New Cytotoxic Peroxylactones from the Marine Sponge, Plakinastrella onkodes

Paul A. Horton, Ross E. Longley, Michelle Kelly-Borges, Oliver J. McConnell, and Larry M. Ballas

J. Nat. Prod., **1994**, 57 (10), 1374-1381• DOI: 10.1021/np50112a006 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 10, pp. 1374-1381, October 1994

NEW CYTOTOXIC PEROXYLACTONES FROM THE MARINE SPONGE, *PLAKINASTRELLA ONKODES*

PAUL A. HORTON, ROSS E. LONGLEY, MICHELLE KELLY-BORGES, OLIVER J. MCCONNELL,*

Division of Biomedical Marine Research, Harbor Branch Oceanographic Institution, Inc., 5600 U.S. Highway 1 North, Ft. Pierce, Florida 34946

and LARRY M. BALLAS

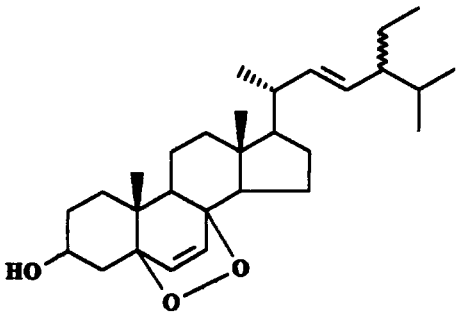
Sphinx Pharmaceuticals Corporation, Two University Place, Post Office Box 52330, Durham, North Carolina 27717

ABSTRACT.—Three new peroxylactones, plakortolides B [**6**], C [**7**], and D [**8**], and a new peroxy ester, epiplakinic acid E methyl ester [**9**], were isolated and characterized from a previously unstudied marine sponge, *Plakinastrella onkodes*. A mixture of steroidal peroxides was also found in this organism. Plakortolides B [**6**] and D [**8**], and epiplakinic acid E methyl ester [**9**], were evaluated for biological activity and found to show cytotoxicity against the A549 human lung carcinoma and P388 murine leukemia cell lines, and to effect adhesion in an assay employing the EL-4.IL-2 cell line, which correlates with signal transduction activity.

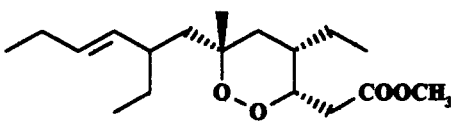
During our ongoing investigation of sessile marine organisms for new cytotoxic compounds (1–3), an extract from the marine sponge *Plakinastrella onkodes* (family Plakinidae) (4) showed cytotoxicity in whole-cell assays against both A549 non-small cell lung carcinoma and P388 murine leukemia cell lines and induced cell adhesion in the EL-4.IL-2 adherence assay (5). Although the natural products chemistry of *Plakinastrella* has not been studied previously, this genus is taxonomically related to representatives in the family Plakinidae that have yielded a wide variety of peroxide-containing metabolites (6–20), including steroidal peroxides, such as **1** (21,22), and polyketide peroxides, inclusive of the cyclic peroxides plakortin [**2**] from *Plakortis halichondroides* (12) and chondrillin [**3**] from *Plakortis lita* (16). In several of these compounds, the cyclic peroxide is linked to an aromatic ring via an aliphatic chain, as in plakinic acid B [**4**] (15) and the peroxylactone plakortolide [**5**] (20). The latter compounds are characterized by two sites of unsaturation in the aliphatic chain. We wish to report herein the isolation and structure elucidation of three new peroxylactones from *Plakinastrella onkodes*, plakortolides B [**6**], C [**7**], and D [**8**], the peroxy ester epiplakinic acid E methyl ester [**9**], and a mixture of steroidal peroxides, inclusive of **1**.

RESULTS AND DISCUSSION

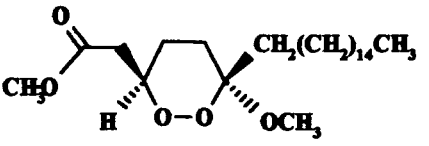
A specimen of the sponge was collected by trawling in the Gulf of Mexico. A portion of the crude EtOH extract from this specimen was partitioned between EtOAc and H_2O;



