

Plakortides I–L, four new cyclic peroxides from an undescribed Jamaican sponge *Plakortis* sp. (Homosclerophorida, Plakinidae)

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Abstract—Four new polyketide-derived cyclic peroxides, plakortides I (1), J (2), K (3) and L (4), were isolated from an undescribed sponge of the genus *Plakortis* collected at Discovery Bay, Jamaica. The structures were elucidated by spectral analysis including the 2D NMR techniques. The relative stereochemistry of the plakortides was established by NOESY NMR experiments. Plakortide I (1) represents the first report of a polyketide-derived cyclic peroxide with an α ,β-unsaturated ketone moiety in the side chain and exhibits significant antimalarial activity against the W2 Clone of *Plasmodium falciparum* with an IC₅₀ of 570 ng mL⁻¹ and a selectivity index of >8.4. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Marine sponges have proven to be a valuable resource for naturally-occurring and bioactive cyclic peroxides. ¹⁻⁴ In

recent years, our research has focused in part on peroxide containing marine natural products due to their activity against the AIDS opportunistic parasitic infection *Toxoplasma gondii* as well as the malarial parasite

Keywords: biologically active compounds; marine metabolites; peroxides; stereochemistry.

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Table 1. ¹³C NMR Data for plakortides I-L (in CDCl₃)

No.	I (1)	J (2)	K (3)	L (4)
1	172.1 (s)	172.6 (s)	172.5 (s)	176.6 (s)
2	31.4 (t)	31.8 (t)	31.7 (t)	36.3 (t)
3	79.1 (d)	79.3 (d)	79.2 (d)	82.1 (d)
4	35.4 (d)	35.5 (d)	35.3 (d)	36.9 (d)
5	33.4 (t)	33.2 (t)	33.1 (t)	36.3 (t)
6	83.9 (s)	83.9 (s)	83.7 (s)	84.4 (s)
7	147.7 (d)	133.5 (d)	132.5 (d)	131.6 (d)
8	130.3 (d)	130.7 (d)	131.6 (d)	131.6 (d)
9	201.1 (s)	42.4 (t)	35.0 (t)	34.8 (t)
10	34.0 (t)	28.8 (d)	22.8 (t)	22.7 (t)
11	8.2 (q)	22.7 (q)	13.9 (q)	13.8 (q)
12	25.2 (t)	25.4 (t)	25.4 (t)	24.2 (t)
13	11.1 (q)	11.5 (q)	11.3 (q)	10.7 (q)
14	32.9 (t)	33.6 (t)	33.5 (t)	33.2 (t)
15	7.2 (q)	7.6 (q)	7.4 (q)	7.5 (q)
16		22.7 (q)		
OMe	52.2 (q)	52.3 (q)	52.1 (q)	

Plasmodium falciparum. 5,6 The polyketide-derived cyclic peroxides have been identified primarily from the genus Plakortis of the family Plakinidae, 7-15 and we wish to report here four new polyketide-derived cyclic peroxides, plakortides I (1), J (2), K (3) and L (4), ¹⁶ isolated from a sponge of the genus *Plakortis*. The sponge was collected using closed circuit rebreathers from inside caves at a depth of -37.5 m off the mouth of the Rio Buenos, Discovery Bay, Jamaica. The sponge is unique, and thus is easily recognizable in the field, in that it produces a sweet wintergreen or acetone-like smell, and has a thin translucent deep blue exudate that contrasts markedly with the sponge body which remains largely bright mustardgreen. The sponge is probably closest to Plakortis halichondrioides (Wilson 1902) but it is so distinct that it is best kept as an undescribed species. The four new plakortides were assayed for their antifungal and antibacterial activities and found inactive. Plakortide I (1) exhibits significant antimalarial activity in vitro against the W2 Clone of P. falciparum with an IC₅₀ of 570 ng mL $^{-1}$ and a selectivity index of >8.4.

