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Polyketide-peroxides from a Species of Jamaican *Plakortis* (Porifera: Demospongiae)

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A new cyclic peroxide plakortisinic acid (**1**), and a new ketone derivative (**2**), in addition to six known compounds, an α,β-unsaturated ester (**3**), plakortide N (**4**), plakortide F (**5**) and its free acid (**6**), plakortone D (**7**), and a furan-containing molecule (**8**), were isolated from a species of *Plakortis* from Jamaica. The structures were elucidated by interpretation of 1D and 2D NMR spectra, and mass spectrometry data and by comparison with data from the literature. Comparison between experimental and calculated optical rotations allowed the assignment of absolute configuration of **1** and **2**. The isolated compounds have been evaluated for their antimicrobial, antimalarial, anticancer, anti-Mtb, and anti HIV-1 activity.

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Introduction

Marine organisms have attracted enormous interest from scientists in recent decades. The large numbers of structurally diverse compounds that can be obtained from marine organisms have been populating an ever-expanding library of research literature. Sponges are among the most studied marine organisms,[1] and sponges from the Family Plakinidae (Class Demospongiae: Order Homosclerophorida) have revealed a small portion of a vast array of biosynthetically diverse natural products.

In particular, metabolites from the genus *Plakortis* have been found to exhibit a diverse range of bioactivities including anti-tumour, anti-fungal, and other important pharmacological activities. Plakortides are a group of hexacyclic endoperoxides that constitute the major class of metabolites isolated from many *Plakortis* species. Plakortides I and J, isolated from the sponge *P. simplex* Schulze, 1880, were found to inhibit cell growth of the murine fibrosarcoma WEHI 164 cell line with IC_{50} values ranging from 7 to 9 μg mL^{-1}.[2] The first naturally occurring epoxide of this series of metabolites, isolated from the sponge *P. nigra* Lévi, 1953, was active as a cytotoxic agent against the murine lymphocytic leukaemia cell line with an ED_{50} of 0.91 μg mL^{-1}.[3] Plakortones B-F have shown in vitro cytotoxicity against the murine fibrosarcoma WEHI 164 cell line with IC_{50}s ranging from 8.0 to 11.0 μg mL^{-1}.[4] Lehualides, another class of polyketide derived metabolites, were recorded as cytotoxic against both ovarian cancer and leukaemia cell lines.[5] The novel polyketide spiculoic acid and its derivatives, isolated from *P. angulospiculatus* (Carter, 1882) and *P. zygompha* (de Laubenfels, 1934) and occurring in *Plakortis* sp., were cytotoxic against human breast cancer MCF-7 cells.[6–8] Other metabolites, such as the peroxide acids isolated from *P. angulospiculatus*, showed antifungal activity against *Candida albicans* with MICs of 1.6 μg mL^{-1}.[9] Several *Plakortis* metabolites have also been explored for their potential use as antiprotozoal drug candidates. Plakortide F isolated from a Jamaican species of *Plakortis* exhibited significant activity against *Plasmodium falciparum* in vitro with an IC_{50} of 480 ng mL^{-1} for the D6 Clone and IC_{50} of 390 ng mL^{-1} for the W2 Clone.[10] Plakortide Q and plakortin 1 are peroxide metabolites isolated from *P. simplex*, which showed significant antimalarial activity.[11–13] The flagellate protozoan *Leishmania mexicana*, which causes leishmaniasis, was inhibited by a cyclic peroxide isolated from the marine sponge *P.* aff. *angulospiculatus*.[14] Plakortolide and plakortolide G isolated from the marine sponge *Plakinastrella onkodes* (Uliczka, 1929) both exhibited potent inhibitory activity against the protozoan *Toxoplasma gondii* in HFF cells.[15] Although the cyclic peroxides are among the most common secondary metabolites from the *Plakortis* sp. quite a few bioactive alkaloids belonging to different chemical classes such as pyrroloacridine, piperidine, and pyridinum alkaloids were isolated from some

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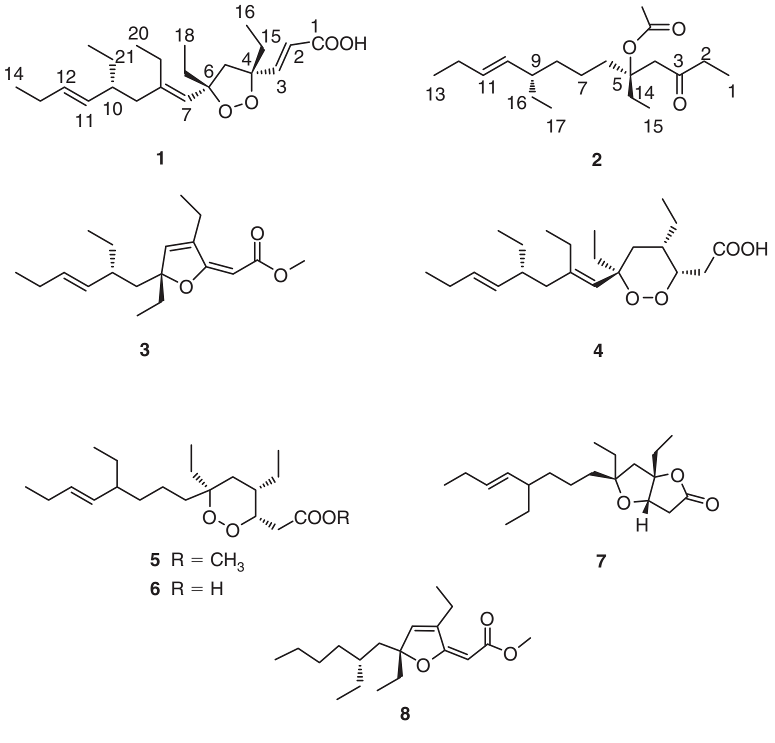
Plakortis species.[16–18] Plakohypaphorines, the first iodoindole alkaloids from *P. simplex*, showed anti-histaminic activity.[19] Other metabolites isolated from different *Plakortis* species have shown other interesting pharmacological and biological activities.[20–26]

