New Cyclic Peroxides from the Philippine Sponge *Plakinastrella* sp.

Asfia Qureshi, Javier Salvá,† Mary Kay Harper, and D. John Faulkner*

Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92093-0212

Received June 24, 1998

Three new cyclic peroxides **5**−**7** and a new carboxylic acid ester **8** were isolated as minor metabolites from the hexane extract of a *Plakinastrella* species from the Philippines. The structures of compounds **5**−**8** were elucidated by interpretation of spectral data and by chemical interconversion, and the absolute stereochemistry of peroxide **6** was determined by application of Mosher's method to a derivative. Although the major compounds in the sponge showed activity against *Candida albicans* prior to decomposition, the minor metabolites **5**−**8** are essentially inactive.

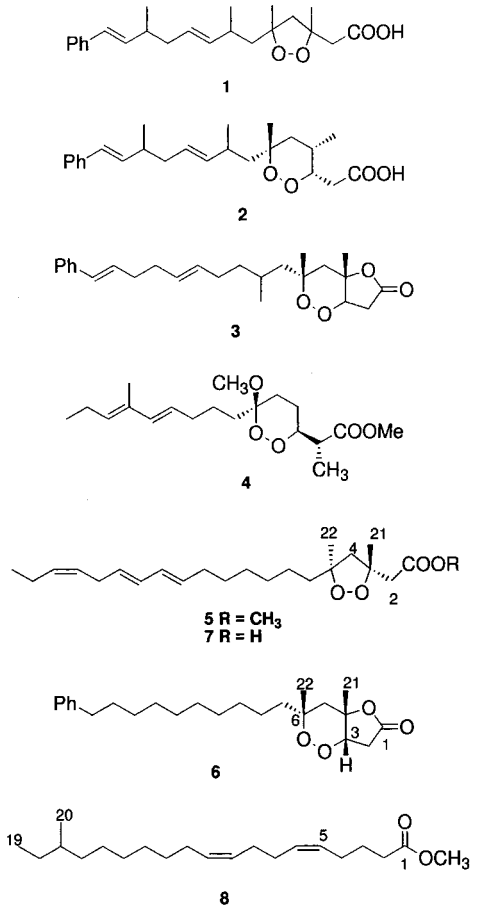
Cyclic peroxides have been isolated previously from a number of marine organisms, especially from sponges of the family Plakinidae.[1] These sponges are rich sources not only of cyclic peroxides, but also of peroxylactones and peroxyketals. Examples include plakinic acids A (**1**) and B (**2**),[2] plakortolide (**3**),[3] and peroxyplakoric acid A1 methyl ester (**4**).[4a] The peroxides often exhibit antimicrobial, ichthyotoxic, and cytotoxic activities,[4] and some activate cardiac SR Ca^{2+} pumping ATPase.[5] Recently, a number of peroxides have been shown to be active against the protozoan *Leishmania mexicana*.[6]

During the course of our investigations, we isolated four new metabolites from a Philippine marine sponge of the genus *Plakinastrella*. This genus is taxonomically related to other representatives of the Plakinidae that yield peroxide-containing metabolites. A crude methanolic extract was toxic to brine shrimp and was active in antimicrobial assays; however, due to the unstable nature of the majority of these compounds, only minor metabolites of this sponge could be isolated, and these showed no bioactivity.

