(s, 22 H, alkyls), 0.88 (t, 3 H, J = 6.7 Hz, CH_3); IR (KBr) 2920, 2850, 2240, 1798, 1469, 1459, 1170, 765, 750 cm^{-1}. Anal. Calcd for $C_{33}H_{41}NO_2$: C, 81.94; H, 8.54; N, 2.90. Found: C, 81.85; H, 8.46; N, 2.95.

(±)-9,10-Dihydro-11-(*cis*-1-cyano-2-phenylvinyl)-9,10-ethanoanthracenecarboxylic Acid (19). To 0.14 g (0.37 mmol) of **14a** in 10 mL of anhydrous nitrogen purged THF was added a mixture of 0.39 mmol of LiOMe (formed by addition of 0.28 mL of 1.40 M *n*-BuLi to 0.39 mmol of anhydrous MeOH) in 10 mL of dry THF at −78 °C. The reaction was slowly warmed to −10 °C over 3 h whereupon a 10-mL aliquot was removed and worked up as described for **14a**. NMR examination of the crude product from this aliquot revealed the presence of **19**, a small amount of **14a** being detected as well. Pure **19** was obtained by allowing the remainder of the reaction mixture to warm slowly to 24 °C and to stand for 24 h. After isolation and workup, **19** was recrystallized from hexane to afford a white powder: mp 190–193 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 6.97–7.69 (m, 14 H, aromatics, H13), 5.11 (s, 1 H, H9), 4.38 (t, 1 H, J = 3.3 Hz, H10), 2.97 (dd, 1 H, J = 13.0, 3.3 Hz, H11), 2.20 (dd, 1 H, J = 13.0, 3.3 Hz, H12), IR (KBr) 3030, 2960, 2210, 1700, 1465, 1455, 1440, 1255, 1235, 740 cm^{-1}; mass calcd for $C_{26}H_{19}NO_2$ 377.1416, observed 377.1425. Anal. Calcd for $C_{26}H_{19}NO_2$: C, 82.74; H, 5.07; N, 3.71. Found: C, 82.34; H, 5.22; N, 3.60.

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Phospholipid Studies of Marine Organisms. 22.[1] Structure and Biosynthesis of a Novel Brominated Fatty Acid from a Hymeniacidonid Sponge[†]

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A new long-chain fatty acid, (5*E*,9*Z*)-6-bromo-5,9-hexacosadienoic acid (**1a**), was isolated from the phospholipids of a marine sponge of the family Hymeniacidonidae. Structure elucidation was accomplished by means of mass spectrometry and 2D-homonuclear (COSY-45) ^1H NMR spectroscopy. The configuration of the bromo-substituted double bond was established by lithiation followed by protonolysis. Incorporation experiments with radiolabeled precursors revealed that biological bromination was the terminal step in the biosynthesis of this unusual acid.

Introduction

Since the original studies of Litchfield and collaborators,[2] numerous unusual fatty acids have been isolated from marine sponges featuring exceptionally long carbon chains (C_{24}–C_{30}) with novel branching, unsaturation, and substituent patterns.[3] It is assumed[4-7] that phospholipids with such acyl components serve special biological functions in modulating the membrane properties of the organism. The recent discoveries[8-11] of brominated fatty acids in marine sponges are of particular interest, as the number of halogenated fatty acids found in marine organisms[8-12] is extremely small in comparison with the wide spectrum of halometabolites from the same source.[13] Unique physiological properties may, therefore, be associated with such acids.