In this report, the bioassay guided isolation of a Jamaican species of *Plakortis* afforded a new five-membered ring containing endoperoxide, plakortisinic acid (**1**), and a new ketone derivative (**2**), in addition to the known α,β-unsaturated ester (**3**), the known plakortide N (**4**), plakortide F (**5**) and its free acid (**6**), plakortone D (**7**), and a furan-containing molecule (**8**). *Ab initio* calculations of optical rotations were compared with experiment to help assign the absolute configuration of **1** and **2** (Scheme 1).

Results and Discussion

Plakortisinic acid (**1**) (Table 1) was obtained as a yellow oil (4 g), $[\alpha]_D^{25}$ +120 (*c* 0.1, CHCl$_3$), and gave a molecular formula of $C_{22}H_{36}O_4$ by HRESI-MS: $[M-H]^-$ *m/z* 363.2623 (calc. 363.2613). The IR spectrum shows a broad hydroxyl band at 3435 cm^{-1} and a strong carbonyl band at 1713 cm^{-1} indicating the presence of carboxylic acid functionality. Close inspection of ^{1}H, ^{13}C, and DEPT NMR experiments allowed the assignment of seven CH$_2$, one CH, six olefinic, five CH$_3$, and two oxygenated quaternary carbons. The molecular formula indicates that there are five degrees of unsaturation (three double bonds for the six olefinic carbons, one for the carboxylic acid functionality and one ring). The presence of two oxygenated carbons suggested the existence of a peroxide functionality. ^{1}H and COSY NMR experiments showed five ethyl groups. HMQC, COSY, and HMBC NMR experiments allowed the assignment of two major partial structures. An HMBC correlation was observed between the C-15 protons at δ 1.72 and 1.80 and C-4 and C-3 at δ 87.3 and 152.4, respectively. The C-2 proton at δ 6.10 gave an HMBC correlation to C-1, C-3, C-4, and C-5. The C-5 protons at δ 2.47 and 2.52 gave an HMBC correlation to both C-4 and C-6. C-15 gave an HMBC correlation to the C-5 protons at δ 2.47 and 2.52, as well as the C-3 proton at δ 6.86. Finally, the COSY correlation between the C-15 protons at δ 1.72 and 1.80 and the C-16 proton at δ 0.91, as well as the COSY between the C-2 proton at δ 6.10 and the C-3 proton at δ 6.86 completed the first partial structure. The C-12 proton at δ 5.24 showed an HMBC correlation to C-11 and C-13, as well as C-10 and C-14. The C-21 protons at δ 1.12 and 1.34 showed COSY correlations to both C-10 and C-22 protons at δ 1.96 and 0.81, respectively. The C-7 proton at δ 5.18 exhibited a ^2J correlation to C-8 and C-6, as well as a ^3J correlation to C-9, C-19, and C-17. C-6 showed an HMBC correlation to the C-17 protons at δ 1.62 and 1.88, which in turn showed a COSY correlation to the C-18 protons at δ 0.92. A COSY correlation between the protons of C-10 and C-9, as well as an HMBC correlation between the C-10 proton and C-8 allowed the assignment of the second partial structure. The two partial structures completed the final suggested structure through an HMBC correlation between the C-7 proton and C-5 and between the C-5 proton and C-6. The two oxygenated quaternary carbons at δ 87.3 and 89.5 became part of a pentacyclic ring containing the peroxide functionality. The *E* configuration of the double bond between C-2 and C-3 was assigned by the coupling constants of 15.6 and 16.2 Hz for the doublets at δ 6.10 and 6.86 in the ^{1}H NMR spectrum, respectively. The large coupling constant between the C-11 and C-12 protons at δ 5.14 (dd, *J* 7.8, 14.4) and 5.24 (dt, *J* 6, 15), respectively, allowed the assignment of the *E* configuration to the second double bond in the side chain of the structure. The relative configuration of the peroxide ring and the configuration of the double bond between C-7 and C-8 were assigned by interpretation of the NOESY spectrum (Fig. 1). The α proton of C-5 at δ 2.52 showed an NOE correlation with the C-3 proton at δ 6.86 and the C-7 proton at δ 5.18, while it



Scheme 1.

did not show any correlation to the C-15 or C-17 protons. The proton of C-7 also exhibited NOE correlation with the proton of C-9 at δ 2.01. This allowed the assignment of an *E* configuration between C-7 and C-8, as well as a *cis* configuration between the two ethyl groups at C-4 and C-6. The ^{1}H, ^{13}C NMR and selected HMBC, COSY, and NOESY correlations are tabulated in Table 1.

