4α-Carboxy-10β-hydroxy-1β,4β-dimethyl-11-methylene-8β,9β-oxido-(2α,6α)-tricyclo[6.3.0.0^{2,6}]undecane (Hirsutic Acid C) (1). The one-pot method of Greene[10f,g] was used to prepare hirsutic acid C (1) from the dienone acid **38**. In this manner, the dienone acid **38** (50 mg, 0.20 mmol) was converted to *dl*-hirsutic acid C (1) (26 mg, 51%), mp 169–171 °C after recrystallization from ether (lit. mp[10a] 168–169 °C): NMR ($CDCl_3$, 200 MHz) δ 1.02 (s, 3 H), 1.18–1.24 (m, 2 H), 1.36 (s, 3 H), 1.46 (m, 1 H), 1.85 (d, 2 H, J = 8.6 Hz), 2.18–2.70 (m, 4 H), 3.45 (d, 1 H, J = 1.8 Hz), 4.58 (m, 1 H), 4.98 (d, 1 H, J = 2.1 Hz), 5.25 (d, 1 H, J = 2.1 Hz); IR (KBr) 3390, 3150–2890 (br), 1695, 1410, 1210, 1160, 1095 cm^{-1}. Anal. Calcd for $C_{15}H_{20}O_4$: M_r, 264.1362. Found: M_r 264.1360. A sample of synthetic hirsutic acid C (1) was converted to the methyl ester for further comparison purposes (vide infra). In addition, the 300-MHz NMR and infrared spectra were identical with those obtained from a sample of *dl*-hirsutic acid C (1) kindly provided by Professor Barry M. Trost.

4α-Carbomethoxy-10β-hydroxy-1β,4β-dimethyl-11-methylene-8β,9β-oxido-(2α,6α)-tricyclo[6.3.0.0^{2,6}]undecane (Methyl Hirsutate) (41). Hirsutic acid C (1) (10 mg, 0.04 mmol) was dissolved in 3 mL of ethyl acetate and the resulting solution was treated with 3–4 mL of diazomethane/ether. After 5 min, the volatiles were evaporated in vacuo and the residue was purified by flash chromatography (5% ethyl acetate in petroleum ether as eluant) to afford methyl hirsutate (41) (8 mg, 80%) as a white crystalline solid: mp 135–136 °C (after crystallization from ether) (lit. mp[10a] 137–138 °C); NMR ($CDCl_3$, 300 MHz) δ 1.05 (s, 3 H), 1.46 (m, 1 H), 1.35 (s, 3 H), 1.44 (m, 1 H), 1.65 (br s, 1 H), 1.86 (d, 2 H, J = 8.5 Hz), 2.20–2.35 (m, 2 H), 2.40–2.63 (m, 2 H), 3.44 (d, 1 H, J = 1.9 Hz), 3.64 (s, 3 H), 4.60 (br s, 1 H), 4.98 (d, 1 H, J = 2.6 Hz), 5.25 (d, 1 H, J = 2.3 Hz). This data is in excellent agreement with that reported in the literature for this compound (See lit. ref 10a for published spectroscopic data).

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Biosynthetic Studies of Marine Lipids. 5.[1] The Biosynthesis of Long-Chain Branched Fatty Acids in Marine Sponges

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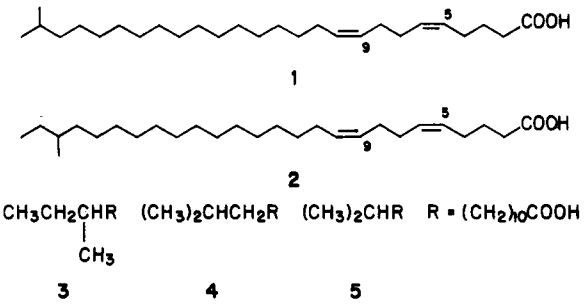
The very long chain branched fatty acids 25-methyl-5,9-hexacosadienoic ($\Delta^{5,9}$-iso-27:2) and 24-methyl-5,9-hexacosadienoic ($\Delta^{5,9}$-anteiso-27:2) acids as well as the straight-chain 5,9-hexacosadienoic ($\Delta^{5,9}$-26:2) acid were shown to originate from the short-chain precursors 13-methyltetradecanoic (iso-15:0), 12-methyltetradecanoic (anteiso-15:0), and palmitic (n-16:0) acids, respectively, by means of ^{14}C incorporation experiments in the marine sponge *Jaspis stellifera*. These results confirm that methyl branching does *not* occur after chain elongation. The unusual $\Delta^{5,9}$ unsaturation probably takes place after chain elongation of short branched or straight-chain fatty acids.