The earliest report[12] of halogenated long-chain fatty acids in marine organisms dates back to 1977 when several chlorohydrins of palmitic and stearic acids were found in the total lipids of a jellyfish. Subsequently a dibrominated straight-chain C_{16} acetylenic acid—the first recorded representative of this class—was found in the marine sponge *Xestospongia muta*.[8] This was followed by the discovery of a monobrominated straight-chain C_{18} bisacetylenic acid from *Xestospongia testudinaria*[9] and six mono- and dibrominated straight-chain unsaturated C_9, C_{16}, and C_{18} acids from the same genus.[10] However, there was so far only one report[11] of brominated demospongic acids existing in the phospholipids of living organisms. Two C_{27} acids with the unique 5,9-diene pattern, iso/anteiso methyl branching, and the bromovinyl functionality were isolated from the marine sponges *Petrosia ficiformis* and *Petrosia hebes*, which appeared to contain also traces of the C_{28} homologues of the above two acids. It is significant that all marine brominated fatty acids identified to date contain a bromovinyl or dibromovinyl moiety.

Despite a growing list of around 1000 isolated marine halometabolites, the mechanisms whereby halogens (mainly chlorine and bromine) are incorporated into these molecules have received attention only in recent years,[14-21]

[†] Dedicated with respect and affection to the memory of Professor Edgar Lederer—a pioneer in the lipid field.

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and the present knowledge is still inadequate to account for their chemical diversity. Biohalogenation in most cases is an enzymatic process,[14-16] which takes place in the presence of halide ions, suitable substrates, and hydrogen peroxide as produced by metabolism. Two major reaction steps can be distinguished, the first one being halide oxidation by hydrogen peroxide catalyzed by an enzyme broadly described as haloperoxidase. The ionic intermediate, which has been proposed[14,16,17,20] variously as free hypohalous acid or an enzyme-bound species (EOX^+), is then transferred to the substrate by electrophilic substitution or addition. Haloperoxidases in nature can be classified according to halide selectivity as chloroperoxidases (for Cl^-, Br^- and I^-), bromoperoxidases (for Br^- and I^-), and iodoperoxidases. Many in vitro studies of the first two classes have been reported: bromoperoxidases have been isolated from numerous marine algae, marine worms, bacteria, and a lichen;[14,19] and chloroperoxidases from a range of fungi including the classic example *Caldariomyces fumago*.[14] Only one marine demosponge, however, has been found to contain two haloperoxidases of uncertain identity.[15]

Biohalogenation is characterized by wide substrate specificity. A variety of organic functionalities that are nucleophilic can be halogen acceptors, in particular alkenes, alkynes, α,β-unsaturated acids, aromatics, β-diketones, and β-keto acids. Mechanisms and products of these reactions are diverse, but of apparent relevance to bromodemospongic acid biosynthesis are the well-known pathways via interhalogen or hypohalous acid addition to unsaturation sites followed by molecular rearrangements or sequel reactions such as dehydrohalogenation and decarboxylation.[14,21] In vivo studies of biohalogenation are sparse, however, and the kinetic and mechanistic information on individual haloperoxidases has not yet led to any unified biosynthetic schemes. The mechanism of vinylic bromide formation in demospongic acids, for instance, remains unknown.

It is the objective of this paper to describe the isolation and structural elucidation of a novel bromodemospongic acid from a marine sponge and to examine at what stage of fatty acid biosynthesis bromine is incorporated into the carbon chain.

Results and Discussion

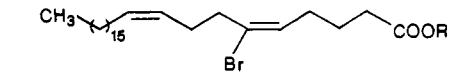
The major fatty acids from the phospholipids of the sponge were identified in the standard fashion[22] from the GC equivalent chain length values (ECL) and EI mass spectra of the methyl esters and the *N*-acylpyrrolidides. As shown in Table I, the phospholipid fatty acid composition of the sponge is characteristic of marine demosponges,[2] the most abundant acids being (5*Z*,9*Z*)-$\Delta^{5,9}$-26:2 (**2a**) and (5*Z*,9*Z*,19*Z*)-$\Delta^{5,9,19}$-26:3 (**3a**). There was, however, an additional major acid (**1a**) methyl ester with GC retention time much longer than those of the respective $\Delta^{5,9,19}$-26:3 and $\Delta^{5,9}$-26:2 acid methyl esters (**3b** and **2b**), and

