on a silica gel column

(26) The radioactivity of ^{14}C and ^{3}H was adjusted based on the assumption that one third of the ^{3}H counts was due to the penetration of ^{14}C counts.
(27) Fulco, A. J.; Mead, J. F. *The Unsaturated and Polyunsaturated Fatty Acid in Health and Disease*; Thomas: Springfield, IL, 1976; pp 63–79.
(28) Quint, J. F.; Fulco, A. J. *J. Biol. Chem.* **1973**, *248*, 6885–6895.
(29) Bligh, E. G.; Dyer, W. J. *Can. J. Biochem. Physiol.* **1959**, *37*, 911.
(30) Bestmann, H. J.; Vostrowsky, O.; Platz, H. *Chem.-Ztg.* **1974**, *98*, 161–162.
(31) Huang, W.; Pulaski, S. P.; Meinwald, J. *J. Org. Chem.* **1983**, *48*, 2270–2274.

to give 1.0 g of 1-bromoeicosane (96% yield).

General Procedure for Acetylenic Coupling Reaction.[32] A solution of 2-(4-pentanyloxy)tetrahydro-2*H*-pyran (0.672 g, 4 mmol) in 6 mL of dry THF was treated with 2.5 mL of 1.6 M *n*-butyllithium in *n*-hexane at −78 °C under nitrogen and stirred at that temperature for 2 h. A solution of 1-bromoeicosane (0.721 g, 2 mmol) in 2 mL of HMPA was then added, and the mixture was stirred at room temperature for 18 h. Addition of water, extraction with ether, evaporation of the solvent, and purification on 100 g of silica gel, with hexane/ether (25:1) as eluent, gave the pure product **4** (0.7 g, 59% yield).

General Procedure for Lindlar Reduction.[32] A mixture of 2-(4-pentacosanyloxy)tetrahydro-2*H*-pyran (90 mg), Lindlar catalyst (10 mg), and quinoline (0.02 mL) in 5 mL of *n*-hexane was stirred under hydrogen for 40 min at room temperature. The reaction mixture was filtered and the filtrate evaporated under reduced pressure. The crude product was purified by silica gel column chromatography to give 83 mg of the olefinic alcohol **5** (92% yield).

General Procedure for the Preparation of Labeled Nitriles.[18] The appropriate alcohol (4 mmol) was dissolved in distilled methylene chloride (20 mL) and triethylamine (1 mL). To this mixture was added dropwise a solution of methanesulfonyl chloride (0.45 mL) and a catalytic amount of 4-(dimethylamino)pyridine (20 mg) at 0 °C. The reaction mixture was stirred at room temperature for 8 h, washed successively with water and 5% sodium bicarbonate, and extracted with dichloromethane. The organic layer was dried over sodium sulfate and evaporated, and the crude mesylate was purified by column chromatography on silica gel (yield 80%). The mesylate (0.34 mmol) was dissolved in 12 mL of tetrahydrofuran/dimethyl sulfoxide (1:1, v/v), and $K^{14}CN$ (1.3 mg, specific activity 57.6 mCi/mmol), with oridinary KCN (15 mg), was added as carrier. The reaction mixture was heated under reflux for 8 h under nitrogen. After the usual workup with ether, the desired nitrile was obtained and purified by silica gel column chromatography with hexane/ether (4:1, v/v) as eluent (yield 85%).

General Procedure for the Preparation of the Labeled Acids.[18] The radioactive nitrile was hydrolyzed by heating under reflux for 16 h in ethanolic aqueous potassium hydroxide solution (6%), extracted with ether, and purified by silica gel column chromatography with hexane/ether (4:1, v/v). Each acid was then transformed into the sodium salt with sodium carbonate.

Heptadecanal. A solution of heptadecanol (1 g, 8.6 mmol) in 20 mL of dichloromethane was stirred with pyridinium dichromate (4.86 g, 12.9 mmol) for 12 h at room temperature. The resulting mixture was filtered through Florisil and further purified by silica gel chromatography with hexane/ether (9:1, v/v) as eluent.

8-Pentadecenol (**2**) was obtained following the general procedure: ^{1}H NMR (400 MHz) δ 0.880 (3 H, t, J = 6.2 Hz, CH_3), 3.639 (2 H, t, J = 6.72 Hz, CH_2OH), 5.345 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 208 (M^+ − 18, 21.4), 152 (8.7), 138 (9.9), 124 (19.1), 109 (32), 96 (68.3), 82 (39.8), 79 (13.3), 69 (55.0), 67 (83), 55 (100), 43 (45.3), 41 (92.0).