Results and Discussion

Specimens of *Plakinastrella* sp. were collected by hand at Hagakhak Island, Philippines, and were kept frozen until extraction. A concentrated methanolic extract, which was obtained after soaking the diced sponge tissue (300 g wet wt), was partitioned between hexane and water. The aqueous phase was further partitioned against ethyl acetate. The hexane extract (490 mg) showed strong activity against *Candida albicans*, and this material was subjected to Si gel flash chromatography. The more polar compounds that eluted with ethyl acetate also showed strong antimicrobial activity and, from initial examination of the crude ^{1}H NMR spectra of all the fractions, appeared to be the corresponding acids of those compounds that eluted earlier from the column. However, on standing, these major polar metabolites decomposed, and therefore examination of only the nonpolar fractions could be pursued. Extensive Si gel HPLC was carried out on the nonpolar fractions to yield methyl (3*R**,5*S**,12*E*,14*E*,17*Z*)-3,5-dimethyl-3,5-peroxydodeca-12,14,17-trienoate (**5**, 7.4 mg, 2.4×10^{-3} % yield); (3*S*,4*S*,6*R*)-4,6-dimethyl-4-hydroxy-3,6-peroxy-16-phenylhexadecanoic acid 1,4-lactone (**6**, 1 mg, 3×10^{-4} % yield); (3*R**,5*S**,12*E*,14*E*,17*Z*)-3,5-dimethyl-3,5-peroxydodeca-12,14,17-trienoic acid (**7**, 4 mg, 1.3×10^{-3} % yield), and (5*Z*,9*Z*)-17-methylnonadeca-5,9-dienoate (**8**, 2 mg, 7×10^{-4} % yield) (Chart 1).

Chart 1



Methyl (3*R**,5*S**,12*E*,14*E*,17*Z*)-3,5-dimethyl-3,5-peroxydodeca-12,14,17-trienoate (**5**) was isolated as a colorless oil. The molecular formula of $C_{23}H_{38}O_4$ was established from HRCIMS and ^{13}C NMR data. The IR spectrum of **5** showed a band at 1740 cm^{-1}, typical of a methyl ester. The ^{13}C spectrum (Table 1) displayed 23 distinct signals of which those at δ 171.1 and 51.7 could easily be distinguished as an ester carbonyl and a methoxy group, respectively. The signals at δ 83.9 and 86.5 were assigned to two oxygenated quaternary carbons. Together with the results of the ^{1}H NMR, DEPT, and gradient HMQC experiments, the presence of six methine, 10 methylene, and four methyl groups was established. Of the four methyl groups, one was the

* To whom correspondence should be addressed. Tel.: (619) 534-2348. Fax: (619) 534-2997. E-mail: jfaulkner@ucsd.edu.

† Permanent Address: Departamento de Química Orgánica, Facultad de Ciencias del Mar, Universidad de Cádiz, Apdo. 40, Puerto Real, 11510 Cádiz, Spain.

10.1021/np9802724 CCC: $15.00
Published on Web 10/31/1998

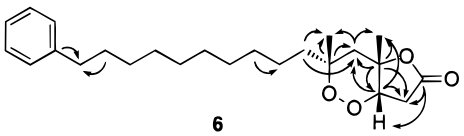
Table 1. ^{13}C (100 MHz, $CDCl_3$) and ^1H (300 MHz, $CDCl_3$) NMR Data for **5** and ^1H (300 MHz, $CDCl_3$) NMR Data for **7**

	5				**7**	
C no.	δ_C	δ_H	mult, *J* (Hz)	HMBC	δ_H	mult, *J* (Hz)
1	171.1			C-2		
2	44.0	2.65	d, 1H, 14.7	C-1, C-3, C-4, C-2	2.70	d, 14.7
		2.78	d, 1H, 14.7		2.82	d, 14.7
3	83.9			C-2, C-4, C-21		
4	55.4	2.23	d, 1H, 12.6	C-2, C-4, C-5, C-6,	2.15	d, 12.3
		2.48	d, 1H, 12.6	C-21	2.54	d, 12.3
5	86.5			C-4, C-8, C-22		
6	39.6	1.68	m, 2H		1.73	m
7	24.5	1.55	m, 2H		1.57	m
8	29.1	1.36	m, 2H	C-5, C-6	1.28	m
9	29.3	1.31	m, 2H	C-8	1.28	m
10	29.9	2.14	m, 2H	C-9	2.08	m
11	32.5	2.04	m, 2H	C-8, C-12	2.00	m
12	130.5	5.58	m, 1H	C-13	5.62	m
13	130.2	6.02	m, 1H	C-15, C-16	6.04	m
14	130.7	6.02	m, 1H	C-11, C-12	6.04	m
15	132.8	5.58	m, 1H	C-13, C-14	5.62	m
16	35.5	2.75	m, 2H		2.75	m
17	126.8	5.45	m, 1H	C-16, C-19	5.44	m
18	133.1	5.45	m, 1H	C-16, C-19	5.44	m
19	25.5	2.04	m, 2H	C-17, C-18, C-20	2.00	m
20	13.8	0.98	t, 3H, 7.6	C-18, C-19	1.01	t
21	24.1	1.44	s, 3H	C-2, C-3, C-4	1.49	s
22	23.2	1.29	s, 3H	C-4, C-5, C-6	1.34	s
23	51.7	3.70	s, 3H	C-1		

