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New Cyclic Peroxides from a Sponge, *Plakortis* sp.

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Two new cyclic peroxides were isolated from an undescribed sponge of the genus *Plakortis* sp. collected at Discovery Bay, Jamaica. The molecular structures were elucidated by interpreting 1D and 2D NMR and HRMS data. The cyclic peroxides, Compound 1 and 2, exhibited significant antimicrobial activity against pathogenic bacteria and fungi with IC_{50} values of 0.9-5.0 μg/mL and 0.7-8.0 μg/mL, respectively.

Key words: Peroxide, Antifungal, Antibacterial, *Plakortis*

Introduction

Marine sponges are a source of a number of biosynthetically diverse natural products (Stierle and Faulkner, 1980; Davidson, 1991; Compagnone et al., 1998; Perry et al., 2001). Specially, cyclic peroxides from the genus *Plakortis* exhibited a diverse range of bioactivities, including anticancer (Fontana et al., 1998), antibacterial (Chen et al., 2002), and antifungal (Gunasekera et al., 1990) activities. In addition, they affect Ca^{2+} uptake by the cardiac sarcoplasmic reticulum (Patil et al., 1996a).

In the recent years, we have focused in part on peroxide containing marine natural products due to their activity against the phethogenic microbes as well as the malarial parasite *Plasmodium falciparum* (Peng, et al., 2002; Perry, et al., 2001). The polyketide-derived cyclic peroxides have been identified primarily from the genus *Plakortis* of the family Plakinidae.

Here we discussed to isolation and structural elucidation for two new cyclic peroxides that have activities against bacteria and funges from a Jamaican sponge, *Plakortis* sp.

Materials and Methods

Sponge collection and identification

The sponge was collected from inside caves at a depth of 37.5 m off the mouth of Rio Bueno, Discovery Bay, Jamaica, on June 2000. The sponge forms a moderately thick encrustation that is very soft, liver-like and very easily torn. It is avocado green in life, emits a bright deep blue watery exudates on exposure to air, and emits a very strong, characteristic sweet Oil of Wintergreen odor. The spicules are mesosclere diods averaging 190 μm long and extremely rare triods. The sponge is thus an undescribed species of *Plakortis* (Subclass Homoscleromorpha, Order Homosclerophorida, Family Plakinidae). A voucher specimen was deposited at the Natural History Museum, London, United Kingdom.

Extraction and Isolation

The frozen sponge (1.3 kg) was extracted with ethanol (3×2 L). The extract was subjected to silica flash column chromatography using a gradient of hexane to ethyl acetate and finally methanol. We obtained 10 fractions, and fractions 2-6 contained active compounds. The fractions were chromatographed using a gel permeation chromatography on Sephadex LH-20 column (4.9×76 cm) in methanol, and the resulting fractions were purified further using reverse-phase HPLC (Phenomenex, C_{18} 5 μM ODS 3100A, 10×250 mm) and eluting with a linear gradient of CH_3CN-H_2O (flow rate, 10 mL/min UV detection at 230 nm). This yielded Compound 1 (235 mg) and Compound 2 (163 mg).

Structure analysis

IR and UV spectra were obtained using an AATI

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Mattson Genesis Series FTIR and a Perkin-Elmer Lambda 3B UV/Vis spectrophotometer. Optical rotations were measured on an AUTOPOL IV autopolarimeter. Both 1D and 2D NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer. Chemical shift (δ) values were expressed in ppm and were referenced to the residual solvent signals with resonances at δ_H/δ_C 7.26/77.0 ($CDCl_3$). The samples were processed on an ESI-FTMS 30es ion cyclotron HR HPLC-FT spectrometer with direct injection onto an eletrospray interface and positive or negative mode. The silica gel (230-400 mesh) and Sephadex LH-20 (Pharmacia) used for column chromatography were obtained from Natland International Corporation and Sigma Chemical Co. (USA), respectively.