E-5,9-Diethyl-3-oxotridec-10-en-5-yl acetate (**2**) (Table 2) was obtained as a colourless oil (2 g), $[\alpha]_D^{25}$ +22 (*c* 0.1, $CHCl_3$), and gave a molecular formula of $C_{19}H_{34}O_3$ by HRESI-MS: $[M + Na]^+$ *m/z* 333.2228 (calc. 333.2400). Close inspection of ^{1}H, ^{13}C and DEPT 135°C NMR spectra showed one methine, eight methylene, five methyls, two disubstituted olefinic carbons, two carbonyls, and one oxygenated quaternary carbon. COSY and HMBC correlations gave two primary partial structures. Four ethyl groups were confirmed from COSY correlations. Protons at C-1 resonating at δ 1.00 gave an HMBC correlation to the carbonyl carbon at δ 208.8, which in turn gave an HMBC correlation to the protons of C-4 at δ 2.89. The protons of C-14 at δ 1.81 gave a COSY correlation to the protons of C-15 at δ 0.80 and resulted in the first partial structure. The two olefinic protons of carbons C-12 and C-11 at δ 5.24 and 5.14, respectively, gave mutual COSY correlations. C-11 gave an HMBC correlation to the proton of C-9 at δ 1.67 and another ^3J correlation to the protons of C-13 at δ 0.98, which gave a COSY correlation to the C-12 protons at δ 1.99. The protons of C-9 at δ 1.67 gave COSY correlations to the protons of C-16 at δ 1.06 and 1.25 and gave an HMBC correlation to C-17 at δ 11.84. The C-9 protons at δ 1.67 showed a COSY correlation to the C-8 protons at δ 1.19 and completed the second partial structure. C-6 gave an HMBC correlation to the C-4 protons at δ 2.39, and its protons at δ 1.23 gave a COSY correlation to the C-7 protons at δ 1.90. The CH_3 group at δ 22.4 showed only one ^2J HMBC correlation to the carbonyl carbon at δ 175.8 suggesting an acetyl functionality. According to the molecular formula, only three degrees of unsaturation are present (two for the carbonyls and one for the olefinic bond). In addition, the structure lacks the peroxide-containing ring (only one oxygenated carbon resonating at δ 87.8 ppm), so the final structure was proposed to be *E*-5,9-diethyl-3-oxotridec-10-en-5-yl acetate. The *E* configuration was assigned by comparison of ^{13}C and ^{1}H NMR chemical shifts to the similar side chain found on a known peroxide [27] and also from the coupling constants of the proton of C-11 at δ 5.33 (dt, *J* 6, 15) and the proton of C-12 at δ 5.04 (dd, *J* 8.4, 15). The ^{1}H, ^{13}C NMR and selected HMBC and COSY correlations are tabulated in Table 2.

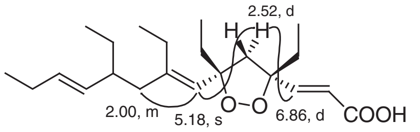


Fig. 1. NOESY correlations of plakortisinic acid (**1**).

Recent advances in computational tools have allowed for *ab initio* methods of varying accuracy and computational complexity such as Hartree–Fock (HF), density functional theory (DFT), and coupled cluster methods to be used to calculate optical rotations to compare with experimental optical rotations to assist in assigning the absolute stereochemistry.[28,29] DFT methods are considered best for flexible medium-sized molecules, since they include electron-correlation effects but are also computationally tractable. Computational techniques are able to handle very flexible molecules by including all low-energy conformations. Often theoretical studies on optical rotation calculation for flexible natural products have only used molecular mechanics (MM) geometries and coupled-HF optical rotations. While MM is relatively inexpensive computationally for geometry determination, it lacks the accuracy that *ab initio* methods provide. It is important also to determine the relative energies accurately, another task to which *ab initio* methods are more amenable than MM ones.

