Three New Cytotoxic Metabolites from the Marine Sponge Plakortis halichondrioides

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THREE NEW CYTOTOXIC METABOLITES FROM THE MARINE SPONGE *PLAKORTIS HALICHONDRIOIDES*

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ABSTRACT.—Three new cytotoxic compounds, one a cyclic-peroxide-containing acid (**5**) and the two others alkylated dihydroxy α,β-unsaturated C_{22} and C_{21} acids (**6** and **7**), were isolated from the marine sponge *Plakortis halichondrioides*, which was collected off the coast of Jamaica. The structures were elucidated through mass and mainly 1D and 2D nmr spectral analysis. All three acids are cytotoxic against P-388 murine leukemia.

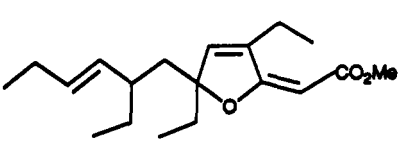
Sponges of the genus *Plakortis* are known to produce oxygenated polyketides, cyclic peroxides, and related metabolites (1–3). During our search for biologically active metabolites from marine sources we have examined the sponge *Plakortis halichondrioides* Schulze (family Plakinidae) which was collected off the coast of Jamaica. The EtOAc extract of the freeze-dried sponge afforded, after Sephadex LH-20 and Si gel chromatographies, seven metabolites **1–7**. Among the seven, four compounds turned out to be known compounds, namely, the furano α,β-unsaturated methyl ester **1**, plakortin [**2**], 3-*epi*-plakortin [**3**], and plakortic acid [**4**] (1,2). The structures of the three new compounds, a C_{20}-cyclic peroxide (**5**) and two differently alkylated dihydroxy C_{14}-unsaturated acids (**6** and **7**), are reported.

Eims determined the molecular formula of $C_{21}H_{38}O_4$ for the ester **8** and $C_{20}H_{36}O_4$ for the parent acid **5** (*m/z* 354 $[M]^+$ for **8** and *m/z* 324 $[M-16]^+$ for **5**). The ^{13}C-nmr spectrum of the parent acid **5** confirmed the 20 carbon atoms (**5**, Table 1) and accounted for all the protons of the molecule, except for the carboxylic proton (4 Me, 9 CH_2, 5 CH, and 2 C; altogether $C_{20}H_{35}$). Furthermore, the ^{13}C-nmr spectrum indicated the carboxylic group (δ_c 173.6, s) and suggested a disubstituted double bond (δ_c 132.1, d; 133.4, d). Two oxygen-bearing carbon atoms (δ_c 82.6, s and 78.3, d), the two remaining oxygen atoms, and the requisite for one ring (the third degree of unsaturation of **5**) suggested a cyclic peroxide for **5**. The nmr data (COSY and HMQC) also indicated four ethyl groups and several other spin systems summarized in Table 1. Most informative for the structure elucidation were the HMBC correlations (Table 1). From the latter data and comparison of the chemical shifts of the various resonances with those of related compounds (1–3) the planar structure of **5** was assigned.

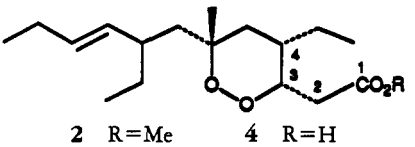
The relative cis configuration of C-3 and C-4 was determined from the 5.2 Hz coupling constant between H-3 and H-4, a value which is in good agreement with the cis configuration of plakortin (against a 9 Hz coupling in the case of epiplakortin, the trans isomer). The relative configuration of C-6 and C-10 remains to be established.

The cims spectrum of compound **6** provided *m/z* 367 for a molecular formula of $C_{22}H_{38}O_4$. The ^{13}C-nmr spectrum of **6** (Table 2) confirmed the presence of 22 carbon atoms and suggested a carboxylic group (δ_c 170.7, s) and three double bonds (δ_c 119.4, d; 126.1, d; 131.9, d; 132.8, d; 142.1, s; and 152.2, d) accounting together for the four degrees of unsaturation of the molecule. Furthermore, the ^{13}C-nmr spectrum exhibited two oxygen-bearing carbon atoms for two tertiary hydroxy groups (δ_c 89.2, s, and 87.1, s). The two hydroxyl protons together with the carboxylic proton completed the 38 protons of **6**.

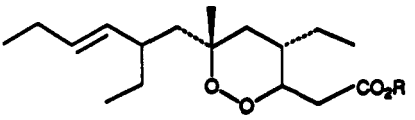
Among the three double bonds of **6**, one is conjugated with the carboxylic acid (δ_H 6.88 d and 6.05 d, J=16.5 Hz).



