New Bioactive Peroxides from Marine Sponges of the Family Plakiniidae

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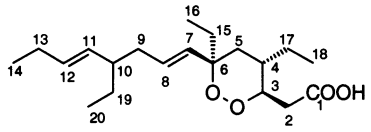
In our continuing program to identify compounds with antifungal properties, the ethanol extracts of two sponges of the family Plakinidae were found to inhibit the growth of the fungal pathogens *Candida albicans* and *Aspergillus fumigatus*. From these organisms three new compounds and five known compounds have been identified. A new 1,2-dioxane ring peroxide acid, **1**, has been isolated from the sponge *Plakortis halichondrioides* along with five known compounds. Two new 1,2-dioxolane peroxide acids, **3** and **4**, have been isolated from the sponge *Plakinastrella onkodes*. The structures were established by interpretation of spectral data. The three new compounds exhibit moderate activity against the fungal pathogen *C. albicans* with MICs of 5, 1.6, and 1.6 μg/mL respectively, for **1**, **3**, and **4**. Compound **1** also showed in vitro inhibition of the fungal pathogen *A. fumigatus* with an IC_{90} value of 5.6 μg/mL.

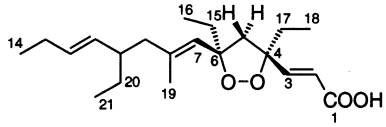
Cyclic peroxides, many of which exhibit antifungal, antibacterial, or antitumor activity, have been reported previously from a number of marine organisms, especially from sponges of the family Plakinidae.[1,2] The majority of these natural products contain 1,2-dioxanes, while a growing number possess the more rare 1,2-dioxolane ring system.[3−6]

In our continuing program to identify compounds with antifungal properties, two ethanol extracts of sponges of the family Plakinidae were found to inhibit the growth of the fungal pathogens *Candida albicans* and *Aspergillus fumigatus*. The first specimen was identified as *Plakortis halichondrioides* and was collected by hand using scuba at a depth of 25 ft of seawater (fsw) off the west coast of Long Bay, Negril, Jamaica. Bioassay-guided purification of the EtOH extract led to the isolation of five known metabolites, plakortides E,[7] F,[8] G,[8] and H[8] and 4,6-dihydroxy-8-methyl-4,6,10-triethyltetradeca-2,7,11-trienoic acid,[9] and one new 1,2-dioxane peroxide acid, **1**. The second specimen was identified as *Plakinastrella onkodes* and was collected by trawling at a depth of 217 fsw, approximately 75 nautical miles off Sanibel Island, FL, in the U.S. Gulf of Mexico. Bioassay-guided purification of the EtOH extract led to the isolation of two new 1,2-dioxolane peroxide acids, **3** and **4**. This paper reports the isolation, structure elucidation, and antifungal activity of these compounds.

Compound **1** was isolated as a colorless oil (9.4 mg, 0.021% yield of wet weight) by vacuum column chromatography of the *n*-BuOH partition on a silica gel stationary phase followed by reversed-phase HPLC of the active fractions. The ^{13}C NMR spectrum and HRFABMS suggested a molecular formula of $C_{20}H_{34}O_4Na$ for **1** ($[M + Na]^+$ *m*/*z* 361.2377 obsd, 361.2346 calcd). Inspection of the NMR spectra suggested that the compound was closely related to plakortides F−H,[8] which possess a 1,2-dioxane ring bearing an acetic acid functionality at the C-3 position, multiple ethyl substituents throughout the molecule, and an octadiene substituent at C-6. The presence of a carboxylic acid functionality is supported by the observation



1



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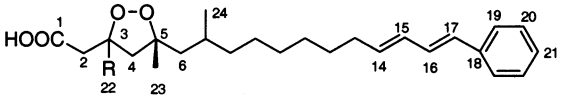
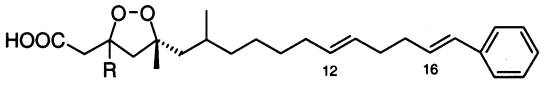