Table 1. ^{13}C and ^{1}H NMR data of plakortisinic acid 1

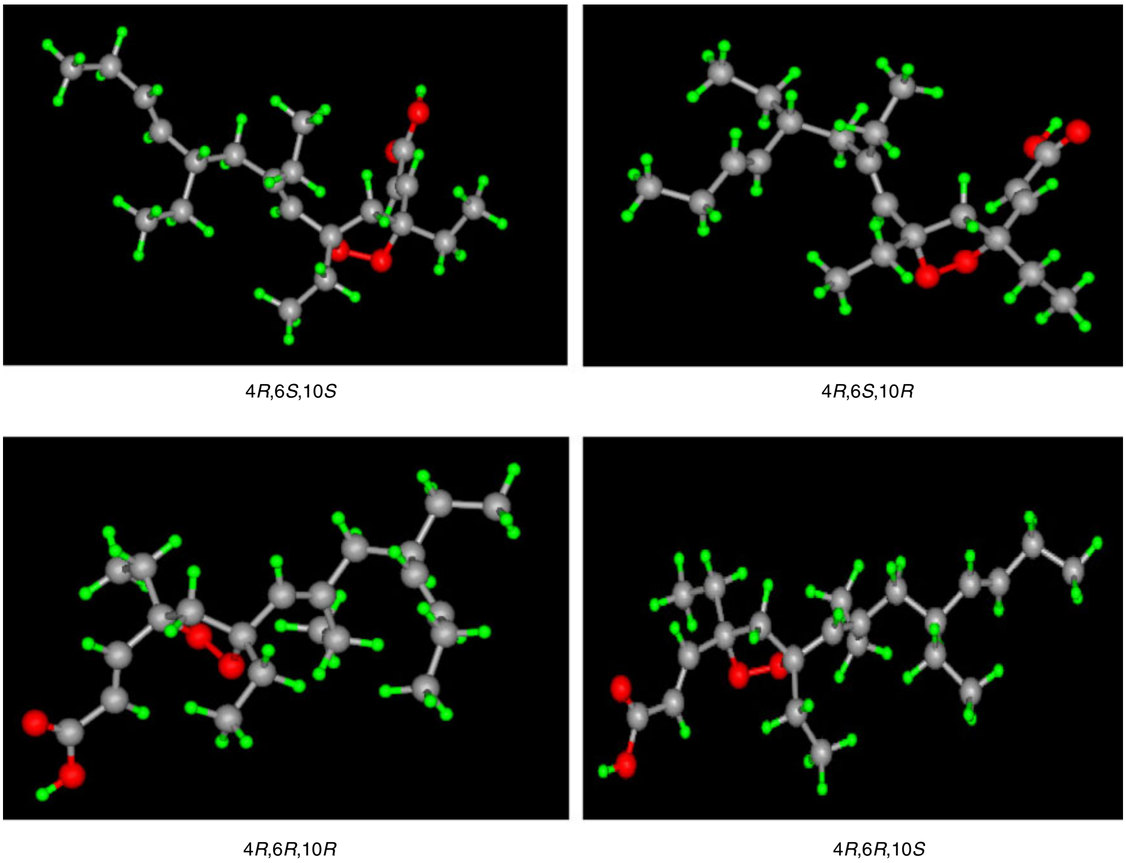
In $CDCl_3$, 600 MHz for ^{1}H and 150 MHz for ^{13}C NMR. Carbon multiplicities were determined by DEPT experiments. Coupling constants (*J*) are in Hz

Position	δ_C	δ_H (mult., *J* in Hz)	HMBC (^{1}H–^{13}C)	COSY ($\delta_{H\ ppm}$–$\delta_{H\ ppm}$)	NOESY ($\delta_{H\ ppm}$–$\delta_{H\ ppm}$)
1	171.9, qC				
2	119.9, CH	6.10 d (15.6 Hz)	C-1, C-4	6.86 ppm	
3	152.4, CH	6.86 d (16.2 Hz)	C-5, C-15		2.52 ppm
4	87.3, qC				
5	56.5, CH_2	2.47, 2.52 q (12.0 Hz)	C-3, C-4, C-6, C-15		
6	89.5, qC				
7	126.4, CH	5.18 s	C-6, C-8, C-17		2.52 ppm, 2.06 ppm
8	142.3, qC				
9	42.2, CH_2	1.86 m, 2.06 m	C-7, C-10	1.96 ppm	
10	42.8, CH	1.96 m	C-12, C-22	1.12 ppm	
11	133.1, CH	5.14 dd (7.8, 14.4 Hz)	C-12	5.24 ppm	
12	132.2, CH	5.24 dt (6.0, 15.0 Hz)	C-10, C-11, C-13, C-14	1.93 ppm	
13	25.8, CH_2	1.93 m	C-12	0.89 ppm	
14	14.2, CH_3	0.89 t (6.0, 13.8 Hz)	C-12		
15	30.9, CH_2	1.72 m, 1.80 m	C-4, C-5	0.91 ppm	
16	9.0, CH_3	0.91 t (12.6, 6 Hz)	C-4, C-15		
17	33.0, CH_2	1.62 m, 1.88 m	C-6, C-7	0.92 ppm	
18	9.1, CH_3	0.92 t (12.6, 6 Hz)	C-6, C-17		
19	24.2, CH_2	1.92 m, 2.05 m	C-8, C-20	0.95 ppm	
20	12.6, CH_3	0.95 t (15.5, 8.4 Hz)	C-8		
21	28.1, CH_2	1.12 m, 1.34 m	C-10	0.81 ppm	
22	11.8, CH_3	0.81 t (7.2, 15 Hz)	C-10		

Table 2. ^{13}C and ^{1}H NMR data of *E*-5,9-diethyl-3-oxotridec-10-en-5-yl acetate 2

In $CDCl_3$, 600 MHz for ^{1}H and 150 MHz for ^{13}C NMR. Carbon multiplicities were determined by DEPT experiments. Coupling constants (*J*) are in Hz