Sponges are the most primitive of the mutlicellular animals with a long, separate evolutionary history. Recent reports[2-5] have shown that they are rich sources of C_{24}–C_{30} fatty acids in contrast to the C_{14}–C_{22} fatty acids typically found in higher animals. Their phospholipids also include numerous branched fatty acids which have been isolated in our laboratory from *Petrosia ficiformis*,[5] *Calix niceaencis*,[6] *Petrosia hebes*,[7] *Aplysina fistularis*,[8] and *Strongylophora durissima*.[9] The first three sponges contain iso and anteiso acids, i.e., (*Z*,*Z*)-25-methyl-5,9-hexacosadienoic (**1**) and (*Z*,*Z*)-24-methyl-5,9-hexacosadienoic (**2**) acids respectively, as their major components, while the other sponges contain fatty acids with branching in the middle of the chain.[5-7]

The origin of terminal methyl branching in short-chain fatty acids has been studied thoroughly.[10,11] The terminal methyl group is generally derived from the three branched amino acids valine, leucine, and isoleucine via transamination to their α-keto acids and oxidative decarboxylation to the branched acyl-CoA primers. Consequently, there are three families of branched fatty acids derived directly from amino acids, i.e., anteiso-odd **3**, iso-odd **4**, and iso-even **5**.



The origin of terminal methyl branching in the very long chain branched fatty acids of marine sponges has so far not been investigated. Terminally branched, short-chain fatty acids, possibly of bacterial origin, may serve as primers for these long-chain acids.[5] However, the terminal methyl group might also arise from unsaturation after chain elongation at the ω3 carbon followed by *S*-

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0022-3263/86/1951-2751$01.50/0

Table I. Major Fatty Acids from the Phospholipids of *Jaspis stellifera*[a]

compd	ECL[b]	fatty acid	abundance,[c] %
1	14.00	tetradecanoic (n-14:0; myristic)	1.9
2	14.62	13-methyltetradecanoic (iso-15:0)	7.3
3	14.70	12-methyltetradecanoic (anteiso-15:0)	6.2
4	15.60	14-methylpentadecanoic (iso-16:0)	2.6
5	15.72	9-hexadecenoic (Δ^9-16:1; palmitoleic)	2.5
6	16.00	hexadecanoic (n-16:0; palmitic)	9.8
7	16.60	15-methylhexadecanoic (iso-17:0)	6.8
8	16.69	14-methylhexadecanoic (anteiso-17:0)	5.2
9	17.74	11-octadecenoic (Δ^{11}-18:1; vaccenic)	4.4
10	18.00	octadecanoic (n-18:0)	4.4
11	25.08	24-methyl-5,9-pentacosadienoic ($\Delta^{5,9}$-iso-26:2)	5.2
12	25.42	5,9-hexacosadienoic ($\Delta^{5,9}$-26:2)	7.1
13	26.15	25-methyl-5,9-hexacosadienoic ($\Delta^{5,9}$-iso-27:2)	3.8
14	26.37	24-methyl-5,9-hexacosadienoic ($\Delta^{5,9}$-anteiso-27:2)	4.7

[a] Capillary gas chromatography on fused silica (30 m × 0.32 mm) containing SE-54 (J&W Scientific, Inc.); program temperature: 130–290 °C, 5.0 °C/min. [b] Equivalent chain length values are those of the methyl esters of these acids. [c] Some unidentified minor fatty acids were also present in the mixture.

adenosylmethionine (SAM) methylation at either the ω2 or the ω3 carbons.

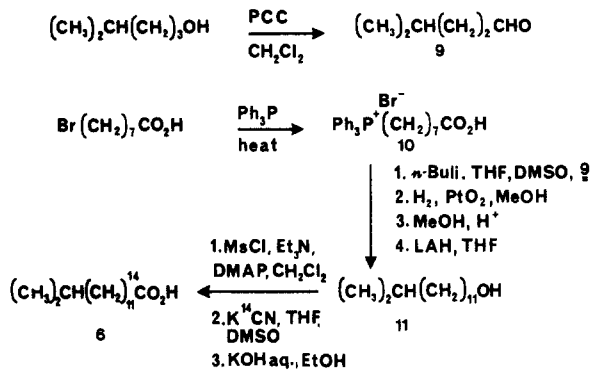
In order to distinguish between these two alternatives, we examined the fate of straight (16:0) and branched (iso-15:0; anteiso-15:0) short-chain acids in the Australian sponge *Jaspis stellifera*, which contains, in its phospholipids, substantial amounts of these acids plus other very long- and short-chain iso and anteiso acids. We now report on a series of ^{14}C incorporation studies which provide, for the first time, direct evidence on certain stages of demospongic (C_{24}–C_{30}) iso- and anteiso-$\Delta^{5,9}$ fatty acid biosynthesis. The results support our previous hypothesis[5] that *branched* short-chain fatty acids serve as precursors to the branched demospongic acids and that terminal methyl branching does *not* occur after chain elongation. The results also confirm the hypothesis of Morales and Litchfield[3] that *straight*-chain demospongic acids are formed by a chain elongation mechanism.

Results and Discussion

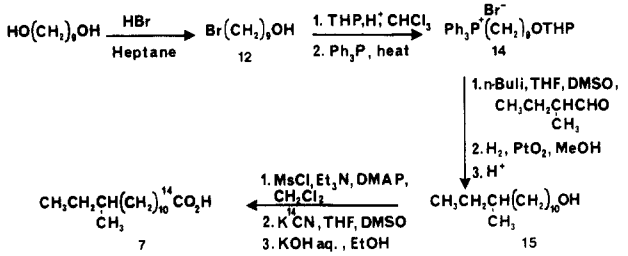
The major identified fatty acids from the total phospholipids of *Jaspis stellifera* are listed in Table I. Comparison of the capillary GC retention times and equivalent chain length values (ECL) of these fatty acid methyl esters with those of known compounds and the interpretation of their mass spectra, and those of the *N*-acylpyrrolidinide derivatives, provided the necessary information for identification. Pyrrolidinides of the hydrogenated fatty acid methyl esters were also prepared and investigated by capillary GC/MS, especially to locate branching.