3 R= α–CH_3
4 R= β–CH3



5 R= β-CH3
6 R= α-CH3

of a ^{13}C NMR resonance at δ 175.93 (s) and an absorption at 1713 cm^{-1} in the IR spectrum. The carbons observed at δ 84.26 (s) and 81.88 (d) are characteristic of C-3 and C-6 of the 1,2-dioxane ring. The ^1H NMR spectrum (Table 1) of **1** contained signals that were typical for a 3,4,6,6-tetrasubstituted 1,2-dioxane ring similar to that found in plakortides F−H[8] and 3-epiplakortin.[10] A proton assigned as H-3 (δ 4.16, ddd, J = 9, 9, 3 Hz) was coupled with both a methine signal observed at δ 1.58 (H-4) and the methylene protons observed at δ 2.64 and 2.29 (H-2ab). H-4 was further coupled to two mutually coupled signals observed at δ 2.00 ($H\text{-}5_{eq}$) and 1.26 ($H\text{-}5_{ax}$). The presence of multiple ethyl functionalities in **1** was apparent from the observation of four methyl triplets in the ^1H NMR spectrum [δ 0.81 (t, J = 7.5 Hz), 0.83 (t, J = 7.5 Hz), 0.89 (t, J = 7.5 Hz), and 0.95 (t, J = 7.5 Hz)], all of which were coupled to methylene groups from the COSY data. The carbons observed at 130.03 (d), 132.08 (d), 132.71 (d), and 132.94

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1** and **2** in $CDCl_3$

compound **1**					compound **2**		
atom	δ_H mult (J)	δ_C (mult)	COSY[a]	HMBC[b]	atom	δ_H mult (J)	δ_C (mult)
1		175.9 (s)			1		172.0 (s)[c]
2A	2.64 dd (3, 16)	36.1 (t)	2B, 3	1, 3, 4	2	6.09 d (15)	120.5 (d)[d]
2B	2.29 dd (9, 16)		2A, 3	1, 3	3	6.93 d (15)	152.1 (d)
3	4.16 ddd (3, 9, 9)	81.9 (d)	2A, 2B, 4	1, 2, 4	4		87.2 (s)
4	1.58 m	36.8 (d)	3, 5eq, 5ax	3, 5	5	2.53 d (12)	56.0 (t)
5eq	2.00 m	36.2 (t)	4, 5ax	3, 4		2.43 d (12)	
5ax	1.26 m		4, 5eq	4, 6	6		89.3 (s)
6		84.3 (s)			7	5.10 (s)	126.6 (d)
7	5.42 d (15)	130.0 (d)	8	6, 8	8		136.7 (s)
8	5.44 dt (6.5, 15)	132.7 (d)	7, 9A, 9B	7, 9	9	1.99 m	46.6 (t)
9A	2.12 m	38.2 (t)	8, 10	7, 8		1.86 m	
9B	2.05 m		8, 10	7, 8, 10	10	1.99 m	42.6 (d)
10	1.90 m	44.5 (d)	11, 19	9, 11	11	5.04 dd (8, 15)	132.8 (d)
11	5.14 dd (8.5, 15)	132.9 (d)	10, 12	10, 13	12	5.33 dt (6.5, 15)	132.0 (d)
12	5.38 dt (6.5, 15)	132.1 (d)	11, 13	13, 14	13	1.95 m	25.6 (t)
13	1.97 2H, q (7)	25.7 (t)	12, 14	11, 12, 14	14	0.92 3H, t (7.5)	14.1 (q)
14	0.95 3H, t (7.5)	14.1 (q)	13	12, 13	15	1.86 m	32.2 (t)
15A	1.38 m	27.5 (t)	15B, 16	6, 16		1.64 m	
15B	1.18 m		15A, 16	6, 16	16	0.86 3H, t (7.5)	8.9 (q)
16	0.83 3H, t (7.5)	7.3 (q)	15A, 15B	6, 15	17	1.78 m	30.8 (t)
17A	1.46 m	24.0 (t)	17B, 18	4, 18		1.74 m	
17B	1.09 m		17A, 18	4, 18	18	0.88 3H, t (7.5)	8.9 (q)
18	0.89 3H, t (7.5)	10.5 (q)	17A, 17B	4, 17	19	1.60 3H, s	17.8 (q)
19A	1.46 m	33.0 (t)	19B, 20	10, 20	20	1.37 m	27.7 (t)
19B	1.26 m		19A, 20	10, 20		1.24 m	
20	0.81 3H, t (7.5)	11.6 (q)	19A, 19B	10, 19	21	0.80 3H, t (7.5)	11.6 (q)