Position	^{13}C δ	^{1}H δ (mult., *J* in Hz)	HMBC (^{1}H–^{13}C)	COSY ($\delta_{H\ ppm}$–$\delta_{H\ ppm}$)
1	7.9, q	1.00, t	C-3	2.39 ppm
2	37.7, t	2.39, q (7.2, 14.4 Hz)	C-3, C-1	
3	208.8, s			
4	46.8, t	2.98, s	C-3, C-5, C-6	
5	87.8, s			
6	35.4, t	1.23, m	C-4	1.90 ppm
7	35.5, t	1.90, m	C-10	
8	28.7, t	1.19, m		
9	44.3, d	1.67, m	C-16, C-17, C-10, C-11	1.06 ppm
10	133.5, d	5.04, dd (8.4, 15.5 Hz)	C-9	5.33 ppm
11	132.5, d	5.33, dt (6.5, 15.5 Hz)	C-12, C-13	
12	25.8, t	1.99, m	C-11, C-13	0.98 ppm
13	14.4, q	0.98, t	C-11	
14	28.5, t	1.81, m; 2.00, m	C-5, C-4	0.80 ppm
15	11.8, q	0.80, t	C-5	
16	21.1, t	1.06, m; 1.25, m	C-9	0.08 ppm
17	7.9, q	0.80, t	C-9	
18	175.8, s			
19	22.4, q	1.89, s	C-18	



4*R*,6*S*,10*S* 4*R*,6*S*,10*R*

4*R*,6*R*,10*R* 4*R*,6*R*,10*S*

Fig. 2. HF/6–31G* optimized lowest-energy structures for **1**.

To assign the absolute configuration of **1** and **2**, we chose to use the reliable DFT hybrid functional and basis set combination B3LYP/6–31G* for the actual optical rotation calculations, but tested the effects of using either MM or HF geometries, and either MM, HF, or DFT for the relative energies of the conformations (Figs 2, 3). Table 3 shows the computed optical rotation angles calculated at 589.3 nm using B3LYP/6–31G* method for diastereomers of **2**. At the MMFF94 optimized

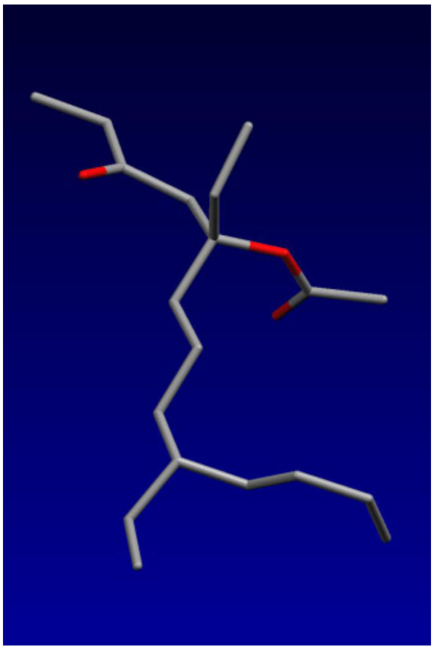


Fig. 3. HF/6–31G* optimized lowest-energy structures 5*R*,9*R* configuration for **2**.

Table 3. Computed optical rotation angles calculated at 589.3 nm using B3LYP/6–31G* method for *E*-5,9-diethyl-3-oxotridec-10-en-5-yl acetate (2), having experimental $[\alpha]^{25}_{D} = +22$ (*c* 0.1, $CHCl_3$)
Best agreement in bold

Geometry energy	Boltzmann-averaged angles[A]			
	MMFF94 MMFF94	MMFF94 B3LYP[B]	HF[B] HF[B]	HF[B] B3LYP[B]
5*R*,9*R*	+19	+9	−19	−25
5*S*,9*S*	−19	−9	+19	**+25**
5*R*,9*S*	+31	+30	−6	−1
5*S*,9*R*	−31	−30	+6	+1

[A] deg $[\alpha]_D$.
[B] With the 6–31G* basis set.

geometries using either MMFF94 or B3LYP relative energies, the optical rotation values differed in sign and order of magnitude compared with those at the HF/6–31G* geometries. At the HF optimized geometries of **2**, the B3LYP optical rotations calculated using B3LYP relative energies, which in general are more accurate predictions,[30] were not much different from those calculated using HF relative energies. Based on typical accuracy of methods, the best results are those with B3LYP calculated relative energies at the HF geometries, and at that level there is a very good agreement between the predicted value of +25 and the experimental optical rotation of +22. We therefore assigned the absolute configuration of **2** as 5*S*,9*S*.

Plakortisinic acid, **1**, is another flexible biologically active molecule. Table 4 shows the computed optical rotation angles calculated at 589.3 nm using the B3LYP/6–31G* optical rotations and relative energies. For **1**, the optical rotation values obtained at MMFF94 geometries do not differ in sign compared with those at the HF geometries. Assuming that the HF geometries are more accurate, the best agreement with the experimentally determined value was found to be +119. We therefore assigned the absolute configuration of **1** as 4*R*,6*S*,10*R*.