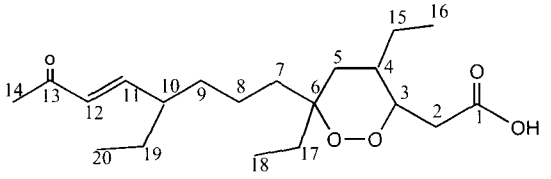
Results and Discussion

Compound 1 was obtained as a colorless oil $[\alpha]_D^{25}$ of -62° (*c* 0.18, $CHCl_3$), and its molecular formula was determined to be $C_{20}H_{34}O_5$, by using positive ion HR-ESIMS [M+H] 355.2406 (calcd. 355.2398). The IR and UV spectra indicated that it was an α, β-unsaturated ketone [IR: 1689, 1647/cm; UV λ_{max} (log ε) nm: 238 (4.25)] with a hydroxy band (3440 /cm). The ^1H, ^{13}C, DEPT, and HMQC NMR experiments allowed the assignment of six methine, seven methylene, and four methyl groups (Table 1). The remaining quaternary centers consisted of a carbonyl (177.7), and oxygenated (82.7) and double-bond oxygenated carbon signals (199.4). The chemical shift values of the two oxygenated carbons suggested a peroxide functionality. A series of downfield ^{13}C NMR resonances were assigned a disubstituted olefins [131.5(CH) and 153.0 (CH)]. The proton-carbon connectivity was determined from the HMQC and HMBC experiments and allowed the connection of spin systems to establish the complete structure of Compound 1. In the HMBC spectrum, one 2*J* and two 3*J* correlations were observed between H-11 at δ 6.54 and the methine carbon C-10, the ketone carbonyl C-13, and the methylene C-9 signals at 45.0, 199.4, and 35.5, respectively. Two 2*J* and 3*J* correlations were observed between the proton H-3 (δ 4.44) and the methine C-4, methylene C-2, ketone carbonyl C-1, and methylene C-5 signals at 34.8, 31.7, 177.7,and 33.2, respectively. The 2*J*, 3*J*, and 4*J* correlations were observed between the proton H-12 (δ 6.03) and the ketone carbonyl C-13, methine C-10, and the methylene C-19 signals at 199.4, 45.0 and 27.6, respectively.

Table 1. ^{13}C NMR data for compounds 1 and 2 ($CDCl_3$)

No.	Compound 1	Compound 2
1	177.7 (s)	178.2 (s)
2	31.7 (d)	31.9 (t)
3	78.7 (d)	79.1 (d)
4	34.8 (d)	35.3 (d)
5	33.2 (t)	33.1 (t)
6	82.7 (s)	84.0 (s)
7	32.2 (t)	133.3 (d)
8	21.1 (t)	130.4 (d)
9	35.0 (t)	38.1 (t)
10	45.0 (d)	44.9 (d)
11	153.0 (d)	133.3 (d)
12	131.5 (d)	132.5 (d)
13	199.4 (s)	26.0 (t)
14	27.5 (q)	14.5 (q)
15	25.5 (t)	25.3 (t)
16	12.1 (q)	11.3 (q)
17	29.8 (t)	33.5 (t)
18	7.5 (q)	7.5 (q)
19	27.6 (t)	27.9 (t)
20	11.1 (q)	12.0 (q)

Compound 1



The ^1H NMR (400 MHz, $CDCl_3$) spectrum (Table 2) showed signals indicating a -CH=CH-moiety [δ 6.54 (1H, dd, *J*=15.8, 9.0 Hz) and 6.03 (1H, d, *J*=16.0 Hz)], terminal methyl groups [δ 0.86 (3H, t, *J*=16.4 Hz) and δ 2.22 (3H, s)], and two isolated moieties at δ 1.48 (1H, m), 1.32 (1H, m), 0.85 (3H, m) and at δ 1.53 (1H, m), 1.35 (1H, m), 0.93 (3H, m), respectively. Five proton spin systems were included based on the analysis of the ^1H-^1H COSY data and include a -CH=CH- (H-11, H-12), CH_3CH_2-[(H-15, H-16), (H-17, H-18), and (H-19, H-20)], and $CH_3CH_2CH(CH_2$-$)CHCH_2$-(H-2, H-3, H-4, H-5, H-15, H-16).