2. Results and discussion

Plakortide I (1) was isolated as an oil, $[\alpha]_D^{25} = -131^\circ$ (c 0.2, CHCl₃), and its molecular formula was determined to be C₁₆H₂₆O₅ by HR-ESIMS (m/z 316.2124, [M+NH₄]⁺). The IR and UV spectra indicated an α,β-unsaturated ketone [IR: 1697, 1643 cm⁻¹; UV λ_{max} (log ε) nm: 238 (4.27)] and an ester carbonyl (1740 cm⁻¹) group. The ¹³C NMR spectrum contained 16 signals, which could be classified by using the DEPT data as four methyl, five methylene and four methine leaving three quaternary carbons (Table 1).

The ¹H NMR (400 MHz, CDCl₃) spectrum (Table 2) showed signals indicating a -CH=CH- moiety [δ 6.82 (1H, d, J=16.4 Hz) and 6.28 (1H, d, J=16.4 Hz); an $-OCH_3$ [δ 3.72 (3H, s)]; terminal methyl groups [δ 0.90 (3H, t, J=7.4 Hz) and two isolated ethyl moieties at δ 2.64 (2H, q, J=7.3 Hz), 1.11 (3H, t, J=7.3 Hz) and 1.57 (2H, q, J=7.3 Hz)J=7.6 Hz), 0.85 (3H, t, J=7.6 Hz), respectively. Five proton spin systems were identified by the analysis of the ¹H-¹H COSY data and include a -CH=CH- (H-7, H-8), H-13) and terminal methyl group at δ 0.90 ppm. The chemical shifts and the coupling constants of this proton spin system together with the ¹³C NMR signals (carbon atoms bearing oxygen) showed 1 contained a plakortintype six-membered cyclic peroxide ring system [¹H NMR: δ 3.01 (1H, dd, J=15.5, 9.6 Hz, H-2), 2.39 (1H, dd, J=15.5, 3.4 Hz, H-2'), 4.48 (1H, ddd, J=9.6, 5.1, 3.4 Hz, H-3e), 2.01(1H, m, H-4a), 1.79 (1H, dd, *J*=13.6, 4.3 Hz, H-5e) and 1.42 (1H, dd, J=13.6, 13.1 Hz, H-5a); ¹³C NMR: δ 79.1 (CH–O, C-3) and 83.9 (C–O, C-6)]. ^{7-9,12,13}

The proton–carbon connectivity was determined by the HMQC and HMBC experiments and allowed the connection of spin systems to establish the complete structure of **1**. In the HMBC spectrum, three 3J correlations were observed between the olefinic proton H-7 at δ 6.82 and the methylene C-5 and C-14 as well as the ketone carbonyl C-9 signals at δ 33.4, 32.9 and 201.1, respectively. A 2J and two 3J correlations

Table 2. ¹H NMR Data for plakortides I–L (in CDCl₃)

No.	I (1)	J (2)	K (3)	L (4)
2	3.01 (1H, dd, 15.5, 9.6)	3.05 (1H, dd, 15.5, 9.6)	3.03 (1H, dd, 15.5, 9.6)	2.71 (1H, dd, 15.4, 9.6)
	2.39 (1H, dd, 15.5, 3.4)	2.41 (1H, dd, 15.5, 3.3)	2.38 (1H, dd, 15.5, 3.3)	2.31 (1H, dd, 15.4, 3.4)
3	4.48 (1H, ddd, 9.6, 5.1, 3.4)	4.46 (1H, m)	4.46 (1H, m)	4.19 (1H, m)
4	2.01 (1H, m)	2.11 (1H, m)	2.09 (1H, m)	1.62 (1H, m)
5	1.79 (1H, dd, 13.6, 4.3)	1.78 (1H, dd, 13.5, 4.5)	1.77 (1H, dd, 13.6, 4.2)	2.03 (1H, dd, 13.7, 4.6)
	1.42 (1H, dd, 13.6, 13.1)	1.30 (1H, dd, 13.5, 12.7)	1.27 (1H, dd, 13.6, 13.1)	1.28 (1H, dd, 13.7, 13.0)
7	6.82 (1H, d, 16.4)	5.48 (1H, d, overlapped)	5.49 (1H, d, overlapped)	5.47 (1H, d, overlapped)
8	6.28 (1H, d, 16.4)	5.48 (1H, m, overlapped)	5.49 (1H, m, overlapped)	5.47 (1H, m, overlapped)
9		1.99 (2H, t, 6.5)	2.06 (2H, m)	2.05 (2H, m)
10	2.64 (2H, q, 7.3)	1.50 (1H, m)	1.38 (2H, m)	1.40 (2H, m)
11	1.11 (3H, t, 7.3)	0.84 (3H, d, 6.8)	0.90 (3H, t, 6.7)	0.91 (3H, t, 6.7)
12	1.19 (2H, m)	1.20 (2H, m)	1.18 (2H, m)	1.15 (2H, m)
13	0.90 (3H, t, 7.4)	0.92 (3H, t, 6.9)	0.92 (3H, t, 6.7)	0.93 (3H, t, 6.8)
14	1.57 (2H, q, 7.6)	1.46 (2H, q, 6.8)	1.41 (2H, q, 6.5)	1.49 (2H, q, 6.5)
15	0.85 (3H, t, 7.6)	0.82 (3H, t, 6.8)	0.82 (3H, t, 6.5)	0.83 (3H, t, 6.5)
16	, , , ,	0.88 (3H, d, 6.7)		, ,
OMe	3.72 (3H, s)	3.70 (3H, s)	3.69 (3H, s)	