[a] Correlations are from the atom number to the proton resonances listed. [b] Correlations are from the proton designated under atom to the carbons listed. [c] Not observed in ^{13}C NMR, but by correlation in the HMBC spectrum. [d] Assignment and multiplicity based on HMQC.

(d) in the ^{13}C spectrum suggested the presence of two disubstituted olefins. The corresponding ^{1}H NMR resonances [δ 5.42 (d, 1H, J = 15 Hz), 5.44 (dt, 1H, J = 15, 6.5 Hz), 5.38 (dt, 1H, J = 6.5, 15 Hz), and 5.14 (dd, 1H, J = 15, 8.5 Hz)] confirmed the presence of the olefin functionality. The presence of a 4-ethyl-1,5-octadiene substituent could be readily defined from the COSY spectrum. Both double bonds of the 4-ethyl-1,5-octadiene were assigned as *trans* based upon the ^{1}H−^{1}H scalar coupling constants (J = 15 Hz for both double bonds). The planar structure of **1** was fully established by interpretation of the 2D NMR data (^{1}H−^{1}H COSY, HMQC, and HMBC, Table 1).

Axial conformation was assigned for both H-3 (δ 4.16) and H-4 (δ 1.58) on the basis of a mutual coupling of 9 Hz observed between these protons. The NOESY spectrum of **1** showed strong correlations between H-3 and the H-5 proton observed at δ 1.26, suggesting that the latter resonance is H-5$_{ax}$. H-5$_{ax}$ in turn showed correlations to H-15A (δ 1.38) and H-17B (δ 1.09), which are methylene protons of each of the ethyl substituents on the 1,2-dioxane ring. Strong correlations observed between H-4 and H-2B (δ 2.29), H-7 (δ 5.42), and H-5$_{eq}$ (δ 2.00) suggested that these protons are on the same face of the ring. The H-4 to H-7 correlation suggests an axial orientation for the 4-ethyl-1,5-octadiene side chain.

Peroxide acid **2** was also isolated from *P. halichondrioides* and was identified as plakortide E by interpretation of the NMR data (Table 1), including 2D NMR (COSY, HMQC, HMBC, and NOESY). This compound was first reported from the same species collected in Jamaica.[7] The majority of the NMR data observed for this isolation of the compound was identical to that reported; however, a chemical shift difference at C-3 was observed in both the ^{1}H and ^{13}C NMR spectra, although they were both measured in $CDCl_3$. In the published report the proton and carbon were observed at δ 6.69 (d, J = 15.8 Hz) and 146.9 respectively, while in **2** these resonances were shifted downfield to δ 6.93 (d, J = 15 Hz) and 152.07. In addition, the published data reports H-2 as a sharp doublet observed at δ 6.09. In the ^{1}H NMR spectrum of **2**, this resonance was observed as a very broad doublet. Additionally, in **2**, the carbonyl resonance was not directly observed but was inferred by observation of a long-range correlation in the HMBC spectrum between H-3 and a carbon observed at δ 172.05. Both the Δ2 and Δ11 double bonds were assigned as *E* stereochemistry on the basis of the scalar coupling constants (J = 15 Hz in both cases). The Δ7 olefin was also assigned as *E* on the basis of NOESY correlations observed between H-7 and H$_2$-9. The NOESY data recorded for **2** was identical to that reported for plakortide E, indicating the same relative stereochemistry of the ring substituents.[7] The optical rotation of **2** was observed to be $[\alpha]^{24}_D$ +63° (*c* 0.001, $CHCl_3$), suggesting that it is the same stereoisomer as that reported previously. The isolation procedures used in both instances were similar, and it is difficult to explain the observed differences in NMR chemical shifts for **2**. The broadness of the peaks suggests that some form of tautomerism is occurring, and it is possible that this isolation is of the sodium or other salt.