The major fatty acids in the phospholipids of *J. stellifera* (Table I) are very similar to those reported by us earlier from *Petrosia ficiformis*,[5] *Petrosia hebes*,[7] and *Calyx niceaencis*.[6] Approximately 65% by weight of the total major fatty acids found in the phospholipids of *J. stellifera* are branched. The branched demospongic acids are the 24-methyl-5,9-pentacosadienoic ($\Delta^{5,9}$-iso-26:2), 25-methyl-5,9-hexacosadienoic ($\Delta^{5,9}$-iso-27:2) (**1**), and 24-methyl-5,9-hexacosadienoic ($\Delta^{5,9}$-anteiso-27:2) (**2**) acids. The major short chain fatty acids are the 13-methyltetradecanoic (iso-15:0), 12-methyltetradecanoic (anteiso-15:0), hexadecanoic (n-16:0; palmitic), 14-methylpentadecanoic (iso-16:0), 15-methylhexadecanoic (iso-17:0), and 14-methylhexadecanoic (anteiso-17:0) acids.

Scheme I



Scheme II



The very long chain iso and anteiso acids of *J. stellifera* are almost certainly sponge constituents, while the shorter acids may be contributed by the extensive symbiotic populations of bacteria and Cyanophyceae contained within this tropical sponge.[12,13] Bacterial phospholipids are usually characterized by the occurrence of normal chain fatty acids with methyl branching at the ω2 or ω3 carbons; they do not contain notable amounts of very long chain phospholipid acids. On the other hand, all sponges so far studied in this and other laboratories have displayed large amounts of either straight-chain, branched, or substituted fatty acids with 24 to 30 C atoms. Further, we have shown[8] in the past that the very long fatty acids of *Aplysina fistularis* are bona fide sponge metabolites.

For the biosynthetic experiments, [1-^{14}C]-13-methyltetradecanoic (**6**) and [1-^{14}C]-12-methyltetradecanoic (**7**) acids were synthesized as depicted in Schemes I and II. The key step for the iso-15:0 acid (**6**) involved a Wittig reaction of 4-methylpentanal (**9**) with (7-carboxyheptyl)-triphenylphosphonium bromide (**10**), affording (*Z*)-12-methyl-8-tridecenoic acid, which was immediately hydrogenated to 12-methyltridecanoic acid and then reduced to the desired 12-methyl-1-tridecanol (**11**). Its mesylate was treated with 1 mCi (57.6 mCi/mmol) of K^{14}CN in dimethyl sulfoxide to afford the corresponding nitrile, which was then converted to the desired [1-^{14}C]-13-methyltetradecanoic acid (**6**). The anteiso-15:0 acid (**7**) was synthesized from 9-bromo-1-nonanol (**12**), which was coupled via its tetrahydropyranyl derivative **14** in THF/Me_2SO with 2-methylbutyraldehyde to afford, in excellent yield, (*Z*)-11-methyl-9-tridecen-1-ol (after acidic deprotection). The remaining steps to the anteiso-15:0 acid (**7**) were patterned after the synthesis of the lower homologue **6**.

The sodium salts of these ^{14}C-labeled precursors and [1-^{14}C]palmitic acid were incorporated into live, whole

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Table II. Distribution of Radioactivity (dpm) in *Jaspis stellifera*

^{14}C precursor[a]	neutral lipids	glycolipids	phospholipids
iso-15:0 (6)	<500	19089	69493
anteiso-15:0 (7)	<500	65365	404545
n-16:0	<500	57424	490207

[a] Each precursor (20 μCi) was incorporated in a separate experiment. See Experimental Section for the details of incorporation.

Table III. Distribution of Radioactivity (dpm) in the Phospholipid Fatty Acid Fraction

^{14}C precursor[a]	recovered precursor	iso-27:2 (1) + anteiso-27:2 (2)	iso-26:2 (16)	n-26:2 (17)
iso-15:0 (6)	59381	8933	350	283
anteiso-15:0 (7)	359477	37357	1156	585
n-16:0	410141	1093	1127	70658

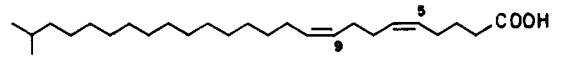
[a] Each precursor (20 μCi) was incorporated in a separate experiment. See Experimental Section for the details of incorporation.

sponges by aquarium incubation in a precursor–seawater solution for 11.5 h, followed by replacement into the natural, marine environment for 30 days.[14] The isolation and analysis of the different fractions were carried out as detailed in the Experimental Section. The radioactivity in the different fatty acid methyl esters was assessed after isolating the individual components. The results for specimens with the highest efficiency of recovery are shown in Table II, although similar results were obtained for all incorporation experiments.