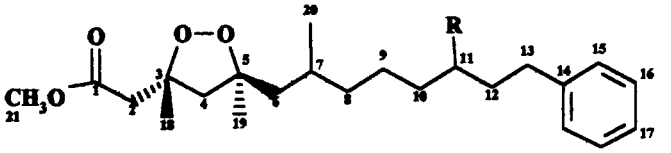
1



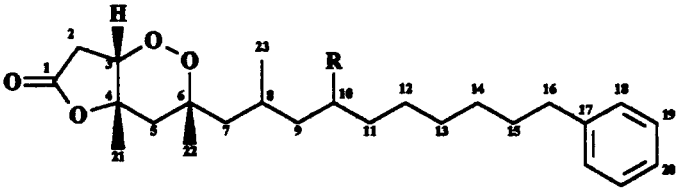
2



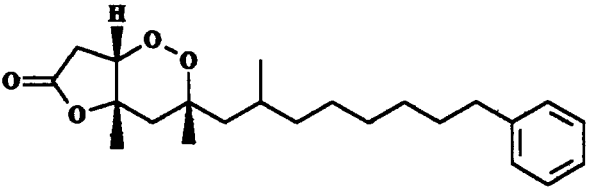
3



4 R=CH_3, $\Delta^{8,12}$
9 R=H



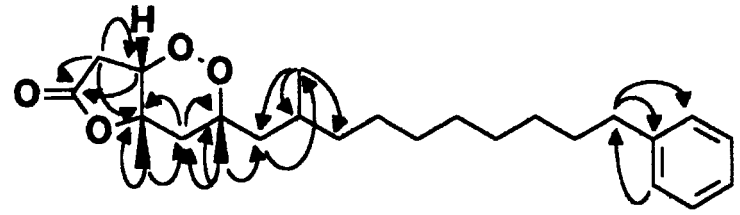
5 R=H, $\Delta^{11,15}$
6 R=H
8 R=CH_3



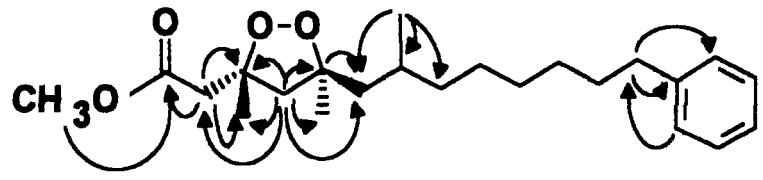
7

the organic phase was then successively partitioned between equal volumes of aqueous MeOH (% adjusted to give a biphasic solution) and a solvent series of hexane and CH_2Cl_2. Assay results against the A549 and P388 cancer cell lines revealed that cytotoxicity was concentrated in the hexane partition. Using biological activity as a guide, the hexane layer was purified further by vlc (Si gel) and then hplc (both silica and RP C_{18} columns) to yield the pure compounds **6–9** and a mixture including **1**.

The molecular formula of plakortolide B [**6**] was deduced as $C_{25}H_{38}O_4$ from high-resolution lsims (*m/z* 403.2887 $(M+H)^+$, Δ 3.9 mmu), which requires seven degrees of unsaturation. The proton-decoupled ^{13}C-nmr spectrum clearly exhibited 23 carbon resonances, two of which represented the degenerate positions of a monosubstituted benzene ring (δ 128.2 and 128.4). The ^{13}C-nmr and DEPT (23) spectra of **6** also contained signals that were attributed to a carbonyl (δ 174.1), three methyl groups (δ 21.0, 24.9, and 25.9), two oxygenated quaternary carbons (δ 82.8 and 80.8), one oxygenated methine carbon (δ 81.0), one aliphatic methine, and ten methylene carbons. The ir spectrum of **6** showed a strong absorbance at 1777 cm^{-1} suggesting the presence of a γ-lactone. These data account for six of the seven degrees of unsaturation, therefore **6** must possess an additional ring. Correlations present in the COSY (24) and HMQC



6



9

H ⟶ C

FIGURE 1. Key HMBC nmr correlations for plakortolide B [**6**] and plakinic acid E methyl ester [**9**].

(25) nmr spectra of **6** led to the identification of several substructures which were connected after analyzing cross-peak connectivities in an HMBC nmr experiment (26) and by comparing our data with previously reported values for plakortolide [**5**] (20) to yield the completed structure **6** (HMBC correlations are shown in Figure 1).

By analogy to the structure elucidation of plakortolide [**5**] (19,20), the relative stereochemistry of the peroxylactone ring system in **6** was deduced using a combination of data from ^{1}H- and proton-decoupled ^{13}C-nmr, and nOe difference (27) experiments; the relative stereochemistry for **6** is the same as reported for plakortolide [**5**] (20).