Compounds **3** and **4** were obtained as colorless oils from *P. onkodes* by chromatography of the EtOAc partition using vacuum flash column chromatography on silica gel followed by reversed-phase HPLC of the antifungal active fractions. Their isomeric relationship was evident from the mass spectral data, which indicated a molecular formula of $C_{26}H_{38}O_4$ by HRFABMS (*m/z* 414.2761 and 414.2770 found, respectively; 414.2771 calcd), and also from their nearly identical NMR spectra. The ^{1}H NMR, ^{13}C NMR, and DEPT spectra were reminiscent of the corresponding spectral signals of plakinic acid C (**5**) and epiplakinic acid C (**6**), isolated from a *Plakortis* sp. collected in the Fiji Islands.[3] The NMR data of compounds **3** and **4** (Table 2) were consistent with a 1,2-dioxolane bearing two methyl groups at C-3 and C-5 and an acetic acid group at C-3. Their NMR (Table 2) also contained signals for a monosubstituted benzene ring, two *trans* disubstituted double bonds, and a

Table 2. ^{1}H and ^{13}C NMR Data for Compounds **3** and **4** in $CDCl_3$

	compound **3**				compound **4**			
atom	δ_H mult (*J*)	δ_C (mult)	COSY[b]	HMBC[c]	δ_H mult (*J*)	δ_C (mult)	COSY[b]	HMBC[c]
1		173.2 (s)		2		171.2 (s)		2
2	2.73 2H, s	43.6 (t)		4A, 4B, 22	2.72 2H, s	43.8 (t)		4A, 4B, 22
3		83.6 (s)		2, 4A, 4B, 22		83.8 (s)		2, 4A, 4B, 22
4A	2.45 d (12.5)	57.3 (t)	4B	2, 22, 23	2.46 d (12.5)	57.4 (t)	4B	2, 22, 23
4B	2.21 d (12.5)		4A		2.20 d (12.5)		4A	
5		87.1 (s)		4A, 4B, 23		87.3 (s)		4A, 4B, 23
6	1.60 m	46.4 (t)	7	4A, 4B, 23, 24	1.64 m	45.9 (t)	7	4A, 4B, 23, 24
7	1.58 m	29.6 (d)	6, 24	6, 24	1.60 m	29.6 (d)	6, 24	6, 24
8A	1.25 m	38.66 (t)	8B	24	1.26 m	38.1 (t)	8B	24
8B	1.14 m		8A, 9		1.14 m		8A, 9	
9	1.25 m	27.06 (t)			1.26 m	27.0 (t)		
10	1.25 m	29.26 (t)[a]			1.26 m	29.2 (t)[a]		
11	1.25 m	29.36 (t)[a]		9, 13	1.26 m	29.3 (t)[a]		9, 13
12	1.40 m	29.76 (t)[a]	11, 13	10, 13	1.40 m	29.7 (t)[a]	11, 13	10, 13
13	2.12 2H, q (7)	32.9 (t)	14	12, 14, 15	2.13 2H, m	32.9 (t)	14	12, 14, 15
14	5.81 dt (15, 7)	135.9 (d)	13, 15	13, 16	5.80 dt (15, 7)	135.9 (d)	13, 15	13, 16
15	6.18 dd (15, 10)	130.5 (d)	14, 16	13, 17	6.18 dd (15, 10)	130.5 (d)	14, 16	13, 17
16	6.72 dd (15.5, 10)	129.5 (d)	15, 17	14	6.73 dd (15.5, 10)	129.5 (d)	15, 17	14
17	6.42 d (15.5)	130.0 (d)	16	15, 19	6.42 d (15.5)	130.0 (d)	16	15, 19
18		137.7 (s)		16, 20		137.7 (s)		16, 20
19	7.34 2H, d (7.8)	126.1 (d)	20	17, 19, 21	7.35 2H, d (7.8)	126.1 (d)	20	17, 19, 21
20	7.27 2H, t (7.5)	128.5 (d)	19, 21	20	7.27 2H, t (7.5)	128.6 (d)	19, 21	20
21	7.16 t (7.0)	127.1 (d)	20	19	7.16 t (7.0)	127.1 (d)	20	19
22	1.45 3H, s	23.9 (q)		2, 4B	1.46 3H, s	23.5 (q)		2, 4B
23	1.31 3H, s	23.3 (q)		4A, 6	1.35 3H, s	24.6 (q)		4A, 6
24	0.93 3H, d (6.2)	21.0 (q)	7		0.90 3H, d (6.2)	20.9 (q)	7	