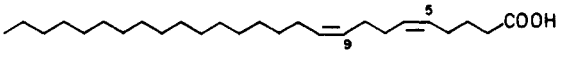
Most of the radioactivity accumulated in the phospholipids of the sponge (Table II). A small, unidentified fraction of the activity was also found in the acetone-soluble glycolipid fractions, while the neutral lipids were essentially cold. The counting had to be performed on the total fatty acid methyl esters derived from the phospholipid and glycolipid fractions, respectively, since a deep yellow color, probably from traces of malabaricane triterpenes,[15] slightly contaminated the fractions and covered the counts. The total fatty acid methyl esters obtained from the phospholipid fractions were subjected to HPLC separation. The three $\Delta^{5,9}$ long chain acid fractions shown in Table III (especially $\Delta^{5,9}$-n-26:2) were found to contain small amounts of monounsaturated and traces of saturated long-chain acids, based on capillary GLC analysis. For further purification, these fractions were subjected to epoxidation with *m*-chloroperoxybenzoic acid, followed by silica gel column chromatography to separate the bisepoxides from saturated compounds and monoepoxides. The pure bisepoxides were used for radioactivity measurements and for degradation studies with periodic acid.

The distribution of radioactivity in the fatty acid methyl esters derived from the *J. stellifera* phospholipids (Table III) showed that saturated short-chain acids served as precursors to the long-chain branched and unbranched dienes and that ω-oxidation to acetate followed by resynthesis is insignificant. Although most of the radioactivity was found in the short-chain fatty acids (mainly the precursors), sufficient incorporation was achieved into the long-chain acids to draw the following conclusions from the data summarized in Table III.

(1) Incorporation of the labeled n-16:0 acid (palmitic acid) led to radioactivity in the $\Delta^{5,9}$-n-26:2 acid **17** but not in the $\Delta^{5,9}$-iso-26:2 analogue **16**.



16



17

(2) When the iso-15:0 fatty acid **6** was incorporated into *J. stellifera*, the $\Delta^{5,9}$-n-26:2 acid **17** was essentially nonradioactive. The radioactive long chain acid fraction consisted of the mixture of $\Delta^{5,9}$-iso- and $\Delta^{5,9}$-anteiso-27:2 acids **1** and **2**. Unfortunately, these two long-chain acids did not give a satisfactory separation, but according to the previous general experimental and theoretical evidence, an iso short-chain acid would generate only an iso long-chain acid and not an anteiso counterpart.

(3) When the anteiso-15:0 fatty acid **7** was incorporated in a separate experiment into *J. stellifera*, the $\Delta^{5,9}$-n-26:2 acid **17** was again essentially cold with the radioactivity residing in the branched $\Delta^{5,9}$-27:2 acids **1** and **2**. In all of the above mentioned three experiments, incorporation of the precursors did not lead to any significant radioactivity in the $\Delta^{5,9}$-iso-26:2 acid **16**.

According to these observations, oxidation of the precursors to acetate and rebiosynthesis of the acids do not seem to take place to a significant extent. As a further experiment, the pure bisepoxides obtained from the radioactive $\Delta^{5,9}$ long-chain normal (**17**), iso (**1**), and anteiso (**2**) acids of the three incorporations were subjected to periodic acid cleavage. As expected, the radioactivity was largely concentrated in the monoaldehydes derived from the cleavage of the epoxide group at C_9: 84.6% of the radioactivity was found in the monofunctional degradation product obtained from the incorporation experiment with iso-15:0. Only 6.6% of the radioactivity was encountered in the polyfunctional products from the cleavage of the epoxides of C_5 and C_9. Some radioactivity (8.8%) was lost during the purification process. This is attributed to the instability and polymerization tendency of the aldehydes produced from the oxidative periodic acid degradation. In the other long-chain monoaldehydes obtained from the incorporation experiments with anteiso-15:0 and n-16:0, the radioactivity was higher (91.6% and 92.3%, respectively). The lack of significant amounts of radioactivity in the bifunctional degradation products close to the carboxylic groups of the long-chain acids clearly establishes the validity of our conclusion.

Analysis of the fatty acid methyl esters from the glycolipid fraction revealed that the radioactivity was associated only with the short-chain fatty acids and that no homologation had occurred of the type observed in the phospholipid fractions. The major constituents of this fraction were palmitic (16:0), palmitoleic (Δ^9-16:1), 13-methyltetradecanoic (iso-15:0), and 12-methyltetradecanoic (anteiso-15:0) acids.

Knowledge of the biosynthesis of long-chain fatty acids in marine sponges is sparse and limited to unbranched acids. One of the first papers regarding sponge metabolism is that of De Rosa et al.,[16] where the biosynthesis of fatty acids in the sponge *Verongia aerophoba* was investigated through administration of [1-^{14}C]acetate, [2-^{14}C]-mevalonate and [CH_3-^{14}C]-L-methionine. Some incorporation of radioactivity was observed, notably with acetate, but since the fatty acids were not separated or identified, information about fatty acid composition and location of

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radioactivity is not available.

Morales and Litchfield[3] continued this work by incorporating [1-^{14}C]acetate into the fatty acids of *Microciona prolifera* using sponge fragments, dissociated cells, and cell-free systems; they proposed that the unusual very long straight-chain fatty acids were formed by a chain elongation mechanism from precursors such as palmitoleic (Δ^9-16:1) and palmitic (16:0) acids. The proposed pathway, acetate → 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 unit used in all fatty acid chain elongations, and no other labeled precursors were examined. Moreover, recent work in our laboratory with the sponge *Tethya aurantia*[17] indicates that the order of double bond introduction postulated by Morales and Litchfield[3] either is not operative or is not the only one in this organism.