The ^{1}H- and proton decoupled ^{13}C-nmr spectra of plakortolide C [**7**] were virtually identical to those of plakortolide B [**6**] with the notable exception of two fewer methylene carbons in the ^{13}C-nmr and DEPT spectra of **7**. The ir spectra were also similar; a strong absorbance at 1777 cm^{-1} was observed in the ir spectrum of **7**, signifying a γ-lactone. These data indicated that plakortolide C [**7**] is a smaller homologue of plakortolide B [**6**]. The expected molecular formula of **7**, $C_{23}H_{34}O_4$, was confirmed by high-resolution lsims (*m/z* 375.2442 $(M+H)^+$, Δ −8.4 mmu).

The nmr spectra of plakortolide D [**8**] contained ^{13}C- and ^{1}H-nmr resonances characteristic of the bicyclic peroxylactone and aromatic groups of **6** and **7**, and much of the aliphatic chain of **6** (compare nmr signals for **6** and **8** associated with C-1–C-7 and C-11–C-22 in Tables 1 and 2). Additional evidence for the γ-lactone was obtained from the ir spectrum which exhibited a strong absorption at 1781 cm^{-1}. The aliphatic chain in **8** differed slightly from the corresponding chain found in plakortolide B [**6**] by the existence of an additional methyl group, which was substantiated by a molecular formula of $C_{26}H_{40}O_4$ from high-resolution lsims (*m/z* 417.3007 $(M+H)^+$, Δ 0.1 mmu). The HMBC nmr spectrum proved especially useful in positioning the additional methyl group (Me-24); correlations were observed from Me-23 to C-7, C-8, and C-9, and from Me-24 to C-9, C-10, and C-11. These correlations were used to place Me-24 on C-10; this placement is corroborated by significant downfield ^{13}C-nmr chemical shifts observed for C-9 and C-10 relative to the analogous chemical shifts in plakortolide B [**6**] (Table 2). The relative stereochemistry of plakortolide C [**7**] and plakortolide D [**8**] was

TABLE 1. ^{1}H-Nmr Chemical Shifts of Compounds **6–9**.[a,b]

Position	**6**	**7**	**8**	**9**
	Attached ^{1}H δ[c]	Attached ^{1}H δ	Attached ^{1}H δ	Attached ^{1}H δ
1				
2	2.90 (dd, J=18.6, 6.1)	2.90 (dd, J=18.6, 6.1)	2.89 (dd, J=18.8, 6.2)	2.75 (d, J=14.4)
	2.60 (d, J=18.6)	2.60 (d, J=18.6)	2.60 (d, J=18.8)	2.50 (d, J=14.4)
3	4.45 (d, J=6.1)	4.45 (d, J=6.1)	4.46 (d, J=5.9)	
4				2.50 (d, J=12.5)
				2.22 (d, J=12.5)
5	2.22 (d, J=14.9)	2.22 (d, J=14.9)	2.21 (d, J=15.0)	
	1.70 (d, J=14.8)	1.70 (d, J=14.8)	1.71 (d, J=14.9)	
6				1.76 (dd, J=14.0, 5.3)
				1.36 (dd, J=14.0, 5.9)
7	1.72 (dd, J=14.3, 8.3)	1.72 (dd, J=14.3, 8.3)	1.71 (dd, J=14.8, 3.3)	1.57 (m)
	1.46 (dd, J=14.4, 3.1)	1.46 (dd, J=14.4, 3.1)	1.42 (dd, J=14.8, 2.3)	
8	1.60 (m)	1.60 (m)	1.61 (m)	1.33 (m)
9	1.25 (m)	1.25 (m)	1.20 (m)	1.35 (m)
10	1.56 (m)	1.56 (m)	1.37 (m)	1.59 (m)
11	1.30 (m)	1.30 (m)	1.30 (m)	1.28 (m)
12	1.30 (m)	1.56 (m)	1.30 (m)	1.61 (m)
13	1.56 (m)	1.61 (m)	1.53 (m)	2.60 (t, J=8.1)
14	1.56 (m)	2.61 (t, J=7.9)	1.56 (m)	
15	1.61 (m)		1.61 (m)	7.17 (d, J=7.0)
16	2.60 (t, J=8.0)	7.17 (d, J=7.3)	2.60 (t, J=8.1)	7.27 (m)
17		7.27 (m)		7.17 (m)
18	7.17 (d, J=7.3)	7.17 (m)	7.17 (d, J=7.5)	1.43 (s)
19	7.27 (m)	1.37 (s)	7.27 (m)	1.31 (s)
20	7.17 (m)	1.25 (s)	7.17 (m)	0.91 (d, J=6.7)
21	1.37 (s)	0.91 (d, J=6.6)	1.37 (s)	3.69 (s)
22	1.25 (s)		1.27 (s)	
23	0.91 (d, J=6.6)		0.91 (d, J=6.1)	
24			0.83 (d, J=6.6)	