[a] Assignments interchangeable. [b] Correlations are from the atom number to the proton resonances listed. [c] Correlations are from the carbon designated under atom to the protons listed.

branching methyl group. Comparison of this spectroscopic data with those described for plakinic acid C and epiplakinic acid C together with a complete 2D NMR study (COSY, HMQC, HMBC) allowed the assignment of all NMR resonances. These data indicated that the benzene ring and the two *trans* double bonds were conjugated in both compounds **3** and **4**. NOESY experiments showed correlations nearly identical to those reported for plakinic acid C and epiplakinic acid C and confirmed an epimeric relationship between **3** and **4**. For compound **3**, H-2 showed strong dipolar coupling to H-22, H-23, and H-4A (δ 2.45). H-4A in turn showed strong dipolar coupling to H-23. H-4B (δ 2.21) showed strong dipolar coupling to H-22 and H-6. All of these couplings are consistent with a *trans* relative stereochemistry for **3**. For compound **4**, a *cis* relative stereochemistry was suggested by the following NOESY data. H-2 showed dipolar couplings to H-4A (δ 2.46) and H-22. H-4A in turn showed dipolar couplings to H-6 (δ 1.64) and H-22. H-4B showed dipolar couplings to both H-22 and H-23. In both compounds, the H-4 proton that is *cis* to the acetic acid side chain appears downfield from the proton *trans* to the acetic acid. As with plakinic and epiplakinic acids C, the assignment of C-3 as the epimeric center is arbitrary.

All three new compounds **1**, **3**, and **4** exhibited moderate antifungal activity against *C. albicans*: Compound **1** gave an MIC of 5 μg/mL; compounds **3** and **4** both gave MICs of 1.6 μg/mL. Compound **1** also showed moderate in vitro inhibition of *A. fumigatus* with an IC_{90} of 5.6 μg/mL.

Experimental Section

General Experimental Procedures. The IR spectra were collected on a Midac M-1200 with Galactic GRAMS/386 software. The ^{1}H COSY and NOESY and the ^{13}C, DEPT90, DEPT135, HMQC, and HMBC (optimized for 10 Hz) spectra were recorded on a Bruker AMX-500 operating at 500 MHz (^{1}H) and 125 MHz (^{13}C). ^{1}H chemical shifts are referenced to $CDCl_3$ observed at 7.24 ppm, while ^{13}C chemical shifts are referenced to $CDCl_3$ observed at 77.0 ppm. The high-resolution FAB mass spectra were obtained on a Finnigan MAT 95 mass spectrometer at the University of Minnesota, Minneapolis, MN.

Animal Material. The specimen of *Plakortis halichondrioides*[11] (phylum Porifera, class Demospongiae, order Homosclerophorida, family Plakinidae) was collected by scuba at a depth of 25 ft off Long Bay, Negril, Jamaica (latitude 18°17.25′ N, longitude 78°22.10′ W). The sponge was thickly encrusting. Color in life was black, both externally and internally. The surface of the sponge was smooth, and the consistency was firm and dense. Spicules are diods. A taxonomic reference specimen is deposited at the Harbor Branch Oceanographic Museum (HBOM catalog number 003:00968, sample number 22-VIII-93-1-002). The specimen of *Plakinastrella onkodes*[11] (class Demospongiae, order Homosclerophorida, family Plakinidae) was collected by trawling at a depth of 217 ft in the U.S. Gulf of Mexico, 0.75 nautical miles SW of Sanibel Island, FL (latitude 26°18.096′ N, longitude 83°34.682′ W). The sponge was a cluster of lobes with apical oscules. Color in life was gray externally and tan internally. The consistency was firm. Spicules are calthrops and diods in at least three size categories. A taxonomic reference specimen is deposited at the Harbor Branch Oceanographic Museum (HBOM catalog number 003:00967, sample number 10-VIII-95-3-004). Both samples were frozen immediately after collection and kept frozen until used.