Table I. Major Fatty Acids of the Phospholipid Fraction[a]

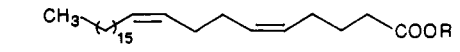
ECL[b]	fatty acid	abundance,[c] %
14.00	tetradecanoic (*n*-14:0)	0.9
14.52	4,8,12-trimethyltridecanoic (4,8,12-tri-Me-13:0)	4.6
15.82	9-hexadecenoic (Δ^9-16:1)	2.0
16.00	hexadecanoic (*n*-16:0)	4.5
16.63	15-methylhexadecanoic (iso-17:0)	0.7
17.00	heptadecanoic (*n*-17:0)	0.7
17.77	9-octadecenoic (Δ^9-18:1)	1.0
17.82	11-octadecenoic (Δ^{11}-18:1)	1.0
18.00	octadecanoic (*n*-18:0)	3.0
19.13	5,8,11,14-eicosatetraenoic ($\Delta^{5,8,11,14}$-20:4)	9.5
20.00	eicosanoic (*n*-20:0)	1.3
25.05	5,9,19-hexacosatrienoic ($\Delta^{5,9,19}$-26:3)	20.3
25.16	5,9-hexacosadienoic ($\Delta^{5,9}$-26:2)	18.3
25.29	9-hexacosenoic (Δ^9-26:1)	0.6
26.38	hexacosanoic (*n*-26:0)	0.5
27.33	6-bromo-5,9-hexacosadienoic (6-Br-$\Delta^{5,9}$-26:2)	12.8

[a] Capillary gas chromatography with Hewlett-Packard Model 5790 on fused silica (30 m × 0.32 mm) containing SE-54 (J&W Scientific, Inc.); program temperature: 170–320 °C, 5.0 °C/min. [b] Equivalent chain length values are those of the methyl esters of these acids. [c] Average values for three sponge samples. Some minor fatty acids (less than 0.5%) were present in the mixture.

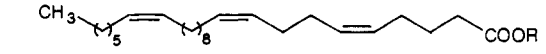
which made up a significant percentage of the total phospholipid fatty acid composition.



1a, R = H
b, R = Me



2a, R = H
b, R = Me



3a, R = H
b, R = Me

A sample of **1b** was obtained by reverse-phase HPLC separation of the total fatty acid methyl esters with absolute methanol as eluent. EI-GC/MS showed a strong mass spectral peak at *m/z* 74 due to McLafferty rearrangement typical of fatty acid methyl esters,[23] and also a series of peaks at *m/z* 405, 373, 355, and 331, which closely corresponded to those obtained with $\Delta^{5,9}$-26:2 methyl ester (**2b**) (*m/z* 406, 374, 357, 332). These data strongly suggested the presence of a labile substituent, which was easily lost under electron-impact conditions. Both CI/EI-GC/MS and positive DCI-MS with ammonia as reagent gas revealed, in addition to a distinct peak at *m/z* 405, a doublet due to $(M + NH_4)^+$ adduct ions with a 1:1 intensity ratio at *m/z* 502 and 504. Under negative DCI-MS conditions with ammonia as reagent gas, a doublet of roughly equal intensity corresponding to the M^- peaks appeared at *m/z* 484 and 486 in addition to another doublet at *m/z* 483 and 485 due to $(M - H)^-$ ions. These mass spectral data clearly indicated the presence of bromine, which had been lost to produce the prominent ion fragment of mass 405.

The similarity between the mass spectra of the methyl esters of $\Delta^{5,9}$-26:2 (**2b**) and the new acid (**1b**) suggested similar carbon skeletons. This hypothesis was tested by catalytic hydrogenation of **1b**, which yielded according to

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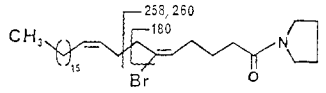

Figure 1. Fragmentation pattern of 6-Br-$\Delta^{5,9}$-26:2 pyrrolidide under EI-MS conditions.