[a]Spectra recorded at 360 MHz in $CDCl_3$, referenced to residual $CHCl_3$ (7.26 ppm).
[b]Coupling constants (J) in Hz.
[c]^{1}H-^{13}C connectivities assigned by HMQC nmr.

assigned based on similarities in ^{1}H- and ^{13}C-nmr chemical shifts with plakortolide B [**6**].

The molecular formula of epiplakinic acid E methyl ester [**9**] was determined to be $C_{23}H_{36}O_4$ by high-resolution lsims (*m/z* 377.2696 $(M+H)^+$, Δ 1.4 mmu), and this formula requires six degrees of unsaturation. The proton decoupled ^{13}C-nmr and DEPT spectra of **9** contained 21 distinct carbon resonances (Table 2); based on the relative size of the signals, two of the methine resonances (δ 128.2 and 128.4) represent two carbons each, suggesting a monosubstituted benzene ring. Although many similarities were observed between the nmr spectra of **9** and **6–8**, especially in the aromatic region, a strong absorption of 1740 cm^{-1} in the ir spectrum suggested that the carbonyl is an aliphatic ester and not a γ-lactone; further, nmr and DEPT data [δ 171.0 (s), 3.69 (s)/51.7 (q)] indicated that the ester is a methyl ester. The ester functionality along with a

TABLE 2. ^{13}C-Nmr Chemical Shifts of Compounds **6–9**.[a,b]

Position	Compound			
	6	**7**	**8**	**9**
1	174.1 (s)	174.0 (s)	174.1 (s)	171.0 (s)
2	34.2 (t)	34.2 (t)	34.2 (t)	43.9 (t)
3	81.0 (d)	81.0 (d)	81.1 (d)	83.8 (s)
4	82.8 (s)	82.7 (s)	82.8 (s)	56.5 (t)
5	42.1 (t)	42.0 (t)	42.3 (t)	86.8 (s)
6	80.8 (s)	80.8 (s)	80.9 (s)	46.3 (t)
7	44.5 (t)	44.4 (t)	44.4 (t)	29.4 (d)
8	28.9 (d)	28.9 (d)	26.3 (d)	37.9 (t)
9	38.9 (t)	38.9 (t)	46.9 (t)	26.8 (t)
10	27.0 (t)	27.0 (t)	36.8 (d)	29.7 (t)
11	29.8 (t)	29.7 (t)	30.0 (t)	29.4 (t)
12	29.6 (t)	29.3 (t)	29.9 (t)	31.5 (t)
13	29.5 (t)	31.5 (t)	29.6 (t)	36.0 (t)
14	29.3 (t)	36.0 (t)	29.4 (t)	142.9 (s)
15	31.5 (t)	142.9 (s)	31.5 (t)	128.2 (d)[c]
16	36.0 (t)	128.4 (d)[c]	36.0 (t)	128.4 (d)[c]
17	143.0 (s)	128.2 (d)[c]	143.0 (s)	125.5 (d)
18	128.4 (d)[c]	125.5 (d)	128.4 (d)[c]	24.4 (q)
19	128.2 (d)[c]	25.9 (q)	128.2 (d)[c]	23.9 (q)
20	125.5 (d)	24.9 (q)	125.5 (d)	21.3 (q)
21	25.9 (q)	21.0 (q)	25.9 (q)	51.7 (q)
22	24.9 (q)		24.9 (q)	
23	21.0 (q)		21.6 (q)	
24			20.1 (q)	

[a]^{13}C-Nmr chemical shifts are reported as δ values in ppm relative to $CDCl_3$ (77.0 ppm).
[b]Multiplicities were determined by DEPT sequence.
[c]Signals represent two degenerate carbons.

monosubstituted benzene ring accounted for five of the six degrees of unsaturation suggesting that **9** contains an additional ring.