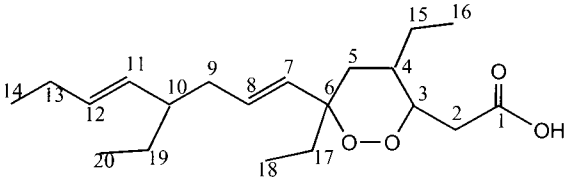
Compound 2 was isolated as an oil and its molecular formula was determined to be $C_{20}H_{34}O_4$ by HR-ESIMS[M-H] 337.1356 (calcd. 337.3641). Comparison of the IR and UV spectra of Compound 2 with those of Compound 1 indicated the absence of the α, β-unsaturated ketone group.

The ESI (positive mode) spectrum of Compound

Table 2. ^{1}H NMR data for compounds 1 and 2 ($CDCl_3$)

No.	Compound 1	Compound 2
2	2.99 (1H, dd, 14.8, 9.6)	3.01 (1H, dd, 16.0, 9.6)
	2.38 (1H, d, 15.2)	2.39 (1H, br d, 16.0)
3	4.44 (1H, br m)	4.41 (1H, dt, 5.6, 4.2)
4	2.10 (1H, m)	2.12 (1H, m)
5	1.52 (1H, m)	1.75 (1H, dd, 13.2, 4.0)
	1.25 (1H, m)	1.25 (1H, dd, 13.2, 12.8)
7	2.01 (1H, t, 11.8)	5.48 (1H, m)
	1.46 (1H, m)	
8	1.32 (2H, m)	5.48 (1H, m)
9	2.15 (1H, m)	2.14 (1H, d, 5.6)
	1.30 (1H, d, 7.6)	2.02 (1H, m)
10	2.01 (1H, br m)	1.88 (1H, m)
11	6.54 (1H, dd, 15.8, 9.0)	5.12 (1H, dd, 15.2, 8.4)
12	6.03 (1H, d, 16.0)	5.37 (1H, 15.2, 6.2)
13		2.05 (1H, dd, 13.4, 3.4)
		1.90 (1H, m)
14	2.22 (3H, s)	0.94 (3H, t, 7.6)
15	1.25 (1H, m)	1.20 (1H, m)
	1.16 (1H, m)	1.12 (1H, m)
16	0.86 (3H, t, 16.4)	0.89 (3H, t, 6.8)
17	1.48 (1H, m)	1.40 (2H, m)
	1.32 (1H, m)	
18	0.85 (3H, m)	0.80 (3H, m)
19	1.53 (1H, m)	1.36 (1H, m)
	1.35 (1H, m)	1.20 (1H, m)
20	0.93 (3H, m)	0.82 (3H, t, 7.2)

Compound 2



2 had pseudo-molecular ion peaks at 356.1237 and 694.9215 ($[M+NH_4]^+$) and ($[2M+NH_4]^+$), respectively, which is comparable to Compound 1. However, the molecular formula of Compound 2 was established to be $C_{20}H_{34}O_4$ by a HR-ESIMS signals at *m/z* 356.1237 ($[M+NH_4]^+$) and 694.9215 ($[2M+NH_4]^+$).

The ^{13}C NMR spectrum contained 20 signals, which were assigned, using the DEPT experiment, as four methyl, seven methylene, and seven methine catbons, leaving two quaternary carbons (Table 1). Comparison of the ^{1}H and ^{13}C NMR spectral data showed a plakotin-type cyclic peroxide ring system with signals at δ 3.01 (1H, dd, *J*=16.0, 9.6 Hz, H-2e) and 2.39 (1H, br d, *J*=16.0, H-2a), 4.41 (1H, m, H-3), 2.12 (1H, m, H-4), 1.75 (1H, dd, *J*=13.2, 4.0, H-5e) and 1.25 (1H, m, H-5a), which was supported by two typical signals for carbon atoms bearing oxygen at 79.1 (CH, C-3) and 84.0 (C, C-6) in the ^{13}C NMR.