Figure 1. Significant NOE correlations found for plakortide I (1). The two olefinic protons (H-7 and H-8) have the same cross peaks with H-3e. H-4a and H-5e in its NOESY spectra due to the free rotation of the C-6–C-7 bond

tions were observed between the olefinic proton H-8 at δ 6.28 and the ketone carbonyl C-9, the methylene C-10 and the quaternary C-6 signals at δ 201.1, 34.0 and 83.9, respectively. Two 2J and three 3J HMBC correlations were observed between the proton H-3 (δ 4.48) and the methylene C-2, methine C-4, ester carbonyl C-1, methylene C-5 and C-12 signals at δ 31.4, 35.4, 172.1, 33.4 and 25.2, respectively. The HMBC data revealed the junction of the four proton spin systems, the location of the α,β -unsaturated ketone moiety and also provided the chemical shift assignments for all carbons (Table 1) and protons (Table 2) in structure 1. The E-configuration of the double bond in the side chain was readily confirmed by the large coupling constant (J=16.4 Hz). The relative stereochemistry of 1 was deduced from the detailed analysis of the coupling constants and NOESY correlations in combination with molecular modeling (Fig. 1). The vicinal coupling constants of the C-5 proton signals at δ 1.42 (1H, dd, J=13.6, 13.1 Hz) and 1.79 (1H, dd, J=13.6, 4.3 Hz) indicated that the C-4 proton must be axial. The orientation of H-3 (equatorial) and H-4 (axial) is supported by the coupling constant of 5.1 Hz $(J_{\text{H-3e,H-4a}})$. Clear NOE correlations (Fig. 1) were observed between H-2 and H-5a, H-3e and H-4a, H-4a and H-5e, H-4a and H-7, H-4a and H-8, H-5e and H-7 as well as H-5e and H-8, which required a 6α (equatorial)-ethyl configuration. Such a configuration at C-6 in polyketide-derived cyclic peroxides was previously reported from other sponges of the genus Plakortis. 12,14

The ESI (positive mode) spectrum of plakortide J (2) provided a pseudo-molecular ion peak at 316 $([M+NH_4]^+)$, which is comparable to compound 1. However, the molecular formula of 2 was established to be C₁₇H₃₀O₄ by a HR-ESIMS signal at m/z 316.2494 ([M+NH₄]⁺). Comparison of the IR spectrum of 2 with that of 1 indicated the presence of an ester carbonyl (1737 cm $^{-1}$) but absence of the α,β -unsaturated ketone carbonyl group. The ¹³C NMR spectrum contained 17 signals, which could be assigned by using the DEPT experiment as five methyl, five methylene and five methine leaving two quaternary carbons (Table 1). Comparison of the ¹H and ¹³C NMR spectral data of 2 with those of 1 (Tables 1 and 2) indicated 2 also contained a plakortintype cyclic peroxide ring system with signals at δ 3.05 (1H, dd, J=15.5, 9.6 Hz, H-2), 2.41 (1H, dd, J=15.5,3.3 Hz, H-2'), 4.46 (1H, m, H-3e), 2.11 (1H, m, H-4a),