The remaining oxygens in **9** must be linked via a peroxide because an absorption in the ir spectrum for a hydroxyl functionality was not observed and only two oxygenated carbon signals remained in the proton-decoupled ^{13}C-nmr and DEPT spectra of **9** [δ 83.8 (s) and 86.8 (s)]. Correlations observed in the COSY and HMQC nmr spectra coupled with signals observed in ^{1}H- and ^{13}C-nmr spectra allowed for the generation of several substructures, which could then be connected through interpretation of HMBC nmr correlations (Figure 1) to produce the complete structure of plakinic acid E methyl ester [**9**], which, in contrast to **6–8**, contains a five-membered rather than a six-membered peroxide ring.

Analysis of correlations observed in a NOESY nmr spectrum (28) led to an assignment of the relative stereochemistry of the peroxide ring in compound **9**. Cross-peak connectivities could be seen from H-2 and H-2′ to Me-18, from Me-18 to H-4′, and from H-4′ to H-6 and H-6′ (diastereotopic hydrogen resonances assigned H′ were high-field relative to H in the ^{1}H-nmr spectrum). This must mean that Me-18, H-4′ and C-6 share a cis-relative orientation. This geometry was further confirmed by the NOESY nmr correlation observed between H-4 and Me-19. Also significant was the lack of an observed cross-peak connectivity from Me-18 to H-6 or H-6′. Based upon examination of a Dreiding model, these data suggest that Me-18 and C-6 are pseudo-equatorial, while H-4′ adopts a pseudo-axial orientation. This trans orientation is consistent with that reported for epiplakinic acids C and D (19).

During the chromatography that yielded **6–9**, a vlc Si gel fraction was obtained that appeared homogeneous by Si gel hplc; however, it yielded greater than ten components by reversed-phase hplc. The major component was identified as **1** by characteristic nmr and mass spectral data, i.e., ^{1}H-nmr signals at δ 3.9 (heptet), 6.0 (doublet), and 6.4 (doublet), ^{13}C-nmr signals at δ 67, 79, 82, 130, 131, 135, and 138, and lreims fragments at *m/z* 442 (M^+) and 410 ($M^+ - O_2$), which are identical to published values (21,22).

Plakortolides B [**6**] and D [**8**], and epiplakinic acid methyl ester [**9**], were evaluated for biological activity and all three were found to show cytotoxicity against the A549 human lung carcinoma and P388 murine leukemia cell lines. For purposes of comparison, the biological activity of chondrillin [**3**] (5) is also provided in Table 3.

TABLE 3. Observed Biological Activities for Compounds **3**, **6**, **8**, and **9**.

Compound	Cell Line		
	A549 IC_{50} (μg/ml)	P388 IC_{50} (μg/ml)	EL-4 IC_{50} (μg/ml)
3	0.3	2.4	0.4 (induction)
6	1.3	0.4	4.4 (induction)
8	3.8	0.8	15.8 (inhibition)
9	2.0	2.5	4.6 (inhibition)

Plakortolide B [**6**] induced cell adhesion in the EL-4.IL-2 cell line, which corresponded to very modest agonistic activity against a suite of protein kinase C isoenzymes (29,30) (activity at 50 μg/ml: alpha −19%, beta I −13%, beta II −27%, delta −9%, epsilon −38%, and gamma −9%). In contrast, chondrillin [**3**] induced cell adhesion in the EL-4.IL-2 cell line but expressed modest antagonistic activity against the PKC isoenzymes (IC_{50} values (μg/ml): alpha +36, beta I +49, beta II +49, delta +23, epsilon +30, gamma >150, and zeta +43). Neither plakortolide D [**8**] nor epiplakinic acid E methyl ester [**9**] was tested in the PKC assays.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All 1D nmr [including nuclear Overhauser enhancement (27)] and COSY (24) spectra were measured on a Bruker AM-360 at 360.13 MHz for ^{1}H nmr and 90.56 MHz for ^{13}C nmr. Inverse 2D nmr spectra, including HMQC (25), HMBC (26), and NOESY (27), were measured non-spinning on a Bruker AMX-500 at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. Ir spectra were recorded on a Midac M Series Ftir spectrometer. Optical rotations were measured on a Jasco DIP-360 Digital Polarimeter. Ms were obtained by Dr. David Powell (University of Florida, Gainesville) using liquid secondary ion mass spectrometry (lsims) on a Finnigan MAT95Q with 3-nitrobenzyl alcohol as the matrix.