methyl ester, two were singlets, and one was a triplet in the ^1H NMR spectrum. In addition to the data presented above, a gradient COSY experiment allowed the construction of several partial structures that could then be interconnected using data obtained from the gradient HMBC experiments. Key long-range correlations are as follows: H_2-4 to C-2, C-4, C-5, and C-6; CH_3-22 to C-4, C-5, and C-6; CH_3-21 to C-2, C-3, and C-4. These results are consistent with a five-membered peroxide ring.[2,7] The relative stereochemistry of the five-membered peroxide ring in **5** was established using a 1D-NOE experiment. The H-4 signal at δ 2.48 showed a strong NOE to CH_3-22, while the other H-4 signal at 2.23 showed a stronger NOE to CH_3-21, and a weak correlation to CH_3-22. Irradiation of CH_3-21 with a narrow bandwidth (10 Hz) showed no NOE to CH_3-22. A molecular model (PC model v 5.0 [MMX]) of the peroxide ring indicates that if both methyls are in a cis orientation, a strong NOE correlation should be observed because the calculated distance between the two methyl groups is 2.6 Å, while the distance between two trans-oriented methyls is 5.4 Å. These results are fully consistent with a trans orientation of the methyl groups.

The remainder of the molecule was established as follows. The overlapping ^1H signals at δ 5.45, 5.58, and 6.02, integrating to six protons, indicated the presence of three double bonds along the alkyl chain, two of which were conjugated as indicated by the gradient COSY data. Corresponding ^{13}C signals between δ 126.8 and 133.1 also provided further evidence for six olefinic carbons. The positioning of the double bonds at C-12, C-14, and C-17 was determined from gradient COSY and HMBC data. The geometry of the double bonds was established from the ^{13}C NMR chemical shift of the adjacent methylene carbons, which occur at ca. 27 ppm for *Z*-olefins and ca. 32 ppm for *E*-olefins.[8]

(3*S*,4*S*,6*R*)-4,6-Dimethyl-4-hydroxy-3,6-peroxy-16-phenylhexadecanoic acid 1,4-lactone (**6**) was isolated as a colorless oil. The molecular formula of $C_{24}H_{36}O_4$, which requires seven degrees of unsaturation, was established



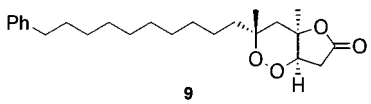
6

Figure 1. HMBC Correlations observed for **6**.

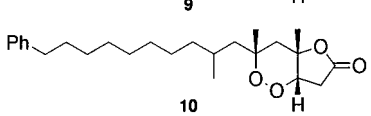
Table 2. ^{13}C (100 MHz, $CDCl_3$) and ^1H (300 MHz, $CDCl_3$) NMR Data for **6**

C no.	δ_C	δ_H	mult, *J* (Hz)	HMBC
1	174.1			C-2, C-3
2	34.1	2.91	dd, 1H, 18.6, 6	
		2.59	dd, 1H, 18.6, 1.5	
3	80.8	4.49	d, 1H, 6	C-2, C-5, C-21
4	82.5			C-2, C-3, C-5, C-21
5	40.2	2.28	d, 1H, 15.3	C-21, C-22
		1.66	d, 1H, 15.3	
6	80.2			C-5, C-22
7	36.9	1.75	m, 2H	C-5, C-22
8	23.7	1.27	m, 2H	
9	29.5	1.30	m, 2H	C-8
10	29.6	1.30	m, 2H	
11	29.9	1.30	m, 2H	
12	29.6	1.30	m, 2H	
13	29.5	1.30	m, 2H	
14	29.3	1.30	m, 2H	
15	31.5	1.57	m, 2H	C-16
16	36.0	2.60	t, 2H, 7.8	
17	143.0			C-16
18	128.4	7.19	m, 2H	
19	128.2	7.27	m, 2H	
20	125.5	7.19	d, 1H, 7.2	
21	25.9	1.38	s, 3H	C-3, C-5
22	24.9	1.20	s, 3H	C-5