EI-GC/MS criteria *n*-hexacosanoic acid (26:0). EI-GC/MS of the brominated acid pyrrolidide showed the weak molecular ion peak as a doublet of equal intensity at m/z 523 and 525 (corresponding to $C_{30}H_{54}ONBr$ as confirmed by high-resolution MS) and a strong peak at m/z 444 due to the loss of bromine. The fragmentation pattern was typical of straight-chain demospongic acid pyrrolidides and similar to that of the $\Delta^{5,9}$-26:2 pyrrolidide.[22] In addition to the base peak at m/z 113 due to McLafferty rearrangement, a peak not so prominent as in the case of $\Delta^{5,9}$-26:2 was observed at m/z 180, which corresponded to a double allylic fragmentation between C-6 and C-9 with the loss of bromine (Figure 1). A much stronger doublet of equal intensity, however, was observed at m/z 258 and 260 due to the same fragmentation with the bromine substituent intact. These facts implied the same double-bond locations in **1a** as in the $\Delta^{5,9}$-26:2 acid and limited the point of attachment of the bromine substituent between C-1 and C-7 of **1a**.

The main feature of the ^{1}H NMR spectrum (see Experimental Section) of **1b** is the presence of two multiplets at 5.32 and 5.41 ppm (δ) corresponding to the two vicinal olefinic hydrogens. An additional downfield triplet at 5.84 ppm (J = 7.6 Hz) suggested the presence of a second double bond with a vinylic substituent. The complete proton coupling network from C-2 to C-11 was resolved from the major cross-peaks in the 2D COSY-45 spectrum (Figure 2).[24] It can be seen that the two olefinic protons at C-9 and C-10 were coupled to each other, as depicted by the two cross-peaks adjoining the respective diagonal peaks in the spectrum. The C-9 proton was further coupled to the methylene protons at C-8, which were in turn coupled to those at C-7, while the C-10 proton was coupled also to the C-11 methylene. The hydrogen at C-5, however, was coupled only to the C-4 methylene, and no proton signal was observed at C-6. This coupling pattern clearly indicated that the bromine substituent was at the vinylic C-6 position, and the connectivity from C-1 to C-11 of **1b** was established.

The absence of prominent IR bands in the region between 960 and 980 cm^{-1} provided evidence for the *Z* configuration of the Δ^9 double bond but not for that at Δ^5, nor did NMR data give clear indications as to the configuration of the trisubstituted olefin. This problem was finally solved by a microscale metal–halogen exchange reaction. After lithium aluminum hydride reduction of **1b** under mild conditions, the product alcohol was stereospecifically debrominated with *sec*-butyllithium followed by protonolysis using methanol.[25,26] Subsequent Jones oxidation and esterification with BF_3–MeOH yielded a major product, which was identified by comparison with an authentic sample on GC to be (5*Z*,9*Z*)-$\Delta^{5,9}$-26:2 methyl ester. The 5*E*,9*Z* isomer, which had previously been synthesized and characterized,[28] was not detected. As the configurational

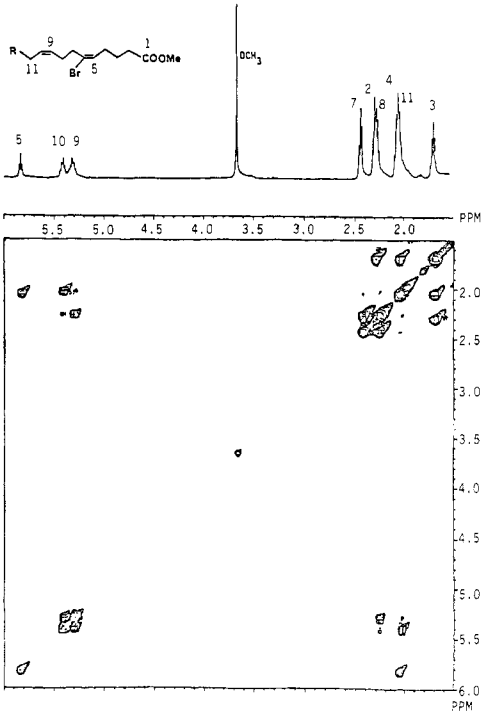

Figure 2. Partial COSY-45 ^{1}H NMR spectrum of 6-Br-$\Delta^{5,9}$-26:2 methyl ester.