Methyl 8-Pentadecene-1-sulfonate was obtained by following the general procedure: ^{1}H NMR (400 MHz) δ 0.880 (3 H, t, J = 6.14 Hz, CH_3), 3.000 (3 H, s, OSO_2CH_3), 4.219 (2 H, t, J = 6.85 Hz, CH_2OMs), 5.344 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 208 (M^+ − 96 ($HOSO_2CH_3$), 15.3), 152 (3.8), 138 (5.9), 124 (12.9), 110 (23.2), 96 (56.6), 82 (73.1), 79 (38.8), 69 (46.3), 67 (81.8), 55 (100).

1-[^{14}C]Cyano-8-pentadecene was obtained by following the general procedure: ^{1}H NMR (400 MHz) δ 0.883 (3 H, t, J = 7.02 Hz, CH_3), 2.335 (2 H, t, J = 7.02 Hz, CH_2CN), 5.347 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 235 (M^+, 6.5), 220 (2), 206 (12.6), 192 (17.9), 178 (13.0), 164 (10.9), 150 (17.9), 136 (45.8), 122 (62.6), 108 (11.95), 97 (21.3), 94 (14.0), 83 (30.6), 81 (18.3), 69 (70), 55 (100).

[1-^{14}C]-9-Hexadecenoic acid (**3**) was obtained by following the general procedure and was converted to the methyl ester for identification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.882 (3 H, t, J = 6.5 Hz, CH_3), 2.301 (2 H, t, J = 7.3 Hz, CH_2CO_2Me), 3.664 (3 H, s, CO_2CH_3), 5.340 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 268 (M^+, 3), 236 (12.1), 194 (10.2), 152 (11.7), 111 (15.2), 96 (31.6), 87 (35.8), 69 (52.2), 55 (100).

Pentacosadecanol was obtained quantitatively by catalytic hydrogenation of *cis*-9-pentacosenol (**2**) with platinum oxide in ehtanol at room temperature: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.876 (3 H, t, J = 6.7 Hz, CH_3), 3.639 (2 H, t, J = 6.8 Hz, CH_2OH); MS (70 eV), m/z (relative intensity) 350 (M^+ − 18 (H_2O), 33.3), 322 (9.0), 278 (3.8), 264 (5.1), 250 (6.4), 236 (9.0), 222 (10.3), 208 (10.3), 194 (10.3), 181 (15.4), 167 (17.9), 153 (26.9), 139 (33.3), 125 (57.7), 111 (73.1), 97 (98.7), 83 (100), 71 (53.8), 69 (82.1), 55 (97.4), 43 (88.4), 41 (52.6).

(32) Golob, N. F.; Yarger, R. G.; Smith, A. B., III *J. Chem. Ecol.* **1979**, *5*, 543–555.

2-(4-Pentacosynyloxy)tetrahydro-2*H*-pyran (**4**) was prepared by following the general procedure: ^{1}H NMR (400 MHz) δ 0.877 (3 H, t, J = 7.2 Hz, CH_3), 2.260 (4 H, m, $CH_2C{\equiv}CCH_2$), 4.598 (1 H, t, OCHO); MS (70 eV), m/z (relative intensity) 448 (M^+, 2.1), 377 (6.5), 181 (8.3), 167 (80.1), 153 (9.4), 139 (6.9), 97 (40.5), 85 (100), 55 (60.4).

4-Pentacosen-1-ol (**5**) was prepared by following the general procedure: ^{1}H NMR (400 MHz) δ 0.877 (3 H, t, J = 7.2 Hz, CH_3), 3.660 (2 H, t, J = 6.4 Hz, CH_2O), 5.380 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 348 (M^+ − 18 (H_2O), 4.9), 320 (2.1), 306 (1.2), 292 (1.8), 278 (1.7), 264 (1.2), 250 (1.6), 222 (1.1), 208 (1.3), 194 (1.3), 180 (1.2), 166 (2.4), 152 (4.4), 138 (11.4), 124 (19.1), 110 (25.6), 96 (80.1), 82 (99.9), 67 (82.3), 55 (100).

[1-^{14}C]-5-Hexacosenoic acid (**6**) was prepared by following the general procedure and was identified as the methyl ester: ^{1}H NMR (400 MHz) δ 0.877 (3 H, t, J = 7.2 Hz, CH_3), 2.314 (2 H, t, J = 7.6 Hz, CH_2CO_2Me), 3.666 (3 H, s, OCH_3), 5.358 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 408 (M^+, 2.5), 376 (49.9), 292 (7.3), 278 (8.3), 152 (6.4), 138 (5.9), 124 (8.0), 110 (19.4), 96 (55.1), 74 (89.5), 55 (100).