1.78 (1H, dd, J=13.6, 4.3 Hz, H-5e) and 1.30 (1H, dd, J=13.6, 13.1 Hz, H-5a), which was supported by two typical signals for carbon atoms bearing oxygen at δ 79.3 (CH, C-3) and 83.9 (C, C-6) in the ¹³C NMR.

The carbon skeleton of 2 differs from 1 only in the C-6 side chain. The ¹H NMR spectrum of 2 revealed two doublet methyl groups at δ 0.84 (3H, d, J=6.8 Hz) and 0.88 (3H, d, J=6.7 Hz). In addition to two proton spin systems which are comparable to 1 [CH₃CH₂CH(CH₂-)CHCH₂- (H-2, H-3, H-4, H-5, H-12, H-13) and CH₃CH₂- (H-14, H-15)]; a third spin system [-CH=CH-CH₂CH(CH₃)₂ (H-7, H-8, H-9, H-10, H-11 and H-16] observed from the ${}^{1}H$ - ${}^{1}H$ COSY spectra required a terminal isopropyl group in the side chain. The position of the double bond at C-7 was also confirmed by the HMQC and HMBC experiments. The two olefinic proton signals (H-7 and H-8) are overlapped at δ 5.48 (2H, m) in the ¹H NMR spectrum. The configuration of the C-7(8) double bond in the side chain was deduced on the basis of 13 C NMR chemical shift (δ) values modeled for C-6 and C-9 in both Z-(C-6: 70.7, C-9: 37.0) and E-(C-6: 76.7, C-9: 43.0) configuration. ¹⁷ The observed chemical shift (δ) values of 83.9 (C-6) and 42.4 (C-9) indicate the thermodynamically more favored E-configuration for the double bond. The relative stereochemistry of 2 was confirmed to be the same as 1 and provided comparable coupling constants (Table 2) and NOESY correlations. Therefore, the longer side chain at C-6 in 2 is also in the β (axial) position.

The structures of plakortides K (3) and L (4) were determined independently by their HR-ESIMS, IR, 1D and 2D NMR spectral data. The relative stereochemistry of the ester 3 and the acid 4 was determined on the basis of the coupling constants (Table 2) and the NOESY correlations, which also proved to be the same as in 1 and 2. Clear NOESY crosspeaks were observed between H-2 and H-5a, H-3e and H-4a, H-4a and H-5e, H-4a and H-7, H-4a and H-8, H-5e and H-7 as well as H-5e and H-8. These data strongly suggested all four compounds (1-4) have a plakortin-type six-membered cyclic peroxide ring with the same stereochemistry at C-3, C-4 and C-6. Therefore, compounds 3 and 4 are the diastereomers of the previously reported peroxides 5 and $6^{8,9}$ with inverted stereochemistry at C-6. Their physical and chemical properties are presented in Section 3 and Tables 1 and 2.

Similar to plakortin, plakortides I–J are also optically active and were not a mixture of diastereoisomers. These data suggests that each compound is generated through enzyme-mediated reactions. The four new plakortides were tested against the W2 Clone of P. falciparum for their malaria activity in vitro, and only plakortide I (1) was found to exhibit significant antimalarial activity at IC_{50} 570 ng mL⁻¹ and a selectivity index of >8.4. This indicated that the α,β -unsaturated ketone moiety in the side chain is essential in those plakortides for the activity against W2 clone of P. falciparum. Plakortides I-L were assayed for their antifungal and antibacterial activities against C. albicans ATCC 90028, C. neoformans ATCC 90113, S. aureus ATCC 29213, MRSA ATCC 43300, P. aeruginosa ATCC 27853, A. fumigatus ATCC 90906 and M. intracellulare ATCC 23068, and found inactive.