Table 4. Computed optical rotation angles calculated at 589.3 nm using B3LYP/6–31G* method for plakortisinic acid (1), having experimental $[\alpha]^{25}_{D}$ +120 (*c* 0.1, $CHCl_3$)
Best agreement in bold

Geometry energy	Boltzmann-averaged angles[A]	
	MMFF94 B3LYP[B]	HF[B] B3LYP[B]
4*R*,6*S*,10*S*	+154	+101
4*S*,6*R*,10*R*	−154	−101
4*R*,6*S*,10*R*	+93	**+119**
4*S*,6*R*,10*S*	−93	−119
4*R*,6*R*,10*R*	+182	+172
4*S*,6*S*,10*S*	−182	−172
4*R*,6*R*,10*S*	+113	+146
4*S*,6*S*,10*R*	−113	−146

[A] deg $[\alpha]_D$.
[B] With the 6–31G* basis set.

Conclusion

In conclusion, both of the cyclohexane endoperoxides, plakortide N, and plakortide F free acid were active in the antimicrobial assays. They both exhibited activity against *C. albicans*, *C. glabrata*, *C. krusei*, and *C. neoformans* with IC_{50}s ranging from 0.25 to 3.0 $\mu g\,mL^{-1}$. Both compounds showed potent activity against *L. donovani*, with IC_{50}s of 3.1 and 2.8 $\mu g\,mL^{-1}$, respectively. In the previously mentioned biological assays, the cyclopentane endoperoxide (plakortisinic acid) was totally inactive. Only plakortide F (methyl ester) exhibited moderate activity against the *P. falciparum*. In general plakortide N and plakortide F acid were moderately cytotoxic against the NCI tumour cell lines. In addition, they were remarkably active against colon cancer (KM12 cell line) and melanoma (LOX IMVI cell line) with GI_{50}s of 0.02–0.3 μM. Plakortisinic acid was completely inactive against all tumour cell lines. Only plakortide N exhibited anti-HIV activity in human PBM cells with an EC_{50} of 7.8 and an EC_{90} 22.1 μM, but was remarkably cytotoxic to CEM and *Vero* cells. Plakortide F acid was moderately active against Mtb with 83% inhibition at 64 $\mu g\,mL^{-1}$.

Experimental

General Experimental Procedures

IR and UV spectra were obtained using an AATI Mattson Genesis Series FTIR and a Perkin–Elmer Lambda 3B UV/vis spectrophotometer. Optical rotations were measured on an AUTOPOL IV autopolarimeter. 1D and 2D NMR spectra were recorded on a Bruker Avance DRX 400 and 600 spectrometer. Chemical shift (δ) values are expressed in ppm and are referenced to the residual solvent signals with resonances at δ_H/δ_C 7.26/77.0 ($CDCl_3$). High resolution MS was obtained using Bruker Daltonics microTOF with microspray ionization resources using positive or negative ion mode. Si gel (200–400 mesh) and Sephadex LH-20 (Pharmacia) for column chromatography was obtained from Natland International Corporation and SIGMA Chemical Co. (USA), respectively. C8 and Si HPLC (Waters 510 model system), Luna 5 μm, C8 (2) 100 Å, 60 × 21.20 mm.

Sponge Collection, Identification, and Taxonomy

The sponge was collected at a depth of 18–20 m at Discovery Bay, Jamaica, on 7 June 2001. The sponge forms a thick

Table 5. In vitro antimicrobial activities against AIDS opportunistic infections for compounds 1–8
NA, not active; NT, not tested

Cpd. #	Antimicrobial (IC_{50}/MIC, $\mu g\,mL^{-1}$)							
	Candida albicans	*Cryptococcus neoformans*	*Candida glabrata*	*Candida krusei*	*Staphylococcus aureus*	MRS[A]	*Mycobacterium intracellulare*	*Aspergillus fumigatus*
1	NA	NA	NA	NA	NA	NA	NA	NA
2	NA	NA	NA	NA	NA	NA	NA	NA
3	NA	NA	NA	NA	NA	NA	NA	NA
4	**3.0/5.0**	**5.5/–**	**0.8/1.25**	**1.5/2.5**	NT	15/–	20/–	NT
5	NA	NA	NA	NA	NA	NA	NA	NA
6	**1.5/2.5**	**2.5/5.0**	**0.25/1.25**	**0.45/1.25**	NT	NA	10/20	5.0/–
7	NA	NA	NA	NA	NA	NA	NA	NA
8	NA	NA	NA	NA	NA	NA	NA	NA
Amphotericin B	NT	0.15	NT	NT	NT	NT	NT	NT
Ciprofloxacin	NT	NT	NT	NT	0.10	0.10	0.25	NT

encrusting pad and is brown-tan in life. The texture is dense and uniform, with a surface that is velvety and soft to the touch. The spicules are diods in two possible size categories, and lacks triods. The sponge is a species of *Plakortis* (Order Homosclerophorida: Family Plakinidae) closely comparable to *Plakortis angulospiculatus* (Carter, 1882) in the sense of Diaz & van Soest (1994) and Lehnert & van Soest (1998). A voucher specimen has been deposited in the Natural History Museum, London (BMNH 2000.7.17.6).