purity of **1b** was apparent from the olefinic proton NMR signals, the overall 5*E*,9*Z* configuration of its carbon skeleton was established and was typical of demospongic acids[2] for the *cis*,*cis*-diene pattern with respect to the longest carbon chain.

As shown in Table I, the new (5*E*,9*Z*)-6-bromo-5,9-hexacosadienoic acid (**1a**) was the third most abundant acid of the total phospholipid fatty acid mixture. Qualitative analysis of different phospholipid fractions isolated by thin-layer chromatography revealed that the bromo acid existed, together with the other two major acids $\Delta^{5,9}$-26:2 (**2a**) and $\Delta^{5,9,19}$-26:3 (**3a**), as the major component of phosphatidylethanolamine (PE). We have shown on numerous occasions[22,27] that demospongic acids are mostly associated with amino-containing phospholipids in cell membranes, and on the basis of present evidence, the halogenated analogue exhibits the same tendency and may play an important role in the membrane physiology of the organism.

The biosynthesis of **1a** was investigated by first feeding to the sponge the sodium salt of the [1-^{14}C]-$\Delta^{5,9}$-26:2 acid.[29] We have shown earlier[29] that the $\Delta^{5,9}$-26:2 acid was biosynthesized in a typical marine demosponge by chain elongation of precursors with seven or more two-carbon units followed by desaturation starting at either the Δ^5 or Δ^9 positions. The aim of the present experiment was to determine whether bromination took place after chain desaturation.

The above precursor (10 μCi) was incorporated into the live, whole sponge by aquarium incubation in an aerated precursor-seawater solution for 3 days. The radioactivity of different fatty acid methyl esters was assessed after extraction and separation by reverse-phase HPLC (see Experimental Section). The fraction containing **1b** was purified four times on the same HPLC system, including the use of nonradioactive $\Delta^{5,9}$-26:2 methyl ester as carrier to remove any remaining labeled precursor, until constant

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Table II. Distribution of Radioactivity from Incorporation Experiments

	observed radioactivity (dpm)					
precursor	26:0	Δ^9-26:1	Δ^5-26:1	$\Delta^{5,9}$-26:2	6-Br-$\Delta^{5,9}$-26:2	$\Delta^{5,9,19}$-26:3
$\Delta^{5,9}$-26:2[a]				890 000	46 000	1300
26:0[b]	82 000	24 000	9700	23 600	5000	400

[a] 5% of the administered radioactivity was recovered from the phospholipid fraction. [b] 0.6% of the administered radioactivity was recovered from the phospholipid fraction. Uptake of this precursor was small because of its low natural abundance in the sponge.

radioactivity was observed. The chemical purity of radioactive fractions was ascertained by GC (flame ionization) analysis of the corresponding nonradioactive fractions, and the 6-Br-$\Delta^{5,9}$-26:2 methyl ester was shown to be over 99% pure. As is apparent from the distribution of radioactivity (Table II), sufficient incorporation and biotransformation had taken place to enable the following conclusions to be drawn.

(1) The 6-Br-$\Delta^{5,9}$-26:2 acid (**1a**) arises from bromination of the $\Delta^{5,9}$-26:2 acid (**2a**).

(2) No significant incorporation of radioactivity was observed into other major fatty acids, in particular the $\Delta^{5,9,19}$-26:3 acid. This observation shows that the $\Delta^{5,9}$-26:2 acid is metabolized only to the bromo derivative but not to the $\Delta^{5,9,19}$-26:3 triene. This observation is in agreement with our earlier report[29] on the marine demosponge *Microciona prolifera* that the $\Delta^{5,9,19}$-26:3 acid arose from homologation of exogenous palmitoleic acid (Δ^9-16:1) followed by eventual desaturation at positions 5 and 9.