from HREIMS, *m/z* 388.2614 (M^+) (Δ 2.5 mmu). The IR spectrum showed a strong absorbance at 1785 cm^{-1}, suggestive of a γ-lactone, and no other carbonyl or hydroxyl band. The major structural features could be deduced from the ^{13}C NMR spectrum (Table 2), which displayed 20 distinct signals, two of which were assigned to the degenerate positions of a monosubstituted benzene ring (δ 128.2 and 128.4). The ^{13}C NMR data, together with the results of ^1H NMR and HMQC experiments, indicated the presence of six methine, 12 methylene, and two methyl groups, both of which appeared as singlets in the ^1H NMR spectrum (δ 1.38 and 1.20). The remaining quaternary carbons were assigned as an *ipso* aromatic carbon (δ 143.0), an ester carbonyl (174.1) and two oxygenated quaternary carbons observed at 82.5 and 80.2. The ^1H NMR spectrum (Table 2) exhibited signals at δ 2.59 (1 H, dd, J = 18.6, 1.5 Hz) and 2.91 (1 H, dd, J = 18.6, 6 Hz) coupled to each other and, in the latter case, to an α-peroxy proton signal at 4.49 (1 H, d, J = 6 Hz). An AB pattern at δ 2.28 (1 H, d, J = 15.3 Hz) and 1.66 (1 H, d, J = 15.3 Hz) is consistent with a bicyclic ring system.[9] A combination of gradient COSY and HMQC data led to the identification of several substructures, which were connected upon analysis of HMBC experiments (Figure 1). The relative stereochemistry of **6** was determined by 1D NOESY experiments and by comparison of the ring methyl NMR signals to similar peroxylactones **9**[9] and **10**[10] (Table 3). Irradiation of the methine



9

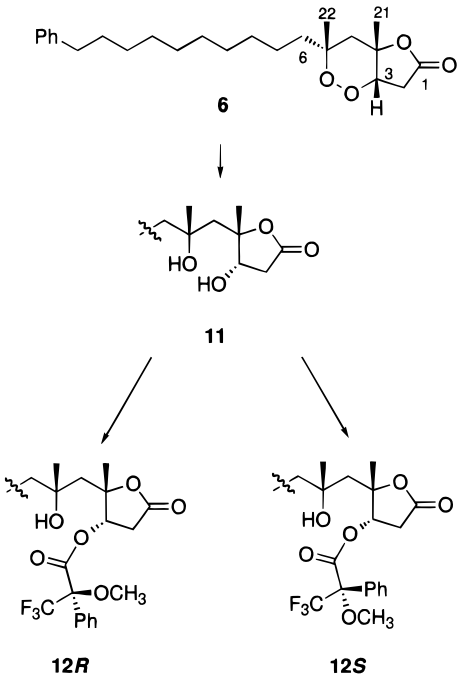


10

Table 3. Selected ^{13}C (100 MHz, $CDCl_3$) and ^1H (300 MHz, $CDCl_3$) NMR Data for **6**, **9**, and **10**

	6		**9**		**10**	
C no.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	174.1		175.2		174.1	
2	34.1	2.91	38.1	2.93	34.2	2.90
		2.59		2.55		2.60
3	80.8	4.49	73.8	4.19	81.0	4.45
4	82.5		90.1		82.8	
5	40.2	2.28	43.9	2.15	42.1	2.22
		1.66		2.09		1.70
6	80.2		72.9		80.8	
7	36.9	1.75	43.6	1.61	44.5	1.72
				1.29		1.46
8	23.7	1.27	24.4	1.29	28.9	1.60
21	25.9	1.38	26.9	1.44	25.9	1.37
22	24.9	1.20	30.0	1.35	24.9	1.25

Scheme 1



proton at δ 4.49 showed a strong NOE to the methyl protons at 1.38, thereby confirming the cis junction of the ring system. Comparison of **6** with **9** and **10** indicated CH_3-21 to be in an axial position, thereby justifying the stereochemistry shown. Molecular modeling depicts the peroxide ring to be in the chair conformation, with the two methyl groups axial and the methine proton at δ 4.49 in an equatorial orientation.