EXTRACTION AND ISOLATION OF COMPOUNDS **1** AND **6–9**.—The sponge, *Plakinastrella onkodes* (4), was collected in July 1992 by trawling at 240 ft in the Gulf of Mexico on the Florida Shelf approximately 84 nautical miles west of Sanibel Island (latitude 26° 15.72′ N, longitude 83° 41.99′ W). A taxonomic voucher is deposited at the Harbor Branch Oceanographic Museum (catalog number 003:00880). A sample of the sponge (102 g wet wt, HBOI sample no. 17-VII-92-2-28) was blended with EtOH (500 ml). The ground sponge material was then extracted twice more with 500-ml portions of EtOH. The EtOH extracts were combined and concentrated to dryness (2.76 g). A portion (2.41 g) of the EtOH extract was partitioned between EtOAc and H_2O. The EtOAc partition was concentrated to dryness (0.32 g) and further partitioned between hexane and 10% aqueous MeOH. The hexane partition was then concentrated to dryness (0.26 g). Sufficient H_2O was added to the aqueous portion to bring the total H_2O to 40%. This was then partitioned with CH_2Cl_2. The CH_2Cl_2 was then concentrated to dryness (0.05 g).

The hexane-soluble material was purified on Si gel using a step gradient beginning with EtOAc-heptane (1:9) and increasing the percentage of EtOAc until 100% EtOAc was reached. Fractions obtained with 10% to 30% EtOAc were further purified by hplc on a Si gel hplc column (Hibar Lichrosorb, 7 μm, 10 mm×25 cm) with 10% EtOAc in heptane to yield a mixture of compounds **6** and **7** and pure **8** (1.6 mg, 0.07% crude extract), and with 25% EtOAc to yield a mixture containing **1**. The mixtures were separated

using reversed-phase hplc [Vydac Protein and Peptide C_{18} column, 10 mm×25 cm, MeOH-H_2O (9:1) eluting solvent] to yield **6** (5.8 mg, 0.24%), **7** (4.9 mg, 0.20%), and **1** (3.7 mg, 0.15%).

The CH_2Cl_2 partition was purified using reversed-phase hplc [Vydac Protein and Peptide C_{18} column, 10 mm×25 cm, MeOH-H_2O (9:1) eluting solvent] yielding pure **9** (6.9 mg, 0.29%).

Plakortolide B [**6**].—Clear, colorless oil; [α] D−4.7° (*c*=0.09, $CDCl_3$); ir ν max (neat) 2925, 2853, 1777, 1450, 1367, 1253, 1165 cm^{-1}; ^{13}C- and ^1H-nmr data, see Tables 1 and 2; hrlsims *m/z* $[M+H]^+$ 403.2887 (requires 403.2848, Δ 3.9 mmu).

Plakortolide C [**7**].—Clear, colorless oil; [α] D−5.3° (*c*=0.05, $CDCl_3$); ir ν max (neat) 2927, 2853, 1777, 1451, 1367, 1253, 1169 cm^{-1}; ^{13}C- and ^1H-nmr data, see Tables 1 and 2; hrlsims *m/z* $[M+H]^+$ 375.2442 (requires 375.2526, Δ −8.4 mmu).

Plakortolide D [**8**].—Clear, colorless oil; [α] D+61.1° (*c*=0.04, $CDCl_3$); ir ν max (neat) 2925, 2853, 1781, 1376, 1254, 1162 cm^{-1}; ^{13}C- and ^1H-nmr data, see Tables 1 and 2; hrlsims *m/z* $[M+H]^+$ 417.007 (requires 417.006, Δ 0.1 mmu).

Epiplakinic acid E methyl ester [**9**].—Clear, colorless oil; [α] D+7.5° (*c*=0.57, $CDCl_3$); ir ν max (neat) 2928, 2854, 1740, 1453, 1207 cm^{-1}; ^{13}C- and ^1H-nmr data, see Tables 1 and 2; hrlsims *m/z* $[M+H]^+$ 377.2696 (requires 377.2682, Δ 1.4 mmu).

ACKNOWLEDGMENTS

We would like to thank John Reed and Shirley Pomponi for collection of the sponge sample, Frank E. Koehn for valuable nmr assistance, and Shirley Pomponi for assistance in sponge taxonomy. Funds for the purchase of the 500 MHz NMR spectrometer that was used extensively in this research were provided by the Robert Wood Johnson, Jr. Charitable Trust Foundation and the Atlantic Foundation. This research was funded by a grant from the National Cancer Institute (CA 55662). This is Harbor Branch Oceanographic Institution Contribution No. 1035.