In order to examine the possibility of earlier stage bromination (e.g., before acyl chain desaturation) and to verify the desaturation pattern in this particular sponge, an additional incorporation experiment was conducted using the sodium salt of the [1-^{14}C]26:0 acid.[29] The precursor (12 μCi) was incorporated into the live, whole sponge for 3 days; subsequent analysis and purification were performed in the same fashion as in the first experiment except that the nonradioactive Δ^5-26:1 and Δ^9-26:1 methyl esters were used as carriers to facilitate HPLC separation of these two fractions.

The results of this particular study (Table II) indicated that the 26:0 acid was converted to Δ^5-26:1, Δ^9-26:1, $\Delta^{5,9}$-26:2, and 6-Br-$\Delta^{5,9}$-26:2 but not the $\Delta^{5,9,19}$-26:3 acid. The absence of other brominated fatty acid intermediates in the phospholipid fraction supports the inference that bromination constitutes the final step in the biosynthesis of the new acid **1a** (see scheme below). Another significant observation was that initial acyl chain desaturation in this sponge occurred also at either the Δ^5 or Δ^9 stages, corroborating our recent similar findings[29] from *M. prolifera*. The unique desaturation sequence in demospongic acids represents a clear exception to the accepted view[30] of polyunsaturated fatty acid formation in eucaryotes, which implies that the first double bond is always introduced at the Δ^9 position.

On the basis of the present knowledge, the following biosynthetic pathway for (5*E*,9*Z*)-6-bromo-5,9-hexacosadienoic acid is proposed:

$$14{:}0 \rightarrow 16{:}0 \rightarrow 26{:}0 \rightarrow \Delta^5\text{- and } \Delta^9\text{-}26{:}1 \rightarrow \Delta^{5,9}\text{-}26{:}2 \rightarrow 6\text{-Br-}\Delta^{5,9}\text{-}26{:}2$$

The detailed mechanism of bromination, however, is still uncertain. It may be a single step or a series of complex reactions via, for example, a bromohydrin or *vic*-dihalide intermediate followed by dehydration or dehydrohalogenation, respectively. It is uncertain whether bromination in this sponge is catalyzed by one of the known haloperoxidases, although the absence of chlorinated analogues of the bromodemospongic acids suggests the existence of a bromoperoxidase. Another intriguing observation is that no 6-bromo-$\Delta^{5,9,19}$-26:3 acid has ever been detected despite the abundance of 6-bromo-$\Delta^{5,9}$-26:2 (**1a**) and similar brominated $\Delta^{5,9}$ acids in two marine sponges.[11] Also the bromine atom in all bromodemospongic acids identified so far is at the vinylic C-6 position, regardless of carbon chain length or methyl branching. These observations point toward the operation of a highly substrate specific and regioselective halogenation reaction.

Experimental Section

General Procedures. The sponge (California Academy of Sciences voucher no. CAS 065611) was collected between March and June at a depth of 10 m in Monterey Bay, California. According to Dr. Welton Lee (W. L. Lee & Associates, Inc., San Francisco, CA; Consultants in Biology, Geology and Palentology), this sponge belongs to a new genus tentatively placed in the family Hymeniacidonidae. The total lipids were extracted by the method of Bligh and Dyer.[31] Neutral and glycolipids were removed by column chromatography on silica gel (60 mesh) with acetone as eluent, and phospholipids were finally collected in 500 mL of absolute methanol. After solvent removal, the fatty acyl components of the phospholipids were obtained as their methyl esters by reaction with 1.8 N methanolic hydrogen chloride followed by chromatographic purification on a 1:1 mixture of silica gel and Florisil with hexane–ether (10:1) as eluent. The resulting methyl esters were analyzed by gas chromatography using a Hewlett-Packard Model 5790 gas chromatograph with a 30 m × 0.32 mm fused-silica column coated with SE-54 (program temperature 170–320 °C at 5 °C/min) and a flame-ionization detector.