The absolute stereochemistry of **6** was determined by reductive cleavage of the peroxide ring in **6** with zinc and acetic acid in ether to afford the diol **11** (Scheme 1), which was esterified at C-3 by (*R*)- or (*S*)-MTPA chlorides in dry pyridine to give the corresponding (*S*)-MTPA ester **12*S*** and (*R*)-MTPA ester **12*R***, respectively. Upon analysis of the ^1H NMR chemical shifts of the MTPA derivatives, the absolute configuration at C-3 was found to be *S*. Coupled with the relative geometry derived above, this established the absolute stereochemistry of the peroxide ring as (3*S*,4*S*,6*R*).

(3*R**,5*S**,12*E*,14*E*,17*Z*)-3,5-Dimethyl-3,5-peroxydodeca-12,14,17-trienoic acid (**7**) was isolated as a pale yellow oil. The molecular formula of $C_{22}H_{36}O_4$ was established from MS and ^{13}C NMR data. LRCIMS of **7** displayed a peak at *m*/*z* 306, consistent with loss of $-CH_2COOH$ from the molecule. The IR spectrum revealed a broad hydroxyl band centered at 3470 cm^{-1} and a carbonyl band at 1710 cm^{-1}. A comparison of the ^1H NMR spectrum with that of methyl ester **5** (Table 1) indicated that **7** was the corresponding acid; however, acid **7** could not be purified by Si gel HPLC, C_{18} HPLC, or GC−MS. Methylation of **7** yielded an inseparable mixture of compounds, the major one of which was shown to be ester **5** by comparison of ^1H NMR spectra. Compound **7** was extremely unstable, decomposing rapidly over short periods of time, thereby rendering purification and characterization difficult.

Table 4. ^{13}C (100 MHz, $CDCl_3$) and ^1H (300 MHz, $CDCl_3$) NMR Data for **8**

C no.	δ_C	δ_H	mult, *J* (Hz)	HMBC
1	174.3			C-21, C-2, C-3
2	33.5	2.34	m, 2H	C-1, C-3, C-4
3	24.9	1.72	m, 2H	C-1, C-2, C-4
4	27.4	2.11	m, 2H	C-2, C-3
5	128.8	5.40	m, 1H	C-4
6	128.9	5.40	m, 1H	
7	29.7	1.28	m, 2H	
8	27.3	2.11	m, 2H	C-5, C-10
9	130.4	5.40	m, 1H	C-8, C-11
10	130.5	5.40	m, 1H	C-8, C-11
11	26.6	2.04	m, 2H	C-10, C-12
12	25.0	1.64	m, 2H	
13	29.3	1.28	m, 2H	
14	29.5	1.28	m, 2H	
15	29.6	1.28	m, 2H	
16	31.9	1.28	m, 2H	
17	28.0	1.64	m, 1H	
18	22.7	1.28	m, 2H	
19	14.1	0.91	t, 3H, 6	C-16, C-17, C-18
20	22.6	0.89	dd, 3H, 14.7, 7.8	C-16, C-17, C-18, C-19
21	51.5	3.70	s, 3H	C-1