Our present work with *Jaspis stellifera*, using the labeled iso-15:0 (6) and anteiso-15:0 (7) acids instead of labeled sodium acetate as precursors, indicates (see Table III) that the biosynthetic pathways iso-15:0 → $\Delta^{5,9}$-iso-27:2 and anteiso-15:0 → $\Delta^{5,9}$-anteiso-27:2 are operative. The incorporation of palmitic acid (Table III) confirms Litchfield's results[3] in that the biosynthetic route n-16:0 → $\Delta^{5,9}$-26:2 is operative in *J. stellifera*.

At least some of the shorter acids, notably the branched ones, are probably of bacterial origin, obtained either through the sponges' diet, that consists largely of bacteria[18] or via symbiotic populations of bacteria and Cyanophyceae.[5,8] Current work in our laboratory seeks to test these two hypothesis. While symbionts likely contribute some fatty acid precursors, especially the more unusual branched acids, they cannot be the sole source of precursors as hexactinellid sponges with very sparse bacterial populations[19] also contain demospongic acids.[20]

The sponge is then likely responsible for chain elongation and double bond introduction with elongation preceding unsaturation at C-5 and C-9. All demospongic acids containing two double bonds close to the carboxyl group found in this[5-9] and other[3,4,21,22] laboratories have these bonds at C-5 and C-9, regardless of the chain length (e.g., $\Delta^{5,9}$-26:2, $\Delta^{5,9,19}$-26:3, $\Delta^{5,9}$-27:2, $\Delta^{5,9}$-28:2, $\Delta^{5,9,21}$-28:3, $\Delta^{5,9,23}$-29:3, and $\Delta^{5,9,23}$-30:3). No unsaturation pattern such as $\Delta^{7,11}$ and/or $\Delta^{9,13}$ has so far been encountered in significant amounts, suggesting that in sponges chain elongation to demospongic acids takes place first, followed by unsaturation at C-5 and C-9. This unusual unsaturation pattern may play an important functional role in sponge phospholipids and membranes. The sequence of double bond introduction is currently under investigation in our laboratory.

Experimental Section

General Procedures. *Jaspis stellifera* (Carter, 1879) was collected on John Brewer Reef, in the central section of the Australian Great Barrier Reef. The total lipids were extracted by the method of Bligh and Dyer.[23] The neutral lipids, glycolipids, and phospholipids were separated by chromatography on ammonium hydroxide treated silicic acid (100–200 mesh) by using the procedure of Privett et al.[24] For the elution of neutral lipids, chloroform/tetrachloromethane (2:1 v/v) was utilized. This was followed by elution of the column with acetone, which is known[24] to give glycolipids. Finally, phospholipids were obtained by methanol elution. The neutral lipid fraction contained primarily carotenoids, sterol esters, triglycerides, and free sterols. The "glycolipid" fraction consisted of three major spots on thin-layer chromatography using silica gel and methylene chloride/acetone (9:1) as eluent. Structure elucidation studies of these components are under way in our laboratory. The fatty acyl components of the neutral lipids, "glycolipids", and phospholipids were obtained as their methyl esters by reaction with methanolic hydrogen chloride followed by purification via silica gel column chromatography and elution with hexane/ether (9:1). The first two fractions were first subjected to saponification with 10% methanolic aqueous potassium hydroxide solution. The resulting methyl esters were analyzed by gas chromatography (GLC) using a Carlo Erba series 4160 Fractovap chromatograph equipped with a 15 m × 0.32 mm fused silica column coated with SE-54, a cooled column injection system, and a flame ionization detector. For mass spectral characterization of the individual fatty acids, *N*-acylpyrrolidinide derivatives were prepared by direct treatment of the methyl esters with pyrrolidine/acetic acid (10:1, v/v) in a capped vial (1 h, 100 °C) followed by ether extraction from the acidified solution and purification by thin-layer chromatography (TLC). The capillary gas chromatography–mass spectrometry (GC/MS) analyses were performed with a Ribermag R10-10 quadrupole mass spectrometer connected to a Carlo Erba series 4160 Fractovap chromatograph.

^{1}H NMR spectra were run in $CDCl_3$ either on a Varian Associates XL-100 NMR instrument or on a Nicolet NT 300 WB (300 MHz) spectrometer. Infrared spectra were recorded on a Nicolet Model 7199 Fourier transform spectrometer. Melting points (mp) were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected.

High-performance liquid chromatography (HPLC) was performed on a Waters Associates HPLC system (M6000 pump, R403 differential refractometer). For reverse-phase chromatography, Altex Ultrasphere ODS2 columns (25 cm × 10 mm i.d., two columns in series) were used with methanol as the mobile phase.