Extraction and Isolation

The frozen sponge material (6 kg) was extracted with EtOH until exhaustion; the dried extract (600 g) was subjected to Si vacuum liquid chromatography (VLC), using a gradient of *n*-hexane to ethyl acetate and finally MeOH. Ten fractions were obtained and all were submitted to cytotoxic assay screening. Only fractions 3 and 4 eluted with 25% and 50% ethyl acetate in *n*-hexane respectively showed remarkable cytotoxic activity against HT29, A549, and SK-MEL-28 cell lines in the PharmaMar cytotoxic screening assay. The two fractions (132 g) showed similar ^1H NMR spectra and TLC patterns and were combined and rechromatographed on Si VLC column using gradient elution technique of *n*-hexane, ethyl acetate then methanol. A total of 20 fractions were collected and screened by TLC. All subfractions were further individually purified on VLC Si columns (using gradient of *n*-hexane to ethyl acetate or acetone and finally MeOH), HPLC (C8 and Si, using gradient of acetonitrile to water and hexane to ethyl acetate and finally MeOH, respectively), Sephadex LH-20 (isocratic of MeOH and MeOH/chloroform 1:1) to produce compounds from **1** to **8**.

α,β-Unsaturated Ester (**3**)

Obtained as an oil (27 g), $[\alpha]_D^{25}$ −246 (*c* 0.2 in $CHCl_3$), and its molecular formula was determined to be $C_{19}H_{30}O_3$ by HRMS (ESI): *m/z* 307.2235 $[M+H]^+$ (calc. 307.2273). The ^1H and ^{13}C NMR chemical shifts were within ±1 ppm of the values reported in the literature.[31,32]

Plakortide N (**4**)

Obtained as transparent oil (500 mg), $[\alpha]_D^{25}$ −146 (*c* 0.2 in $CHCl_3$); its molecular formula was determined to be $C_{22}H_{38}O_4$ by HRMS (ESI): *m/z* 755.5319 $[\text{dimer}+Na]^+$ (calc. 755.5432). The ^1H NMR chemical shifts were comparable to those reported in the literature, and the ^{13}C NMR chemical shifts were within ±1 ppm of the values reported in the literature.[27]

Plakortide F (**5**)

Obtained as pale yellow oil (3 g), $[\alpha]_D^{25}$ −112 (*c* 0.1 in $CHCl_3$). The ^1H NMR chemical shifts were comparable to those reported in the literature, and the ^{13}C NMR chemical shifts were within ±1 ppm of the values reported in the literature.[22,33] Its molecular formula was determined to be $C_{21}H_{38}O_4$ by HRMS (ESI): *m/z* 731.5361 $[\text{dimer}+Na]^+$ (calc. 731.5432).

Plakortide F Acid (**6**)

Obtained as faint yellow oil (9 g), $[\alpha]_D^{25}$ −154 (*c* 0.1 in $CHCl_3$). The ^1H NMR chemical shifts were comparable to those reported in the literature, and the ^{13}C NMR chemical shifts were within ±1 ppm of the values reported in the literature.[33] Its molecular formula was determined to be $C_{20}H_{36}O_4$ by HRMS (ESI): *m/z* 703.5170 $[\text{dimer}+Na]^+$ (calc. 703.5119).

Plakortone D (**7**)

Obtained as faint yellow oil (300 mg), $[\alpha]_D^{25}$ −28 (*c* 0.2 in $CHCl_3$). The ^1H NMR chemical shifts were comparable to those reported in the literature, and the ^{13}C NMR chemical shifts were within ±1 ppm of the values reported in the literature.[23] Its molecular formula was determined to be $C_{20}H_{34}O_3$ by HRMS (ESI): *m/z* 323.2589 $[M+H]^+$ (calc. 323.2632).

Methyl (2Z,6R,8S)-3,6-Epoxy-4,6,8-triethyldodeca-2,4-dienoate (**8**)

Obtained as faint yellow oil (50 mg), $[\alpha]_D^{25}$ −94 (*c* 0.2 in $CHCl_3$). The ^1H NMR chemical shifts were comparable to those reported in the literature, and the ^{13}C NMR chemical shifts were within ±1 ppm of the values reported in the literature.[14] Its molecular formula was determined to be $C_{20}H_{34}O_3$ by HRMS (ESI): *m/z* 331.2162 $[M+Na]^+$ (calc. 331.2243).

In Vitro Evaluation of Antiparasitic, Antimicrobial, and Cytotoxic Activities

Antimalarial activity of the compounds was determined in vitro on chloroquine-sensitive (D6, Sierra Leone) and chloroquine-resistant (W2, Indo-China) strains of *P. falciparum*. The 96-well microplate assay is based on evaluation of the effect of the compounds on growth of asynchronous cultures of *P. falciparum*,

Table 6. In vitro anti-Mtb and antiprotozoal activities against malaria and *Leishmania* parasites for compounds 1–8

IC_{50} is the concentration [μg mL^{-1}] that affords 50% inhibition of growth. $IC_{50} \leq 15$ μg mL^{-1} is considered active. NA, not active; NC, no cytotoxicity; NT, not tested. Selectivity Index (S.I.) = IC_{50} (*Vero* cells)/IC_{50} (*P. falciparum*)