Although 5,9-dienoic acids and dienoates are well established in the literature,[11] methyl (5*Z*,9*Z*)-17-methyl-nonadeca-5,9-dienoate (**8**) appears to be new. The molecular formula $C_{21}H_{38}O_2$ for ester **8** ($[\alpha]_D$ −4°) was established from HREIMS and ^{13}C NMR data. The IR spectrum contained an ester band at 1740 cm^{-1}. The ^{13}C spectrum revealed the expected 21 carbon signals, most of which were assigned using the HMQC and HMBC experiments. The ^{13}C NMR spectrum (Table 4) contained one ester carbonyl signal at δ 174.3 and four olefinic signals at 128.8, 128.9, 130.4, and 130.5, indicating two double bonds, and a methoxy signal at 51.5. The ^1H NMR spectrum (Table 4) contained olefinic proton signals at δ 5.40 (m, 4H), with other signals at 0.89 (3 H, dd, J = 14.7, 7.8 Hz) and 0.91 (3 H, t, J = 6 Hz) due to two methyl groups, one of which was a terminal methyl. Gradient COSY correlations were observed with coupling between the protons at 5.40 and those at 2.11, as well as between 2.11 and 2.34 and between 2.04 and 1.28. Key HMBC correlations were observed between the C-1 carbonyl signal at 174.3 and the proton signals at 3.70 (CH_3-21), 2.34 (H-2), and 1.72 (H-3), thereby establishing the ester end of the chain. Additionally, correlations between the olefinic signals at δ 5.40 and the carbon signals at 27.3 and 26.6, and that at 27.4, allowed the placement of the 9,10 double bond and the 5,6 double bond, respectively. The cis-geometry of the double bonds was established from the ^{13}C NMR chemical shift of the adjacent methylene carbons.[8] The 17-methyl terminus was established by GHMBC correlations between CH_3-20 and C-16, C-17, C-18, and C-19. All other spectral data support the structure assigned to **8**.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Autopol III polarimeter. IR and UV spectra were recorded on Perkin−Elmer 1600 FT-IR and Lambda 3B instruments, respectively. The ^{1}H, gradient COSY, gradient HMQC, gradient HMBC, and 1D NOE spectra were recorded on a Varian Inova 300 MHz spectrometer and the ^{13}C and DEPT spectra on a Varian Gemini 400 MHz spectrometer. All NMR data were recorded in $CDCl_3$. HRMS data were obtained from the UC Riverside Regional Mass Spectrometry Facility. All solvents were distilled prior to use.

Animal Material. The sponge, *Plakinastrella* sp. (Homosclerophorida, Plakinidae), was collected by hand using scuba (at a depth of 30−50 ft) at Hagakhak Island (SSW Dinagat Island), Philippines, in May 1997, frozen immediately after collection, and kept frozen until used. The sponge is mottled brown, forms thick encrustations, and has a smooth surface and dense texture. Our material has been compared with *Plakinastrella* sp.,[12] and the two specimens agree in general physical description and spicule measurements. A voucher specimen has been deposited into the SIO Benthic Invertebrate Collection catalogue #P1175.

Extraction and Purification. The frozen sponge (300 g wet wt) was diced and extracted with MeOH (2 × 1 L). The combined MeOH extracts were concentrated and partitioned between 1:1 hexane−H_2O (2 × 300 mL). The aqueous fraction was further partitioned against EtOAc (2 × 300 mL). The hexane fraction was concentrated under vacuum and chromatographed on Si gel using a gradient of 100% hexane to 100% EtOAc as eluent. Fractions obtained with 10%, 20%, and 70% EtOAc were further purified by HPLC on a Si gel column (Microsorb, 5μ, 10 mm × 25 cm) with 95% hexane in EtOAc to yield (5*Z*,9*Z*)-17-methylnonadeca-5,9-dienoate (**8**, 2 mg, 7 × 10^{-4} % wet wt), and methyl (3*R**,5*S**,12*E*,14*E*,17*Z*)-3,5-dimethyl-3,5-peroxydodeca-12,14,17-trienoate (**5**, 7.4 mg, 2.4 × 10^{-3}% wet wt); with 75% hexane in EtOAc to yield (3*S*,4*S*,6*R*)-4,6-dimethyl-4-hydroxy-3,6-peroxy-16-phenylhexadecanoic acid 1,4-lactone (**6**, 1 mg, 3 × 10^{-4} % wet wt), and with 50% hexane in EtOAc to yield (3*R**,5*S**,12*E*,14*E*,17*Z*)-3,5-dimethyl-3,5-peroxydodeca-12,14,17-trienoic acid (**7**, 4 mg, 1.3 × 10^{-3} % wet wt), respectively, as an impure, inseparable mixture.