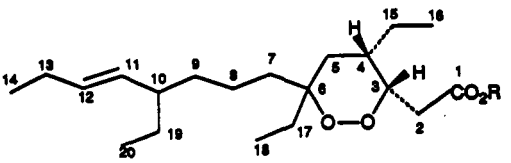
1



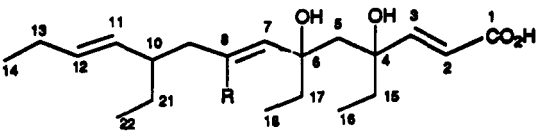
2 R=Me 4 R=H



3 R=Me



5 R=H 8 R=Me



6 R=CH_2CH_3 7 R=Me

Comprehensive studies of the 1D and 2D nmr spectra (COSY, HMQC, HMBC, Table 2) suggested the structure 4,6-dihydroxy-4,6,8,10-tetraethyltetradeca-2,7,11-trienoic acid for **6**.

The last compound, compound **7**,

TABLE 1. Nmr Data of Compound **5**.

Position	δ_C	δ_H[a]	^{2-4}J correlations (HMBC)[b]
1	173.6 s	—	H-2, H-2′
2	31.1 t	2.96 dd (J=15.8, 9.7) 2.3 dd (J=15.8, 3.5)	H-4, H-15′
3	78.3 d	4.39 ddd (J=9.7, 5.2, 3.6)	H-2, H-2′, H-4, H-5, H-15, H-15′
4	34.3 d	2.09 m	H-15, H-15′, H-17
5	25.1 t	1.04 m, 1.17 m	H-4
6	82.6 s	—	H-9, H-17, Me-18
7	31.9 t	1.39 m, 1.84 m	—
8	20.8 t	1.21 m (2H)	—
9	35.6 t	1.32 m (2H)	H-11
10	44.4 d	1.73 m	H-9, H-11, H-12, Me-20
11	133.4 d	5.08 dd (J=15.2, 4.5)	H-9, H-10, H-13
12	132.1 d	5.29 dt (J=15.2, 8.8)	H-10, H-13, Me-14
13	25.6 t	1.91 m (2H)	H-11, H-12, Me-14
14	14.2 q	0.90 t (J=6.5) (Me)	H-11, H-12
15	32.6 t	1.48 m, 1.17 m	H-4, H-5, H-5′
16	11.0 q	0.85 t (J=6.5) (Me)	H-4, H-5, H-15′
17	29.6 t	1.44 m, 1.23 m	Me-18
18	7.1 q	0.80 t (J=6.5) (Me)	H-7
19	28.2 t	1.07 m, 1.04 m	H-11, Me-20
20	11.7 q	0.75 t (J=6.5) (Me)	

[a]CH correlations determined by an HMQC experiment.
[b]Protons with a prime appear at higher field.

TABLE 2. Nmr Data of Compounds **6** and **7**.

Position	Compound			
	6			**7**
	δ_c	δ_H[a]	^{2-4}J correlations (HMBC)[b]	δ_c
1	170.7 s	—	H-3	171.1
2	119.4 d	6.05 d (J=16.5)	H-3	119.4 d
3	152.2 d	6.88 d (J=16.5)	H-5, H-5′, H-15, H-15′	152.0 d
4	89.2 s	—	H-2, H-3, H-5, H-5′, H-17′	89.1 s
5	56.3 t	2.46 & 2.41 AB quartet (J_{AB}=12.5)	H-7	55.8 t
6	87.1 s	—	H-3, H-5, H-15, H-15′	87.0 s
7	126.1 d	5.02 s	H-5, H-9, H-17, H-19	126.4 d
8	142.1 s	—	H-9, H-19, Me-20	136.6 s
9	41.9 t	1.97 m, 1.78 m	H-7, H-19, H-21, H-21′	46.4 t
10	42.5 d	1.87 m	H-9, H-9′, H-12, H-13, Me-22	42.6 d
11	132.8 d	4.96 dd (J=15.0, 8.5)	H-9, H-9′, H-13, H-21, H-21′	132.6 d
12	131.9 d	5.22 dt (J=15.0, 6.5)	H-13, H-13	131.1 d
13	25.6 t	1.92 m, 1.86 m	H-11, H-12	25.4 t
14	14.0 q	0.85 t (J=6.5)	H-12, H-13, H-13′	13.9 q
15	30.7 t	1.72 m, 1.65 m	H-5′	30.6 t
16	8.9 q	0.86 t (J=6.5)	H-15, H-15′	8.7 q
17	32.8 t	1.85 dq, 1.54 dq (J=14.0, 6.5)	H-5′	32.1 t
18	8.9 q	0.83 t (J=6.5)	H-7	8.7 q
19	23.9 t	2.01 m, 1.88 m	H-7, H-9, H-9′, Me-20	—
20	12.4 q	0.89 t (J=6.5)	H-19	C19 17.6 q[c]
21	27.8 t	1.23 m, 1.06 m	H-9, H-9′, Me-22	C20 27.5 t
22	11.6 q	0.75 t (J=6.5)		C21 11.6 q