6-Bromo-1-hexanol was prepared by following the general procedure: ^{1}H NMR (400 MHz) δ 3.415 (2 H, t, J = 7.2 Hz, CH_2Br), 3.652 (2 H, t, J = 6.4 Hz, CH_2OH).

12-Nonadecyn-1-ol (**9**) was prepared in 45% yield by following the general procedure: ^{1}H NMR (400 MHz) δ 0.884 (3 H, t, J = 6.8 Hz, CH_3), 2.135 (4 H, m, $CH_2C{\equiv}CCH_2$), 3.640 (2 H, t, J = 6.6 Hz, CH_2OH); MS (70 eV), m/z (relative intensity) 280 (M^+, 0.1), 163 (0.9), 149 (1.9), 135 (5.1), 121 (9.4), 109 (20.9), 107 (8.2), 96 (38.2), 81 (81.0), 67 (100), 55 (92.2).

***cis*-12-Nonadecen-1-ol** (**10**) was prepared in 85% yield by following the general procedure: ^{1}H NMR (400 MHz) δ 0.880 (3 H, t, J = 7.2 Hz, CH_3), 3.638 (2 H, t, J = 6.4 Hz, CH_2OH), 5.346 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 264 (M^+ − 18 (H_2O), 4.2), 236 (1.2), 222 (0.6), 208 (1.0), 194 (0.9), 180 (1.1), 166 (1.8), 152 (2.9), 138 (6.9), 124 (12.2), 110 (17.6), 96 (49.3), 83 (29.7), 69 (48.8), 55 (100).

1-Bromo-*cis*-12-nonadecene (**11**) was prepared in 90% yield by following the general procedure: ^{1}H NMR (400 MHz) δ 0.878 (3 H, t, J = 6.8 Hz, CH_3), 3.407 (2 H, t, J = 6.8 Hz, CH_2Br), 5.348 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 346 (3.1), 344 (3.5), 164 (4.6), 150 (7.6), 148 (8.6), 111 (17.5), 97 (44.0), 83 (52.6), 69 (70.1), 55 (100).

18-*cis*-Pentacosen-1-ol (**12**).[24] A mixture of 1-bromo-*cis*-12-nonadecene (1.4 g, 4.1 mmol) and 1 mL of a 0.1 M solution of Li_2CuCl_4 (prepared from 8.5 mg of LiCl and 13.4 mg of $CuCl_2$ in 1 mL of THF) was cooled to 0 °C. To this mixture was added slowly the Grignard reagent (**7**) obtained from 2-[(6-bromohexanyl)oxy]tetrahydro-2*H*-pyran (1.2 g, 4.5 mmol) and magnesium turnings (0.5 g, 21 mmol) in 15 mL of THF. The reaction mixture was stirred overnight at room temperature. The mixture was washed successively with saturated ammonium chloride solution and water and then dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure and the crude product purified by silica gel chromatography to give 440 mg of a tetrahydropyran-protected product (23% yield), which was subsequently deprotected by methanol in the presence of a catalytic amount of hydrochloric acid solution to give 18-*cis*-pentacosen-1-ol: ^{1}H NMR (400 MHz) δ 0.881 (3 H, t, J = 6.8 Hz, CH_3), 3.640 (2 H, t, J = 6.4 Hz, CH_2OH), 5.347 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 348 (M^+ − 18 (H_2O), 4.0), 152 (4.2), 138 (7.3), 124 (11.3),110 (18.1), 96 (47.0), 82 (64.3), 69 (63.5), 55 (100).

(4*Z*,8*Z*)-4,8-Pentacosadienoic Acid.[24] A solution of (4*Z*)-1,1-dimethoxy-4-heneicosene (**14**) (0.46 g, 1.3 mmol) in 10 mL of acetone–HCl (20:1) and 0.5 mL of water was stirred at room temperature for 1 h to give a quantitative yield of (4*Z*)-4-heneicosen-1-al (**15**), which was used directly in the following Wittig reaction. A solution of (3-carboxypropyl)triphenylphosphonium bromide in dimethyl sulfoxide (15 mL) was added to a solution of dimsylpotassium, prepared from potassium hydride (0.6 g, 15 mmol) and dimethyl sulfoxide (10 mL), to form a deep red solution. A solution of the aldehyde (**15**, 0.4 g, 1.3 mmol) in 8 mL of DMSO/THF (1:1) was added dropwise to the above Wittig mixture at 15 °C. After stirring at 15 °C for 0.5 h, the reaction mixture was poured into ice water, acidified with 30% H_3PO_4, and extracted with petroleum ether. The extract was purified by column chromatography on silica gel with hexane/ether (7:3) as eluent to yield 0.25 g (51%) of the acid (**16**).