For the epoxidation of the long-chain fatty acid methyl esters, the samples were dissolved in 5 mL of methylene chloride, and excess *m*-chloroperoxybenzoic acid was added portionwise. The reaction products were directly separated by using small silica gel columns. Minor amounts of saturated fatty acid methyl esters and monoepoxides were eluted with hexane/ether (9:1 and 8:2 v/v). The pure bisepoxides were obtained with hexane/ether (1:1), and small aliquots were counted for radioactivity. The labeled bisepoxides were subjected to periodic acid cleavage for further radioactivity measurements. The samples were dissolved in 2 mL, of acetone, and a small excess of periodic acid in 1 mL of water was added. The mixture was refluxed for 30 min. The reaction products were subjected to HPLC separation using methanol as the mobile phase. The bifunctional group containing cleavage products came almost with the solvent peak, while the C_{17} (from $\Delta^{5,9}$-26:2) and C_{18} (from $\Delta^{5,9}$-iso- and $\Delta^{5,9}$-anteiso-27:2) monoaldehydes gave much longer retention times.

Small aliquots (usually 1/50 or 1/10) of the ^{14}C-labeled samples (1–2 mg) were dissolved in 10 mL of organic counting scintillant (OCS), and the radioactivity was measured with a Beckmann LS 7500 liquid scintillation system. All the results were corrected for the background radiation, calculated to the total amount, and were presented as dpm by using a standard solution.

[1-^{14}C]Palmitic acid was obtained from Amersham Corporation (Arlington Heights, IL).

Incorporation of Precursors. Portions of one individual sponge were attached underwater to PVC plastic plaques with nylon cable ties and were left in situ for 1 week to allow reattachment to the plaque and repair of damaged tissues. The ^{14}C-labeled precursors were then incorporated (October 1, 1985) into the sponge transplants with methods modified after Catalan et al.[14] The precursors were transferred to a glass beaker containing 3 L of unfiltered seawater in 2 mL of EtOH/H_2O (70:30).

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The contents of the beakers were continuously aerated by using an aquarium pump with a glass outlet and were maintained under dim natural light at ambient ocean surface temperature (approximately 26 °C). After 30 min of aeration, a single sponge transplant was placed in each beaker for 11.5 h. Next, the sponges were transported to 18 m on the back-reef portion of John Brewer Reef for 30 days. Finally, the sponges were frozen, lyophilized, sealed under argon gas, and shipped to Stanford University for analysis.

Synthesis of Precursors. General Procedure for Wittig Reactions. The phosphonium salt in 25 mL of tetrahydrofuran/dimethyl sulfoxide (1:1 v/v) was placed in a 100-mL, three-necked, round-bottomed flask provided with magnetic spin bar, reflux condenser, pressure-equalizing dropping funnel, and nitrogen inlet. While cooling to ca. 0–5 °C, 2.0 N *n*-BuLi in hexane was added dropwise, resulting in development of a deep orange-red color (ylide). The solution was stirred at room temperature for 30 min before the aldehyde was added, and the reaction mixture was then stirred at room temperature for 3 h before being poured onto ice and acidified with 1 N HCl. The aqueous layer was extracted with ether (2 × 50 mL), and the etheral layer was washed with water (2 × 30 mL) and dried over magnesium sulfate followed by filtration and removal of the solvent. The resulting crude mixture was purified by means of silica gel column chromatography using ether/hexane (1:1 v/v) as eluent. The olefin was hydrogenated in methanol at room temperature with platinum oxide as catalyst.

General Procedure for the Preparation of the Nitriles. The alcohol in 15 mL of distilled methylene chloride was placed in a 25-mL, round-bottomed flask provided with magnetic spin bar. Triethylamine followed by methanesulfonyl chloride and catalytic amounts of 4-(dimethylamino)pyridine (DMAP) were added dropwise with constant stirring. The resulting solution was stirred at room temperature for 14 h. The organic layer was then washed with water (2 × 15 mL), 5% HCl (1 × 15 mL), and finally water (1 × 15 mL). After the organic layer was dried over sodium sulfate and filtered, followed by removal of the solvent, a white solid resulted, which was finally purified by means of HPLC using two Altex Ultrasphere ODS columns in series and methanol as eluent at 3.5 mL/min.

The methanesulfonate in 15 mL of tetrahydrofuran/dimethyl sulfoxide (1:1 v/v) was added to a 25-mL, round-bottomed flask provided with spin bar and reflux condenser. To this stirred solution, $K^{14}CN$ together with cold KCN as carrier was added dropwise in 5 mL of tetrahydrofuran/dimethyl sulfoxide (1:1 v/v). The reaction mixture was refluxed for 16 h at 85–90 °C. The nitrile was extracted with ether (3 × 20 mL), washed with water (3 × 20 mL), and dried over sodium sulfate. The desired nitrile was obtained after evaporation of the solvent and purification of the reaction mixture on silica gel column chromatography with ether/hexane (2:8 v/v) as eluent.

General Procedure for the Preparation of the Acids. The radioactive nitrile was hydrolyzed by heating under reflux for 80 h in 5% ethanolic aqueous potassium hydroxide solution, and the acid was isolated in the standard way.

4-Methylpentanal (9) was prepared by reaction of 4-methyl-1-pentanol with pyridinium chlorochromate (PCC) in dichloromethane.