Cpd. #	*Leishmania donovani*		*Plasmodium falciparum* (D6 Clone)		*Plasmodium falciparum* (W2 Clone)		*M. tuberculosis*		Cytotoxicity (*Vero*)
	IC_{50} [μg mL^{-1}]	IC_{90} [μg mL^{-1}]	IC_{50} [ng mL^{-1}]	S.I.	IC_{50} [ng mL^{-1}]	S.I.	Test conc. [μg mL^{-1}]	% inhibition	TC_{50} [ng mL^{-1}]
1	NA	NA	>4760	>1.0	4700	>1.0	64	72	NC
2	NA	NA	NA	–	NA	–	–	–	NC
3	19	35	NA	–	NA	–	–	–	NC
4	**3.1**	**6.6**	>4760	>1.0	4500	>1.1	64	78	NC
5	16	34	1200	>4.0	900	>5.3	16	34	NC
6	**2.8**	**8.3**	NA	<0.8	NA	<0.8	64	**83**	3800
7	NA	NA	2500	>1.9	2800	>1.7	–	–	NC
8	NA	NA	NA	NA	NA	NA	–	–	NC
Rifampicin	NT	NT	NT	–	NT	–	MIC [μg mL^{-1}] = 0.5		NT
Chloroquine	NT	NT	15.5	–	170	–	–	–	NT
Artemisinin	NT	NT	NT	–	NT	–	–	–	NT
Pentamidine	2.1	10	NT	–	NT	–	–	–	NT
Amphotericin B	0.06	0.15	NT	–	NT	–	–	–	NT

determined by the assay of parasite lactate dehydrogenase (pLDH) activity.[34] The standard serial dilutions for six concentration of the compounds starting with 2 mg mL^{-1} were prepared in RPMI 1640 medium and added to the cultures of *P. falciparum* (2% hematocrit, 2% parasitemia) set up in clear flat-bottomed 96-well plates. The plates were placed in a modular incubation chamber, flushed with a gas mixture of 90% N_2, 5% CO_2, and 5% O_2 and incubated at 37°C for 72 h. Growth of the parasite in each well was determined by pLDH assay using Malstat reagent.[34] The medium and RBC controls were included on each plate. The standard antimalarial agents, chloroquine and artemisinin, were used as the positive controls while DMSO was tested as the negative control.

Antileishmanial activity of the compounds was tested in vitro on a culture of *L. donovani* promastigotes. In a 96-well microplate assay compounds with appropriate dilution were added to the *Leishmania* promastigotes culture (2×10^6 cells mL^{-1}). The plates were incubated at 26°C for 72 h and growth of *Leishmania* promastigotes was determined by the Alamar blue assay.[35] Pentamidine and amphotericin B were used as the standard antileishmanial agents.

Amphotericin B and ciprofloxacin were used as positive drug controls in the antimicrobial assays against AIDS opportunistic pathogens. AZT was used as the positive drug control in the HIV-1 assay, and rifampin was used as the positive drug control in the Mtb assay. Antimicrobial activity against opportunistic pathogens, antituberculosis activity against *M. tuberculosis*,[36] and anti-HIV[37–39] activity were evaluated by previously published procedures.

The in vitro antimicrobial and antiprotozoal, anti-Mtb and anti HIV-1 activities of the identified peroxides are tabulated in Tables 5–7. Plakortisinic acid, plakortide N, and plakortide F free acid were evaluated for their cytotoxic activity in the NCI 60 cell lines (tables are not shown).

Computational Methods

Unique low-energy geometries were obtained by Monte Carlo conformational search using the *SYBYL 7.0* program (Sybyl. 7.0; Tripos: St. Louis, MO, USA; 2006) with the MMFF94 force field.[40] After building the required structure of a specified configuration, energy minimization was performed using the MMFF94 force field using the Random Search procedure implemented in *SYBYL*. Each low-energy conformation was minimized using a 1000 step energy minimization iteration method forcing the newly found structures to be fully relaxed. Further, all the structures were fully optimized at the HF 6–31G* level using *Gaussian* software (g03)[41] to find the lowest-energy conformations.

The optical rotations were computed using B3LYP/6–31G* hybrid DFT calculations with g03, the sodium D-line wavelength, 589.3 nm, was used and no solvent effect was included. The rotations were Boltzmann-weighted (based on the MM and HF relative energies of the conformations) and summed to obtain the optical rotation. 20–30 conformations were computed for each stereoisomer. Rotation angles were computed for each conformation up to 2–3 kcal mol^{-1} above the lowest-energy conformer and these were included in the Boltzmann sum. The Boltzmann-weighted average was done using the equation:

$$\langle A \rangle = \frac{\Sigma_i [\alpha_D] e^{-\varepsilon_i/kT}}{\Sigma_i e^{-\varepsilon_i/kT}}$$

Table 7. In vitro anti-HIV-1 activity in human PBM cells for compounds 1, 3, 4, and 6

Cpd. #	Anti-HIV-1 activity in human PBM cells		Cytotoxicity (IC$_{50}$-μM)		
	EC$_{50}$ [μM]	EC$_{90}$ [μM]	PBM	CEM	*Vero*
1	53.9	$>$100	11.2	44.3	45.0
3	18.2	82.6	–	–	–
4	**7.8**	**22.1**	**<1.0**	**6.8**	**2.3**
6	12.1	40.6	1.5	1.3	1.5
AZT^{23}	0.004	–	–	–	–

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