Different phospholipid headgroups were separated by thin-layer chromatography on silica gel 60 F_{254} plates with a chloroform–methanol–water (75:25:3) mixture as the mobile phase. Phosphatidylethanolamine gave a distinct band (R_f = 0.6) as identified by molybdenum blue and alcoholic ninhydrin sprays in spite of some overlapping with other headgroups. Transesterification with 14% BF_3–methanol and subsequent extraction with a hexane–ether (10:1) mixture gave the major acid methyl esters from the headgroup.

Catalytic hydrogenation of methyl esters was performed by using PtO_2 in absolute methanol (room temperature, 10 h). Pyrrolidization for subsequent confirmation of double-bond positions by EI-MS was achieved by reaction of the methyl esters with pyrrolidine in the presence of a trace of acetic anhydride (90 °C, 1 h).[22] Unreacted methyl esters (less than 10%) were removed by silica gel column chromatography with hexane–ether (10:1) as eluent, and the product was collected in hexane–ether (1:1).

Reverse-phase high-performance liquid chromatography (HPLC) for separation of fatty acid methyl esters was performed on a Waters Associates HPLC system (M6000 pump, R403 differential refractometer) with two Altex Ultrasphere ODS2 columns (25 cm × 10 mm i.d.) in series and absolute methanol as the mobile phase. The flow rate used in all experiments was 3 mL/min.

^{1}H NMR spectra were obtained in $CDCl_3$ on a Varian Associates XL-400 (400 MHz) spectrometer. 2D NMR spectra were obtained in $CDCl_3$ on a Bruker AM-500 (500 MHz) instrument. GC/MS analyses were performed on a Carlo Erba Model 4160 gas chromatograph with on-column injection (column, 30 m × 0.33 mm

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Durabond DB-5; carrier gas, helium at 0.6 bar; column temperature, 130–280 °C at 4 °C/min). Low-resolution mass spectra were obtained with a Ribermag R-10-10 quadrupole mass spectrometer using the "SADR" (simultaneous acquisition and data reduction) data system (source temperature, 200 °C; reagent gas for chemical ionization, NH_3). High-resolution mass spectra were recorded at the mass spectrometry laboratory of the University of Minnesota on a VG 7070E-HF instrument (negative chemical ionization mode, source temperature 150 °C, NH_3 as reagent gas). FTIR spectra were obtained on a Nicolet Model 7199 Fourier transform spectrometer.

Incorporation experiments were conducted on the live, whole sponge in Monterey Bay Aquarium at Hopkins Marine Station by incubation in an aerated precursor-seawater solution for 3 days during November and March. For radioactivity measurements, aliquots (usually $^1/_5$–$^1/_{10}$) of the ^{14}C-labeled material were dissolved in 10 mL of organic counting scintillant (OCS), and the radioactivity was measured with a Beckman LS 7500 liquid scintillation system. All results were corrected for background radiation, calculated to the proportionate amount, and presented as disintegrations per minute by using a standard solution. All precursors used were tested for radioactive purity by means of blank HPLC experiments.

Debromination of (5*E*,9*Z*)-6-Bromo-5,9-hexacosadienoic Acid Methyl Ester (1b). To a suspension of lithium aluminum hydride (1 mg) in dry ether (1 mL) was added dropwise a solution of the ester **1b** (3 mg) in dry ether (1 mL) at 0 °C, and the mixture was stirred at that temperature for 1 h. Addition of a slight excess of oxalic acid dihydrate and filtration yielded a product that was reacted crude, after solvent removal, with excess *sec*-butyllithium in dry THF–hexane–ether (2:1:1 mixture, 2 mL) at 0 °C under nitrogen for 1 h. Absolute methanol was then added to quench the reaction. The crude dried product was dissolved in acetone (2 mL) and titrated with Jones reagent. The reaction mixture was then diluted with water (3 mL) and extracted with ether. The resulting acid was converted to the methyl ester by reflux with 14% BF_3–MeOH. The final product, after being extracted with hexane–ether (10:1) and purified by silica gel–Florisil open column chromatography, was identified to be **2b** by comparison on GC with an authentic sample. The other possible stereoisomer in this case, the (5*E*,9*Z*)-$\Delta^{5,9}$-26:2 methyl ester,[28] had a shorter GC retention time and was not detected.