Extraction and Purification. A frozen sample of *P. halichondrioides* (50 g wet wt) was diced and exhaustively extracted with EtOH (5 × 200 mL). The crude extract was concentrated to dryness by distillation under reduced pressure to yield 2.57 g of a crude oil, which was partitioned between 1:1 EtOAc/H_2O. The aqueous phase was further partitioned between *n*-BuOH and H_2O. The *n*-BuOH partition was concentrated to dryness (536 mg) and was further purified via vacuum flash column chromatography on a Si gel (Kieselgel 60H) stationary phase using a step gradient of heptane and heptane/EtOAc as eluent. Fractions 3 (118.5 mg) and 4 (40 mg), which eluted with heptane/EtOAc (6:4 v/v) and heptane/EtOAc (4:6 v/v), respectively, were responsible for the antifungal activity of the sponge. These fractions were further purified by reversed-phase HPLC (Vydac Protein and Peptide C18 column, 300 Å pore size, 10 × 250 mm, gradient elution,

flow rate = 3 mL/min; solvent A CH_3CN-H_2O (1:9 v/v); solvent B CH_3CN; $t = 0$, A:B (7:3 v/v); $t = 45$, 100% B, hold at 100% B for 5 min; detected by UV absorption at 210 nm). From 35 mg of fraction 4, 9.4 mg of compound **1** was purified; four known compounds, plakortides E (**2**), F, G, and H, were isolated from 90 mg of fraction 3; another known compound, 4,6-dihydroxy-8-methyl-4,6,10-triethyltetradeca-2,7,11-trienoic acid, was purified from fraction 2.

A frozen specimen of *P. onkodes* (50 g wet wt) was diced and extracted exhaustively with EtOH (5 × 200 mL). The crude extract was concentrated to dryness by distillation under reduced pressure to yield 2.76 g of an oil residue. This residue was successively partitioned between EtOAc/H_2O (1:1). The EtOAc partition was concentrated to dryness (262.6 mg) and was further purified via vacuum flash column chromatography on a Si gel (Kieselgel 60H) stationary phase using a step gradient of heptane and heptane/EtOAc as eluent. The fractions 4 (9.6 mg) and 5 (6.8 mg), which eluted with heptane/EtOAc (1:1) and heptane/EtOAc (4:6), respectively, were responsible for the antifungal activity of the sponge. These were further purified by reversed-phase HPLC (Vydac Protein and Peptide C_{18} column, 300 Å pore size, 10 × 250 mm, gradient elution, flow rate = 3 mL/min; solvent A CH_3CN-H_2O (5:95 v/v); solvent B 100% CH_3CN; $t = 0$, A:B (8:2 v/v), $t = 15$ A:B (8:2 v/v), $t = 25$, 100% B, hold at 100% B for 5 min; detected by UV absorption at 254 nm). Compound **3** (1.6 mg, 0.0032% of wet weight) and compound **4** (0.8 mg, 0.0016% of wet weight) were purified from fractions 5 and 4, respectively.

Bioassay. Minimum inhibitory concentrations (MICs) were determined for *C. albicans* by a standard microdilution broth method using buffered RPMI[12] (RPMI-1640, 0.165 M MOPS, pH 7.0) as growth medium. The MIC for the control drug, 5-fluorocytosine, was 1.62 μg/mL. The MIC is defined as the lowest test concentration of drug giving complete inhibition of growth. IC_{90}'s against *A. fumigatus* were determined by standard microdilution broth method using buffered RPMI media (RPMI-1640, 0.165 M MOPS, pH 7.0).[13] Amphotericin B has an IC_{90} of 1 μg/mL against *A. fumigatus* in this system. The IC_{90} is defined as the concentration of drug giving 90% inhibition of growth.