3. Experimental

3.1. General experimental procedures

1D and 2D NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer. Chemical shift (δ) values are expressed in parts per million (ppm) and are referenced to the residual solvent signals with resonances at δ_H/δ_C 7.26/77.0 (CDCl₃). ESI-FTMS analyses were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron HR HPLC-FT spectrometer by direct injection into an electrospray interface. Optical rotations were measured on an AUTOPOL® IV automatic polarimeter. IR spectra were recorded on an AATI Mattson Genesis Series FTIR spectrometer. UV spectra were scanned on a Perkin-Elmer Lambda 3B UV/Vis spectrometer. Silica gel (200-400 mesh) and Sephadex LH-20 (Pharmacia) for column chromatography was obtained from Natland International Corporation (www.natland.com) and SIGMA Chemical Co. (USA), respectively. TLC was performed on aluminum sheets (silica gel 60 F₂₅₄, Merck KGaA, Germany).

3.2. Sponge collection, identification and taxonomy

The sponge was collected from inside caves at a depth of -37.5 m off the mouth of the Rio Bueno, Discovery Bay, Jamaica, on 11-14 July 1998. 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 exudate 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 has been compared to Plakortis zyggompha (de Laubenfels) from deep Puerto Rico waters (100-300 m) and P. halichondrioides (Wilson) from shallow Caribbean waters (3–50 m). It differs from both species in the unique coloration in life and ethanol preservative, the emission of characteristic odor and exudates, in the larger size of the diod mesoscleres, except in the case of *P. halichondrioides*, which has diods that range to 200 μm in length.¹⁸ The sponge is thus an undescribed species of Plakortis (Subclass Homoscleromorpha, Order Homosclerophorida, Family Plakinidae). A voucher specimen has been deposited at the Natural History Museum, London, UK (BMNH 1999.7.20.2).

3.3. Extraction and isolation

The sponge was immediately frozen then freeze-dried (10 g) prior to extraction with CH_2Cl_2 -acetone (1:1, total amount 2 L) in a blender. After filtration and evaporation of the dichloromethane and acetone, the combined extracts were dried to give a brownish gum (ca. 800 mg). This material was chromatographed on Si gel (column: 4.5×62 cm) with a hexane-acetone gradient (15:1–1:1) to yield three fractions: fr. 1 (hexane-acetone 15:1–5:1, ca. 85 mg) was chromatographed on Si gel (column: 2.5×45 cm) with a hexane-ethyl acetate gradient (10:1–5:1) to yield compounds 2 (0.022% dry wt) and 3 (0.15% dry wt). Compound 1 (0.045% dry wt) was obtained from fr. 2 (hexane-acetone 3:1, ca. 30 mg) and was purified by prep. TLC (hexane-acetone 6:1, ×3, R_f =0.40). Compound

4 (0.035% dry wt) was obtained from fr. 3 (hexane–acetone 2:1–1:1, ca. 20 mg) and was further purified by gel permeation chromatography on Sephadex LH-20 (column: 4.9×76 cm) in MeOH.

3.3.1. Plakortide I (1). Pale yellowish oil, $[\alpha]_D^{25} = -131^\circ$ (c 0.2, CHCl₃); IR (dry film) ν_{max} : 2937, 2885, 1740, 1697, 1643, 1461, 1367, 1208, 830 cm⁻¹; UV (MeOH) λ_{max} (log ε): 238 (4.271) nm; HRESI-FTMS m/z: 316.2124 ([M+NH₄]⁺, C₁₆H₃₀NO₅ requires 316.2118, Δ +0.6 mmu), 321.1666 ([M+Na]⁺, C₁₆H₂₆O₅Na requires 321.1672, Δ -0.6 mmu), 619.3453 ([2M+Na]⁺, C₃₂H₅₂O₁₀Na requires 619.3452, Δ +0.1 mmu); ¹³C and ¹H NMR (CDCl₃) data: see Tables 1 and 2.