1-[^{14}C]Cyano-12-methyltridecane. 12-Methyltridecanoic acid was obtained in 98% yield after hydrogenation of 12-methyl-8-tridecenoic acid,[25] which, in turn, was synthesized from 7-(carboxyheptyl)triphenylphosphonium bromide and 4-methylpentanal by following the general procedure. The saturated methyl ester[26] was reduced with lithium aluminum hydride in tetrahydrofuran and transformed in 44% yield to **12-methyl-1-tridecanol methanesulfonate**, mp 52–53 °C, by the reaction of 0.04 g (0.19 mmol) of 12-methyl-1-tridecanol, 0.09 g (0.89 mmol) of triethylamine, and 0.10 g (0.87 mmol) of methanesulfonyl chloride: IR (CCl_4) 2955, 2928, 2853, 1370, 1349, 1177, 1150, 1139 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 0.86 (d, J = 6.30 Hz, 6 H, CH_3), 1.26 (s, 20 H, CH_2), 2.99 (s, 3 H, OSO_2CH_3), 4.22 (t, J = 6.5 Hz, 2 H, CH_2OMs); MS (70 eV) m/e (relative intensity) 196 (16), 168 (19), 153 (11), 140 (27), 125 (17), 112 (22), 111 (44), 98 (20), 97 (64), 96 (13), 85 (16), 84 (32), 82 (30), 79 (25), 71 (25), 70 (49), 69 (83), 68 (19), 67 (14), 57 (86), 56 (100), 55 (86), 54 (10), 43 (72), 42 (19), 41 (76). The reaction of 7.3 mg (0.025 mmol) of 12-methyl-1-tridecanol methanesulfonate with 1.2 mg (0.02 mmol) of 1.0 mCi of $K^{14}CN$ (specific activity 57.6 mCi/mmol) and 1.0 mg (0.01 mmol) of carrier KCN provided in 90% yield 6 mg (0.03 mmol) of the desired cyanide.

[1-^{14}C]-13-Methyltetradecanoic acid (6) was obtained in almost quantitative yield, 3.5 mg (0.01 mmol), in the reaction of 6 mg (0.03 mmol) of 1-[^{14}C]cyano-12-methyltridecane with 50 mg (0.89 mmol) of potassium hydroxide in aqueous ethanol.

9-Bromo-1-nonanol (12) was obtained in 60% yield in the reaction of 1,9-nonanediol with HBr (47–49%) using a continuous extraction method.[27]

2-[(9-Bromononyl)oxy]tetrahydro-2*H*-pyran was obtained almost quantitatively in the reaction of 9-bromo-1-nonanol with dihydropyran and catalytic amounts of *p*-toluenesulfonic acid.[28]

[9-((Tetrahydro-2*H*-pyran-2-yl)oxy)nonyl]triphenylphosphonium bromide (14) was prepared in 45% yield, 2.4 g (4 mmol), in the reaction of 3.0 g (11 mmol) of triphenylphosphine and 2.9 g (9 mmol) of 2-[(9-bromononyl)oxy]tetrahydro-2*H*-pyran by following the general method. The salt could not be crystallized but was homogeneous on TLC (silica gel in chloroform/ethanol (4:1 v/v)) and was used directly in the next step.

11-Methyl-9-tridecen-1-ol was obtained in high yield, 26 mg (0.1 mmol), bp 95 °C (0.05 torr) [lit.[29] bp 95 °C (0.05 torr)], in the reaction of 2.1 g (3.7 mmol) of [9-((tetrahydro-2*H*-pyran-2-yl)oxy)nonyl]triphenylphosphonium bromide with 0.35 g (4.1 mmol) of 2-methylbutyraldehyde.

11-Methyl-1-tridecanol (15) was obtained in quantitative yield in the catalytic hydrogenation of 11-methyl-9-tridecen-1-ol by following the general procedure.[30]

11-Methyl-1-tridecanol methanesulfonate was prepared in 50% yield, 0.015 (0.05 mmol), in the reaction of 0.02 g (0.10 mmol) of 11-methyl-1-tridecanol, 0.10 g (0.99 mmol) of triethylamine, and 0.10 g (0.87 mmol) of methanesulfonyl chloride: IR (CCl_4) 2962, 2927, 2857, 1464, 1365, 1344, 1260, 1176, 1134, 1099, 945 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.84 (d, J = 7.1 Hz, $CHCH_3$), 0.85 (t, J = 7.1 Hz, 3 H, CH_3), 1.27 (s, 18 H, CH_2), 1.77 (q, J = 6.6 Hz, 2 H, CH_2CH_2OMs), 3.00 (s, 3 H, OSO_2CH_3), 4.22 (t, J = 6.6 Hz, 2 H, CH_2OMs); MS (70 eV) m/e (relative intensity) 196 (9), 168 (14), 167 (38), 166 (11), 140 (11), 139 (32), 125 (21), 112 (21), 111 (71), 109 (34), 97 (86), 85 (22), 84 (25), 83 (75), 82 (22), 79 (63), 71 (46), 70 (90), 69 (84), 57 (86), 55 (100), 41 (38).

1-[^{14}C]Cyano-11-methyltridecane was obtained in high yield, 10 mg (0.04 mmol), in the reaction of 13 mg (0.04 mmol) of 11-methyl-1-tridecanol methanesulfonate with 1.2 mg (0.02 mmol) of 1.0 mCi of $K^{14}CN$ (specific activity 57.6 mCi/mmol) and 2 mg (0.03 mmol) of carrier KCN.