The *E*-configuration of the double bond in the side chain was readily confirmed by the large coupling constant (*J*=15.2 Hz). The relative stereochemistry of Compound 1 was deduced from the detailed analysis of the coupling constant and NOESY correlations in the combination with molecular modeling. The vicinal coupling constants of the C-5 proton signals at 1.25 (1H, dd, *J*=13.2, 12.8 Hz) and 1.75 (1H, dd, 13.2, 4.0 Hz) indicated that the C-4 proton must be axial. Significant NOE correlations (Fig. 1) were observed between H-2 and H-5a, H-3e and H-2, H-3e and H-4a, H-4e and H-15, H-4e and H-16, H-5e and H-7, H-8 and H-17, H-8 and H-19, H-8 and H-10, H-10 and H-12, and H-11 and H-19, as well as H-12 and H-19, which required a 6α (equatorial)-ethyl configuration. Such a configuration at C-6 in polyketide-derived cyclic peroxides has previously been reported from sponges in the genus *Plakortis* (Patil et al., 1996b; Hu et al., 2001).

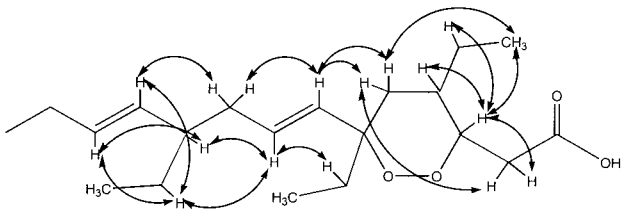


Fig. 1. Significant NOE correlations found for Compound.

The carbon skeleton of Compound 2 was almost similar to that of Compound 1 with exception the carbon signals of the olefinic carbon C-7 (133.3), C-8 (130.4), and methylene C-13 (26.0). The ^{1}H NMR spectrum of Compound 2 revealed two doublet methyl groups at δ 0.94 (3H, t, *J*=7.6) and 0.82 (3H, t, *J*=7.2). In addition to two proton spin systems that are comparable to compound 1[$CH_3CH_2CH(CH_2$-) $CHCH_2$-(H-2, H-3, H-4, H-5, H-15, H-16) and CH_3CH_2- (H-17, H-18)]; a third spin system [-CH=CH-CH_2CH(CH_2 CH_3)CH=$CHCH_2CH_3$ (H-7, H-8, H-9, H-10, H-19, H-20, H-11, H-12, H-13 and H-14)] observed in the ^{1}H-^{1}H COSY spectra required a terminal isopropyl group in the side chain. The position of the double bond was confirmed in the HMQC and HMBC experiments. The two olefinic proton signals (H-7 and H-8) overlapped at δ 5.48 (2H, m), whereas another two olefinic signals (H-11 and H-12) did not overlap at δ 5.12 (1H, dd, *J*=15.2, 8.4) and δ 2.37 (1H, dd, *J*=15.2, 6.2) in the ^{1}H NMR spectrum.

Compound 1 and Compound 2 were assayed for antifungal and antibacterial activities against *Candida*

Table 3. Data of antimicrobial activities by compounds 1 and 2

Compounds	*C. albicans*	*C. neoformans*	*S. aureus*	MRSA	*M. intracellulare*
1	0.9*	0.9	0.7	1.6	2.5
2	5.0	2.5	5.0	8.0	8.0

*Minimum inhibitory concentration (MIC, μg/mL)

albicans ATCC 90028, *Cryptococcus neoformans* ATCC 90113, *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 90906, and *Mycobacterium intracellulare* ATCC 23068. The two compounds exhibited significant antifungal and antibacterial activities *in vitro* with IC_{50} values of 0.9-5.0 μg/mL and 0.7-8.0 μg/mL, respectively (Table 3).

Compound 1-Colorless oil, $[\alpha]_D^{25}$=-62 (*c* 0.18, $CHCl_3$); IR ν_{max} 3440, 2963, 2935, 2877, 1689, 1647, 1460/cm; UV λ_{max} 238 nm (log ε), HR-ESIMS m/z [M+H] 355.2406 (calcd. 355.2398), ^{13}C and ^{1}H and NMR data see Table 1 and 2.

Compound 2-Colorless oil, $[\alpha]_D^{25}$=-163 (*c* 0.19, CHCl3); IR ν_{max} 2962, 2932, 2876, 1443, 1290, 967 /cm; UV λ_{max} 236 nm (log ε), HR-ESIMS *m/z* $[M+NH_4^+]$ 356.1237 (calcd. for $C_{20}H_{34}O_4$ 338.2457), ^{13}C and ^{1}H and NMR data see Table 1 and 2.

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