(5*Z*,9*Z*)-5,9-Hexacosadienoic acid (**18**) was prepared by following the general procedure and was identified as the methyl ester: ^{1}H NMR (400 MHz) δ 0.876 (3 H, t, J = 6.8 Hz, CH_3), 2.314 (2 H, t, J = 7.6 Hz, CH_2CO_2Me), 3.665 (3 H, s, OCH_3), 5.352 (4 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 406 (M^+, 8.6), 374 (6.9), 357 (4.4), 264 (4.7), 164 (10.1), 150 (24.6), 136 (21.9), 109 (69.2), 95 (34.6), 81 (98.0), 67 (89.0), 55 (100).

5-Hexacosyn-1-ol (19). To an ice-cold solution of 5-hexyn-1-ol (1.98 g, 20 mmol) in dry HMPA (20 mL) was added *n*-butyllithium (1.6 M solution in hexane, 27.4 mL, 44 mmol) dropwise under nitrogen. After stirring of the mixture for 5 min, a solution of 1-bromoeicosane (7.94 g, 22 mmol) in dry HMPA (35 mL) was added dropwise over a period of 1 h. Stirring continued for an additional 1.5 h while warming up to 25 °C. The mixture was then diluted with water (200 mL) and extracted with $CHCl_3$ (3 × 200 mL). The extract was washed with water, dried ($MgSO_4$), and evaporated in vacuo. The residue was chromatographed on silica gel with ether/hexane (1:9) as eluent to yield the product, [19] (6.48 g, 86% yield): mp 65–66 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 3.656 (2 H, t, C-1, J = 6.2 Hz), 2.185 (2 H, tt, C-4, J = 6.6, 2.5 Hz), 2.119 (2 H, tt, C-7, J = 6.9, 2.5 Hz), 1.66 (2 H, m, C-2), 1.55 (2 H, m, C-3), 1.459 (2 H, quintet, C-8, J = 7.3 Hz), 0.869 (3 H, t, C-26, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 80.70 and 79.65 (C-6 and C-5), 62.48 (C-1), 32.03 and 31.95 (C-24 and C-2), 25.48 (C-3), 22.80 (C-25), 18.86 and 18.65 (C-4 and C-7), 14.25 (C-26), 29.79, 29.46, 29.26, 29.01 (overlapping remaining signals); MS (70 eV), m/z (relative intensity) 378 (M^+, 2), 349 (2), 335 (2), 334 (3), 313 (1), 292 (3), 153 (4), 139 (3), 135 (9), 111 (39), 94 (53), 81 (49), 68 (100), 57 (32), 43 (27); exact mass calcd for $C_{26}H_{50}O$ 378.3849, found 378.3837.

Methyl 5-Hexacosynoate (20). An ice-cold suspension of 5-hexacosyn-1-ol (4.86 g, 12.86 mmol) in acetone (250 mL) was titrated by dropwise addition of Jones reagent until the brown color persisted (ca. 15 mL). The mixture was then diluted with water (500 mL) and extracted with ether (3 × 300 mL). The ether extract was dried ($MgSO_4$) and evaporated in vacuo. The resulting acid was directly converted to the methyl ester by refluxing for 3 h with methanol (250 mL) containing a catalytic amount of *p*-toluenesulfonic acid (240 mg). The solvent was then evaporated in vacuo and the residue was chromatographed over silica gel. Elution with ether/hexane, 5:95, afforded pure ester **20** (3.87 g, 74%): mp 45–46 °C; GC, ecl = 26.157; ^1H NMR (400 MHz, $CDCl_3$) δ 3.667 (3 H, s, OCH_3), 2.433 (2 H, t, C-2, J = 7.7 Hz), 2.212 (2 H, tt, C-4, J = 7.1, 2.5 Hz), 2.119 (2 H, tt, C-7, J = 6.9, 2.5 Hz), 1.794 (2 H, quintent, C-3, J = 7.4 Hz), 0.873 (3 H, t, C-26, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.51 (C-1), 81.28 and 78.65 (C-6 and C-5), 51.52 (OCH_3), 32.98 and 32.03 (C-2 and C-24), 24.40 (C-3), 22.80 (C-25), 18.85 and 18.37 (C-7 and C-4), 14.23 (C-26), 29.80, 29.47, 29.26, 29.20, 29.00 (remaining carbons); MS (70 eV), m/z (relative intensity) 406 (M^+ 2), 375 (M^+ - 31 (OCH_3), 2), 330 (1), 181 (1), 150 (8), 140 (100), 121 (8), 108 (13), 98 (18), 83 (10), 81 (35), 80 (69), 79 (22), 67 (25), 57 (25), 55 (34), 43 (72); exact mass calcd for $C_{27}H_{50}O_2$ 406.3798, found 406.3792.