Methyl (3*R,5*S**,12*E*,14*E*,17*Z*)-3,5-Dimethyl-3,5-peroxydodeca-12,14,17-trienoate (5):** colorless oil; $[\alpha]_D$ −28.5° (*c* 0.2, $CHCl_3$); UV (hexane) λ_{max} 228 (ϵ 37 000), 275 (4700), 264 (5000) nm; IR (film) ν_{max} 2930, 2850, 1740, 1455, 1435, 1370, 1345, 1210, 990, 965 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; CIMS/NH_3 *m*/*z* 378 $[M]^+$ (<1%), 306 $[MH-CH_2COOCH_3]^+$ (63.7); HRCIMS *m*/*z* 306.2694 (calcd for $C_{20}H_{34}O_2$, 306.2559).

(3*S*,4*S*,6*R*)-4,6-Dimethyl-4-hydroxy-3,6-peroxy-16-phenylhexadecanoic acid 1,4-lactone (6): colorless oil; $[\alpha]_D$ −8° (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} 260 (ϵ 3400) nm; IR (film) ν_{max} 2920, 2850, 1785 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 2; EIMS *m*/*z* 388 $[M]^+$ (16%); HREIMS *m*/*z* 388.2594 $[M]^+$ (calcd for $C_{24}H_{36}O_4$, 388.2614).

(3*R,5*S**,12*E*,14*E*,17*Z*)-3,5-Dimethyl-3,5-peroxydodeca-12,14,17-trienoic acid (7):** pale yellow oil; IR (film) ν_{max} 3470, 2920, 2850, 1710, 1375, 1310, 1225 cm^{-1}; ^{1}H NMR, see Table 1; CIMS *m*/*z* 364 $[M]^+$ (<1%), 306 $[MH - CH_2COOH]^+$ (46.2); HRCIMS *m*/*z* 306.2682 (calcd for $C_{20}H_{34}O_2$, 306.2559).

(5*Z*,9*Z*)-17-Methylnonadeca-5,9-dienoate (8): colorless oil; $[\alpha]_D$ −4° (*c* 0.075, hexane); UV (hexane) λ_{max} 221 (ϵ 4100) nm; IR (film) ν_{max} 2920, 2850, 1740, 1460, 1435 cm^{-1}; ^{1}H NMR, see Table 4; ^{13}C NMR, see Table 4; EIMS *m*/*z* 322 $[M]^+$ (12.3%); HREIMS *m*/*z* 322.2872 $[M]^+$ (calcd for $C_{21}H_{38}O_2$, 322.2872).

Methylation of Mixture Containing Acid (7). Excess CH_2N_2 was distilled into a solution of **7** (2 mg) in Et_2O (1 mL) at 5 °C, and the solution allowed to warm to room temperature. After 60 min, any excess CH_2N_2 was destroyed by addition of HOAc (1 drop) and the solution evaporated. Si gel HPLC did not succeed in giving the methyl ester of **7** as a pure compound. However, the ^{1}H NMR data was identical in all respects to **5**.

Reduction of Lactone (6) to Diol (11). Zinc (30 mg) was added to a solution of the lactone **6** (1 mg, 2.6 μmoL) in anhydrous Et_2O (1 mL) containing HOAc (50 μL), and the reaction mixture was stirred for 24 h at room temperature. The solution was filtered to remove the excess zinc and zinc acetate, and the solvent was removed to obtain the diol **11** (0.9 mg, 90%): IR (film) ν_{max} 3390, 2920, 2850, 2345, 1760, 1460, 1260, 1175, 1075, 940, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27 (2H, m), 7.19 (2H, m), 7.19 (1H, d, J = 6.3 Hz), 4.24 (1H, d, J = 6 Hz), 2.91 (1H, dd, J = 18, 6.3 Hz), 2.60 (1H, dd, J = 18, 1.5 Hz), 2.60 (2H, t, J = 7.8 Hz), 2.39 (1H, d, J = 14.9 Hz), 2.13 (2H, br s), 1.89 (1H, d, J = 14.9 Hz), 1.60 (4H, m), 1.48 (3H, s), 1.37 (3H, s), 1.28 (12H, m), 1.26 (2H, m).