(5*E*,9*Z*)-6-Bromo-5,9-hexacosadienoic Acid Methyl Ester (1b): ca. 5 mg, oil, from 30 g of dried sponge; HPLC relative retention time, 0.97 (**2b**: 1.00) under the above-stated instrumental conditions; ^1H NMR (400 MHz, $CDCl_3$) 5.84 (t, J = 7.6 Hz, 1 H, C-5), 5.41 (m, 1 H, C-10), 5.32 (m, 1 H, C-9), 3.67 (s, 3 H, OMe), 2.45 (t, J = 7.4 Hz, 2 H, C-7), 2.32 (t, J = 7.6 Hz, 2 H, C-2), 2.30 (m, 2 H, C-8), 2.08 (m, 2 H, C-4), 2.04 (m, 2 H, C-11), 1.71 (quin, J = 7.4 Hz, 2 H, C-3), 1.26 (m, 28 H, C-12–C-25), 0.88 (t, J = 7.0 Hz, 3 H, C-26); EIMS m/z (relative intensity) 405 (100), 373 (23.3), 355 (9.6), 331 (9.8), 264 (86.5); CI-MS-NH_3 (positive) 504 (100), 502 (98.9), 405 (25.5); CI-MS-NH_3 (negative) 486 (38.7), 484 (53.6), 485 (58.3), 483 (56.0), 405 (47.1); EI-MS (acid pyrrolidide) 525 (0.3), 523 (0.3), 444 (60.4), 260 (18.4), 258 (17.6), 180 (7.7), 113 (100); IR (CCl_4) (cm^{-1}) 2920, 2850 (=CH, CH_3, CH_2), 1740 (C=O), 1460, 1430 (=CH, CH_3, CH_2), 1210, 1160 (CO); high-resolution MS (negative DCI mode, NH_3 as reagent gas) calcd for $(M - H)^-$ where M = $C_{27}H_{49}O_2Br$ 485.2887 and 483.2907, found 485.2855 and 483.2851 (intensity ratio 1:0.89).

Abbreviations: DCI-MS, desorption chemical ionization mass spectrometry; CI/EI-MS, a combination of electron-impact and chemical-ionization mass spectrometry.

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Synthesis and Characterization of a Novel Betaine Dye: 2,4-Dimethyl-6-(2,4,6-triphenyl-*N*-pyridinio)phenolate

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A pyridinium *N*-phenoxide betaine with the pyridine ring attached at the ortho position of the phenoxide ring was synthesized via the procedure of Reichardt for making para betaines. The dye was found to be highly solvatochromic, which is indicative of a large dipole moment change upon excitation. Crystals of the betaine were grown, and the structure was determined by X-ray analysis. Interestingly, the pyridine and phenoxide rings are orthogonal, in apparent conflict with the observed movement of electron density between the two rings upon excitation. Possible mechanisms for interaction between orthogonal rings are discussed.

Introduction

Pyridinium *N*-phenoxide betaine dyes, first synthesized in the 1920s[1] and studied in depth by Reichardt and Dimroth in the 1960s,[2-4] are of interest for several reasons. They are unusual in that their electronic ground state exists as a dipolar ion, with the nitrogen atom carrying a positive charge and the oxygen atom carrying a negative charge. As a consequence they possess very large ground-state dipole moments, which decrease significantly upon excitation because of intramolecular charge transfer. This causes them to be highly solvatochromic,[5,7] i.e., their

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