3.3.2. Plakortide J (2). Colorless oil, $[\alpha]_D^{25} = -93^\circ$ (c 0.1, CHCl₃); IR (dry film) ν_{max} : 2932, 2889, 2867, 1737, 1462, 1385, 1375, 1211 cm⁻¹; HRESI-FTMS m/z: 316.2494 ([M+NH₄]⁺, C₁₇H₃₄NO₄ requires 316.2482, Δ +1.2 mmu), 321.2008 ([M+Na]⁺, C₁₇H₃₀O₄Na requires 321.2036, Δ -2.6 mmu), 337.1795 ([M+K]⁺, C₁₇H₃₀O₄K requires 337.1775, Δ +2.0 mmu), 619.4175 ([2M+Na]⁺, C₃₄H₆₀O₈Na requires 619.4180, Δ -0.5 mmu); ¹³C and ¹H NMR (CDCl₃) data: see Tables 1 and 2.

3.3.3. Plakortide K (3). Colorless oil, $[\alpha]_D^{25} = -116^{\circ}$ (c 0.5, CHCl₃); IR (dry film) ν_{max} : 2937, 2882, 2863, 1735, 1460, 1313, 1216 cm⁻¹; HRESI-FTMS m/z: 302.2329 ([M+NH₄]⁺, C₁₆H₃₂NO₄ requires 302.2325, Δ +0.4 mmu), 307.1875 ([M+Na]⁺, C₁₆H₂₈O₄Na requires 307.1879, Δ -0.4 mmu), 323.1620 ([M+K]⁺, C₁₆H₂₈O₄K requires 323.1618, Δ +0.2 mmu); ¹³C and ¹H NMR (CDCl₃) data: see Tables 1 and 2.

3.3.4. Plakortide L (**4**). Colorless oil, $[\alpha]_D^{25} = -44^\circ$ (*c* 0.2, CHCl₃); IR (dry film) ν_{max} : 3030 (br), 2927, 2867, 1703, 1641, 1446, 1209 cm⁻¹; ESI-FTMS *m/z*: 271 ([M+H]⁺; ¹³C and ¹H NMR (CDCl₃) data: see Tables 1 and 2.

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- The trivial names of these new compounds are consistent with Patil's work (see Ref. 12).
- The ¹³C NMR chemical shift (δ) values were estimated by the software of CambridgeSoft Corporation, CS ChemDraw Ultra version 5.0.
- 18. The sponge that we have described has extremely distinct field characteristics in terms of coloration in life and death, and in terms of exudate and odor emissions. The spicules average 190 μm long. This sponge occurs in moderately deep water, and has not usually been collected above 35 m in our experience. The sponge is also not very common and usually restricted to caves and under overhangs. *Plakortis zyggompha* (de Laubenfels) was first described in 1934 from Puerto Rico,

and was collected by dredge between depths of 92-180 m. It was first described under the new genus Roosa de Laubenfels, which has since been synonymized with Plakortis Schmidt. The sponge was described as lamellate, 3×20×35 mm in size, and was blue in alcohol (no color in life is given). The spicules were diods and rare triods 50-140 µm long, de Laubenfels (1934) considered this to be 'a well marked species with no close relatives'. P. zyggompha has since been used as a popular name for the species represented by the sponge described here, by various taxonomic workers over the last 10 years. Species of Plakortis are notoriously variable in the field and there are few reliable distinguishing characteristics for known species. When a specific character such as 'blue coloration' and 'deep-water distribution' is noted from the literature, the species name is often applied without a thorough check of the holotype for confirmation. The distinctive characteristics of P. zyggompha (de Laubenfels) however, as reviewed by Diaz and van Soest (1994) and MK, include it being very thin (3–6 mm thick) and light-colored (cream, buff). The sponge described here is not considered to be conspecific with P. zyggompha, as it is relatively uncommon, occurs in much shallower waters, and is extremely distinctive morphologically. In terms of spicule dimensions, a character that is notoriously difficult to use to differentiate plakortid sponges, the sponge is also relatively distinct; the mean length of diods in this species (190 µm) is larger than the range of dimensions for all known Caribbean species (Plakortis angulospiculatus 100-150 μm; P. zyggompha 50-140 μm; P. simplex 60-150 μm) except P. halichondrioides (130–200 μm).