Preparation of (*R*)- and (*S*)-MTPA Esters (12*R*) and (12*S*). To a solution of diol **11** (0.45 mg, 1.15 μmoL) in dry pyridine (500 μL, distilled over KOH) was added either the (*R*)- or the (*S*)-MTPA chloride (10 μL, 45 μmoL), and the solution was allowed to stand overnight at room temperature. MeOH (500 μL, 12 μmol) was added, and the residue obtained after evaporation of the solvent was then chromatographed on Si gel using a disposable pipet column with 50% EtOAc in hexane as eluent to obtain either the (*S*)-MTPA ester **12*S*** (0.4 mg, 57%) or the (*R*)-MTPA ester **12*R*** (0.2 mg, 29%). The $\Delta\delta$ values, where $\Delta\delta = \delta_S - \delta_R$, were measured: H2a, −0.023; H2b −0.0005; H3 +0.007; H5a +0.122; H5b +0.106; CH_3-21 +0.026; CH_3-22 +0.061.

Acknowledgment. The sponge specimens were collected by the staff of the Silliman University Marine Laboratory and by NCNPDDG participants. We thank Shirley Pomponi, Harbor Branch Oceanographic Institute, for providing a museum specimen. This research was supported by grants from the National Institutes of Health (CA 49084 and CA 67775). The Varian Inova 300 MHz NMR spectrometer was purchased using a grant from the National Science Foundation (CHE95-23507).

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1998**, *15*, 113−158, and previous reports in this series.
(2) Phillipson, D. W.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1983**, *105*, 7735−7736.
(3) Davidson, B. S. *Tetrahedron Lett.* **1991**, *32*, 7167−7170.
(4) (a) Kobayashi, M.; Kondo, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1993**, *41*, 1324−1326. (b) Sakemi, S.; Higa, T.; Anthoni, U.; Christopherson, C. *Tetrahedron* **1987**, *43*, 263−268. (c) Gunasekera, S. P.; Gunasekera, M.; Gunawardana, G. P.; McCarthy, P.; Burres, N. *J. Nat. Prod.* **1990**, *53*, 669−674. (d) Rudi, A.; Kashman, Y. *J. Nat. Prod.* **1993**, *56*, 1827−1830. (e) Ichiba, T.; Scheuer, P. J.; Kelly-Borges, M. *Tetrahedron* **1995**, *51*, 12195−12202 (erratum: *ibid*., **1996**, *52*, 14079).
(5) (a) Murayama, T.; Ohizumi, Y.; Nakamura, H.; Sasaki, T.; Kobayashi, J. *Experientia* **1989**, *45*, 898. (b) Patil, A. D.; Freyer, A. J.; Bean, M. F.; Carté, B. K.; Johnson, R. K.; Laharoute, P. *Tetrahedron* **1996**, *52*, 337−394. (c) Patil, A. D.; Freyer, A. J.; Carté, B. K.; Johnson, R. K.; Laharoute, P. *J. Nat. Prod.* **1996**, *59*, 219−223.
(6) Compagnone, R. S.; Pina, I. C.; Rangel, H. R.; Dagger, F.; Suárez, A. I.; Reddy, M. V. R.; Faulkner, D. J. *Tetrahedron* **1998**, *54*, 3057−3068.
(7) Davidson, B. S. *J. Org. Chem.* **1991**, *56*, 6722−6724.
(8) Fusetani, N.; Yasumoto, K.; Matsunaga, S.; Hirota, H. *Tetrahedron Lett.* **1989**, *30*, 6891−6894.
(9) Varoglu, M.; Peters, B. M.; Crews, P. *J. Nat. Prod.* **1995**, *58*, 27−36.
(10) Horton, P. A.; Longley, R. E.; Kelly-Borges, M.; McConnell, O. J.; Ballas, L. M. *J. Nat. Prod.* **1994**, *57*, 1374−1381.
(11) Ando, Y.; Kawabata, Y.; Narukawa, K.; Ota, T. *Fisheries Science* **1998**, *64*, 136−139, and references therein.
(12) Juagdan, E. G.; Kalidindi, R. S.; Scheuer, P. J.; Kelly-Borges, M. *Tetrahedron Lett.* **1995**, *36*, 2905−2908.

NP9802724