[a]CH correlations determined by an HMQC experiment.
[b]Protons with a prime appear at higher field.
[c]C-19 in **7** is a methyl and C-20, -21 the ethyl on C-10.

$C_{21}H_{36}O_4$, *m/z* 353, turned out to be the 8-desethyl-8-methyl homologue of **6**. Comparison of the nmr and especially the ^{13}C nmr data of **7** with those of compound **6** (Table 2 and Experimental) pointed clearly to the similarities and to the differences between the two. The main difference was in the chemical shifts of C-8 and C-9 (due to subtraction of a β effect on C-8 and a γ effect on C-9 in **7**). Compound **7** therefore is 4,6-dihydroxy-8-methyl-4,6,10-triethyltetradeca-2,7,11-trienoic acid.

All three acids **5**–**7** are cytotoxic against P-388 murine leukemia, with IC_{50} values of 0.5, 10, and 10 μg/ml for **5**, **6**, and **7**, respectively. Methylation of **5** reduced its activity to 5 μg/ml (3).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. Low resolution mass spectra were recorded on a Finnigan-4021 mass spectrometer; ^1H- and ^{13}C-nmr spectra were recorded on Bruker AM-360 and ARX-500 spectrometers. All chemical shifts are reported with respect to TMS (δ=0). Hrms was taken on a VG70 VSEQ instrument and lrms on a Finnigan 4021 instrument. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 1 dm microcell.

ISOLATION OF COMPOUNDS **1**–**7**.—A sample of the sponge (PharmaMar SA, Madrid 15-04-90-1-9), collected off the coast of Jamaica in April 1990 and deep frozen immediately after collection, was lyophilized to give 50 g of dry material. A voucher specimen is located in PharmaMar Madrid. Extraction of this with EtOAc afforded 230 mg of crude material. The latter gum was chromatographed on Sephadex LH-20 and eluted with hexane-CH_2Cl_2-MeOH (2:1:1). The major fraction was then chromatographed on Si gel and eluted with petroleum ether-EtOAc (10:1→1:1) to afford compounds **1**–**7**. Compound **1** (10 mg) R_f 0.95 [Si gel, hexane-EtOAc (1:1)]. Compound **2** (5 mg) R_f 0.86. Compound **3** (10 mg) R_f 0.84. Compound **4** (5 mg) R_f 0.72. Compound **5** (30 mg) R_f 0.75. Compound **6** (5 mg) R_f 0.80. Compound **7** (5 mg) R_f 0.78.

Compound **5**.—An oil: $[\alpha]_D$ −98.7° (*c*=1, $CHCl_3$); hrms *m/z* $[M-16]^+$ 338.2780 (calcd for $C_{21}H_{38}O_3$, 338.2811); ir ($CHCl_3$) 3500, 2950, 1715, 1460, 1380, 1300, 980 cm^{-1}; ^1H and ^{13}C nmr see Table 1.

Compound **6**.—An oil: $[\alpha]_D$ +1.2° (*c*=0.33, $CHCl_3$); hrms *m/z* $[M]^+$ 366.2741 (calcd for $C_{22}H_{38}O_4$, 366.2760); ir ($CHCl_3$) 3500, 1950, 1710, 1470, 1130 cm^{-1}; ^1H and ^{13}C nmr see Table 2.

Compound **7**.—An oil: $[\alpha]_D$ +4.8° (*c*=0.46, $CHCl_3$); hrms *m/z* $[M]^+$ 352.2596 (calcd for $C_{21}H_{36}O_4$, 352.2604); ir ($CHCl_3$) 3500, 1950, 1710, 1460, 1120, 1070 cm^{-1}; ^1H and ^{13}C nmr see Table 2.

METHYL ESTER OF COMPOUND **5**.—To compound **5** (3 mg) in Et_2O, a solution of CH_2N_2 in Et_2O (2 ml) was added. After 24 h the solvent was evaporated to give pure **8** (3 mg): oil; ^1H nmr identical with that of **5** except for the OMe group at δ_H 3.40 s.

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