Compound 1: colorless oil, 9.4 mg (0.21% of wet weight); $[\alpha]^{24}_D$ −78.5° (*c* 0.0017, $CHCL_3$); IR ($CHCl_3$) ν_{max} 2962, 2911, 2866, 1713, 1456, 968, 670 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS (MNBA) *m/z* 361.2377 $[M + Na]^+$ (calcd for $C_{20}H_{34}O_4Na$ 361.2346).

Compound 3: colorless oil,[14] 1.6 mg (0.0032% of wet weight); IR ($CHCl_3$) ν_{max} 2930, 2853, 1716, 1702, 1684, 1654, 1635, 1559, 1539, 1507, 1457, 1419, 1374, 1204, 972, 670 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFABMS *m/z* 414.2761 $[M]^+$ (calcd for $C_{26}H_{38}O_4$ 414.2771).

Compound 4: colorless oil,[14] 0.8 mg (0.0016% of wet weight); IR ($CHCl_3$) ν_{max} 2932, 2853, 1716, 1698, 1682, 1652, 1646, 1576, 1558, 1540, 1507, 1457, 1418, 1395, 1204, 972, 670 cm^{-1}; ^1H and ^{13}C NMR, see Table 2;. HRFABMS *m/z* 414.2770 $[M]^+$ (calcd for $C_{26}H_{38}O_4$ 414.2771).

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References and Notes

(1) Faulkner, D. *J. Nat. Prod. Rep*. **2001**, *18*, 1−49, and previous reports in this series.
(2) Casteel, D. A. *Nat. Prod. Rep*. **1999**, *16*, 55−73.
(3) Davidson, B. S. *J. Org. Chem*. **1991**, *56*, 6722−6724.
(4) Qureshi, A.; Salvá J.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod*. **1998**, *61*, 1539−1542
(5) Horton, P. A.; Longley, R. E.; Kelly-Borges, M. K.; McConnell, O. J.; Ballas, L M. *J. Nat. Prod*. **1994**, *57*, 1374−1381.
(6) Chen, Y.; Killday, K. B.; McCarthy, P. J.; Schimoler, R.; Chilson, K.; Selitrennikoff, C.; Pomponi, S. A.; Wright A. E *J. Nat. Prod*. **2001**, *64*, 262−264.
(7) Patil, A. D.; Freyer, A. J.; Bean, M. F.; Carté, B. K.; Westley, J. W.; Johnson, R. K. *Tetrahedron* **1996**, *52*, 337−394.
(8) Patil, A. D.; Freyer, A. J.; Carté, B. K.; Johnson, R. K.; Lahouratate, P. *J. Nat. Prod*. **1996**, *59*, 219−223.
(9) Rudi, A.; Kashman, Y. *J. Nat. Prod*. **1993**, *56*, 1827−1830.
(10) Stierle, D. B.; Faulkner, D. J. *J. Org. Chem*. **1980**, *45*, 3396−3401.
(11) Diaz, M. C.; van Soest R. W. M. In *Sponges in Time and Space*; van Soest, van Kempen, Braekman, Eds.; Balkema Press: Rotterdam, 1994; pp 93−109.
(12) Jones, R. N.; Barry, D. A.; Gavan, T. L.; Washington, J. A., II. In *Manual of Clinic Microbiology*, 4th ed.; Lennette, E. H., Balows, A., Hausler, W. J., Shadomy, H. J., Eds.; American Society for Microbiology: Washington, DC, 1985; p 972.
(13) Galgiani, J.; Bartlett, M.; Ghannoum, M.; Espinel-Ingroff, A.; Lancaster, A.; Odds, F. *NCCLS* **1995**, *15*, 1−29.
(14) Compounds **3** and **4** decomposed before optical rotations could be measured.

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