Methyl (5*Z*)-5-Hexacosenoate (21). A mixture of acetylenic ester **20** (100 mg, 0.24 mmol), Lindlar catalyst (30 mg), and quinoline (0.01 mL) in hexane (5 mL) was stirred under hydrogen at room temperature for 2 h. The catalyst was then filtered off and the solvent was removed under vacuum. Flash chromatography of the residue on silica gel using 0–2% ether/hexane as eluent afforded pure ester **21** (92 mg, 91%); GC, Ecl = 25.838; ^1H NMR (400 MHz, $CDCl_3$) δ 5.406 (1 H, dtt, C-5, J = 10.5, 7.1, 1.3 Hz), 5.311 (1 H, dtt, C-6, J = 10.5, 6.9, 1.3 Hz), 3.667 (3 H, s, OCH_3), 2.316 (2 H, t, C-2, J = 7.5 Hz), 2.069 (2 H, br q, C-4, J = 7.2 Hz), 1.998 (2 *H*, m, C-7, J = 7.0 Hz), 1.685 (2 H, quintet, C-3, J = 7.4 Hz), 0.879 (3 H, t, C-26, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.91 (C-1), 131.03 (C-6), 128.13 (C-5), 51.50 (OCH_3), 33.56 (C-2), 32.02 (C-24), 27.33 (C-7), 26.64 (C-4), 25.00 (C-3), 22.81 (C-25), 14.28 (C-26), 29.81, 29.68, 29.47 (overlapping signals, remaining carbons); MS (70 eV), m/z (relative intensity) 408 (M^+, 18), 378 (19), 376 (21), 359 (19), 347 (10), 334 (60), 292 (49), 278 (24), 250 (19), 222 (18), 166 (30), 165 (28), 152 (65), 141 (75), 137 (78), 127 (47), 124 (92), 112 (62), 109 (76), 97 (100), 83 (85), 75 (82), 68 (98), 59 (82), 53 (93).

Sodium (5*Z*)-[5,6-$^3H_2$]-5-Hexacosenoate. A mixture of acetylenic ester **20** (10.0 mg, 24.5 mmol), Lindlar catalyst (10 mg), and quinoline (0.01 mL) in heptane (1 mL) was exposed to a small amount of tritium-enriched hydrogen gas, generated externally by reaction of NaB^3H4 (ca. 30 mCi; specific activity 1.4 Ci/mol) with a saturated aqueous solution of $CoCl_2$ (0.2 mL). After 24 h, the reaction was completed by exosure to tritium-free hydrogen gas for 2 h. The catalyst was then filtered off and the mixture was chromatographed on silica gel with hexane as an eluent. Tritiated ester **21** obtained was directly hydrolyzed by 5% NaOH in aqueous ethanol to yield the product (700 μCi; specific activity 7.4 mCi/mmol).

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Sterols in Marine Invertebrates. 60.[1] Isolation and Structure Elucidation of Four New Steroidal Cyclopropenes from the Sponge *Calyx podatypa*

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Abstract: Four new sterols (**4**, **8**, **9**, and **10**) with cyclopropene-containing side chains were isolated from the Caribbean sponge *Calyx podatypa*. Their structure elucidation was accomplished by ^1H NMR and mass spectrometry. The major sterol, **4**, was shown to be the 23-epimer of (23*R*)-23*H*-isocalysterol (**3**), previously isolated from the Mediterranean sponge *Calyx nicaeensis*. ^{13}C NMR spectra of cyclopropene-containing sterols were determined and signals were fully assigned. Trends in ^{13}C chemical shifts of the cyclopropene sterols are discussed. The biosynthetic implications suggest the operation of an unprecedented in vivo cyclopropene isomerization process.

The cyclopropene moiety is extraordinarily rare among natural products. Prior to the isolation of the sponge sterol calysterol (**1**),[2] the only known example was the fatty acid sterculic acid[3] and its congeners.[4,5] Since then, two more cyclopropenes (**2** and **3**) have been isolated in our laboratory.[6,7] As part of these studies we investigated the absolute configuration of these steroidal cyclopropenes as well as their photochemical and acid-catalyzed behavior.[8]

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