LITERATURE CITED

1. G.P. Gunawardana, S. Kohmoto, S.P. Gunasekera, O.J. McConnell, and F.E. Koehn, *J. Am. Chem. Soc.*, **109**, 5267 (1987).
2. A.E. Wright, D.A. Forleo, G.P. Gunawardana, S.P. Gunasekera, F.E. Koehn, and O.J. McConnell, *J. Org. Chem.*, **55**, 4508 (1990).
3. O.J. McConnell, R. Longley, and M. Gunasekera, *Experientia*, **48**, 891 (1992).
4. E. Uliczka, in: "Ergebnisse einer zoologischen Forschungsreise nach Westindien." Ed. by W. Kukenthal and R. Hartmeyer, *Zool. Jb.* suppl. **16**, 1929, pp. 35–62.
5. R.E. Longley, O.J. McConnell, E. Esich, and D. Harmody, *J. Nat. Prod.*, **56**, 915 (1993).
6. Y. Kashman and M. Rotem, *Tetrahedron Lett.*, 1707 (1979).
7. M. Albericci, M. Collart-Lempereur, J.C. Braekman, D. Daloze, B. Tursch, J.P. Declercq, G. Germain, and M. van Meerssche, *Tetrahedron Lett.*, 2687 (1979).
8. A. Albericci, J.C. Braekman, D. Daloze, and B. Tursch, *Tetrahedron*, **38**, 1881 (1982).
9. L.V. Manes, G.J. Backus, and P. Crews, *Tetrahedron Lett.*, **25**, 931 (1984).
10. R.J. Capon and J.K. MacLeod, *Tetrahedron*, **41**, 3391 (1985).
11. R.J. Wells, *Tetrahedron Lett.*, 2637 (1976).
12. M.D. Higgs and D.J. Faulkner, *J. Org. Chem.*, **43**, 3454 (1978).
13. D.B. Stierle and D.J. Faulkner, *J. Org. Chem.*, **44**, 964 (1979).
14. D.B. Stierle and D.J. Faulkner, *J. Org. Chem.*, **45**, 3396 (1980).
15. D.W. Phillipson and K.L. Rinehart, Jr., *J. Am. Chem. Soc.*, **105**, 7735 (1983).
16. S. Sakemi, T. Higa, U. Anthoni, and C. Christophersen, *Tetrahedron*, **43**, 263 (1987).
17. S.P. Gunasekera, M. Gunasekera, G.P. Gunawardana, P. McMarthy, and N. Burres, *J. Nat. Prod.*, **53**, 669 (1990).
18. F.S. DeGuzman and F.J. Schmitz, *J. Nat. Prod.*, **53**, 926 (1990).
19. B.S. Davidson, *J. Org. Chem.*, **56**, 6722 (1991).
20. B.S. Davidson, *Tetrahedron Lett.*, **32**, 7167 (1991).
21. A.A.L. Gunatilaka, Y. Gopichand, F.J. Schmitz, and C. Djerassi, *J. Org. Chem.*, **46**, 3860 (1980).
22. A. Malorni, L. Minale, and R. Riccio, *Nouv. J. Chim.*, **2**, 4095 (1978).
23. M.R. Bendall, D.T. Pegg, D.M. Doddrell, and D.H. Williams, *J. Org. Chem.*, **47**, 3021 (1982).
24. W.P. Aue, E. Bartholdi, and R.R. Ernst, *J. Chem. Phys.*, **64**, 2229 (1976).
25. A. Bax and S. Subramanian, *J. Magn. Res.*, **67**, 565 (1986).
26. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).
27. B.K. Hunter, L.D. Hall, and J.K.M. Sanders, *J. Chem. Soc., Perkin Trans. I*, 657 (1983).

28. S. Macura and R.R. Ernst, *Mol. Phys.*, **41**, 95 (1980).
29. D.J. Burns, J. Bloomenthal, M. Lee, and R.M. Bell, *J. Biol. Chem.*, **265**, 12044 (1990).
30. P. Basta, M.B. Strickland, W. Holmes, C.R. Loomis, L.M. Ballas, and D.J. Burns, *Biochem. Biophys. Acta*, **1132**, 154 (1992).

Received 28 February 1994