[1-^{14}C]-12-Methyltetradecanoic acid (7) was obtained in good yield, 10 mg (0.04 mmol), in the reaction of 10 mg (0.04 mmol) of 1-[^{14}C]cyano-11-methyltridecane with 100 mg (1.8 mmol) of potassium hydroxide in aqueous ethanol.

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Registry No. 1, 83474-12-8; 2, 83474-13-9; 4, 2485-71-4; 6, 102537-89-3; 6 (nitrile), 102537-88-2; 7, 102537-91-7; 7 (nitrile), 102573-68-2; 9, 1119-16-0; 9 (alcohol), 626-89-1; 10, 52956-93-1; 11, 21987-21-3; 11 (acid), 2724-57-4; 11 (methyl ester), 5129-58-8; 11 (methanesulfonate), 102537-87-1; 14, 55695-91-5; 15, 20194-46-1; (*Z*)-15 (9-alkene), 58257-54-8; 15 (methanesulfonate), 102537-90-6; 16, 83474-17-3; 17, 52715-55-6; $H_3CCH_2CH(CH_3CHO$, 96-17-3; $Br(CH_2)_9OTHP$, 55695-90-4; (Z)-$(CH_3)_2CH(CH_2)_2CH{=}CH(CH_2)_6CO_2H$, 102537-86-0; tetradecanoic acid, 544-63-8; 12-methyltetradecanoic acid, 5502-94-3; 14-methylpentadecanoic acid, 4669-02-7; 9-hexadecanoic acid, 2091-29-4; hexadecanoic acid, 57-10-3; 15-methylhexadecanoic acid, 1603-03-8; 14-methylhexadecanoic acid, 5918-29-6; 11-octadecenoic acid, 143-25-9; octadecanoic acid, 57-11-4.

Hydrolysis of Mustard Derivatives in Aqueous Acetone–Water and Ethanol–Water Mixtures

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The hydrolyses of two mustard derivatives, 2-chloroethyl ethyl sulfide (CEES) and 2-chloroethyl methyl sulfide (CEMS), were investigated and compared with the hydrolysis of *tert*-butyl chloride (TBC) in aqueous binary mixtures of acetone and ethanol from 0 to 45 °C. The solvent effect on rates and on activation parameters provided further evidence for an S_N1 mechanism with anchimeric assistance of the sulfur atom to form a cyclic sulfonium ion as the reaction intermediate. Lower $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values of both CEES and CEMS relative to that of TBC reflected the S–C bond formation and the strained structure of the intermediate. A finite and negative $\Delta C_p^{\ddagger}$ was detected. The observed $\Delta H^{\ddagger}$ was corrected for the cosolvent effect by adopting Fagley's model and was consistent with the value in pure water.

Mustard, bis(2-chloroethyl) sulfide, hydrolyzes to form mustard chlorohydrin (2-hydroxylethyl ethyl sulfide) and HCl; the chlorohydrin subsequently hydrolyzes to form another mole of HCl and thiodiglycol. Bartlett and Swain[1] showed that the kinetics of the hydrolysis consisted of two consecutive, first-order reactions at 25 °C and concluded that the hydrolysis proceeded via a sulfonium ion intermediate. Their proposed mechanism was only recently verified. McManus and co-workers[2] monitored the hydrolysis of deuterium-labeled chlorohydrin and observed 100% scrambling of the products by NMR. They also agreed with Bartlett and Swain that nucleophilic solvent attack of the substrate was absent. However, no measurements of the activation energies of mustard hydrolysis were available.[3] In a study on anchimeric-assisted hydrolyses of alkyl halides, Blandamer et al.[4] determined a constant $\Delta C_p^{\ddagger}$ of −71 cal/mol, K between 5 and 50 °C for 2-chloroethyl methyl sulfide (CEMS)—a value consistent with those reported for alkyl halide hydrolyses undergoing an S_N1 mechanism.[5] All of the above studies found that it was necessary to measure their rates in the presence of a small amount of acetone, but any solvation effect on kinetics was not examined.

The aim of this study is to investigate the effect of an organic cosolvent on the rates and activation parameters of two mustard derivatives CEES and CEMS. These compounds are more common simulants of mustard than chlorohydrin, and each hydrolyzes in one step to form 1 mol of HCl and 1 mol of 2-hydroxylethyl sulfide. We first verified that the reaction rate was independent of the presence of added HCl up to 2×10^{-3} M and restricted our substrate concentration below 1×10^{-3} M. We also employed a sensitive method[6] to detect whether $\Delta C_p^{\ddagger}$ was constant in a temperature range of 0–45 °C. The solvent systems are in the aqueous regions of both acetone–water and ethanol–water mixtures of mole fractions 0.025–0.20 in the organic cosolvent. These solvent systems have been the subjects of extensive thermodynamic and structural studies. Both acetone and ethanol are "typically aqueous" solutes[7] that promote the structure of water when present in small amounts and were known to affect the kinetics of hydrolysis by revealing large ranges of variations in both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ with composition, as observed for TBC hydrolysis.[8] We performed a comparative study of the hydrolyses of CEES and TBC, which has long been used for developing theories of solvent effects on hydrolysis reaction rates and activation parameters, in the same solvent mixtures. Furthermore, we are able to report kinetic data of CEES hydrolysis in pure water and compare these data with cosolvent-corrected enthalpies of activation (ΔH^{*})

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