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New peroxylactones from the Jamaican sponge *Plakinastrella onkodes*, with inhibitory activity against the AIDS opportunistic parasitic infection *Toxoplasma gondii*

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Abstract—An analysis of the chemical constituents of *Plakinastrella onkodes* collected in Jamaica yielded three cyclic peroxides, including the known plakortolide (**1**) and two new analogs of **1**. The absolute configuration of plakortolide G ((3*R*,4*R*,6*R*,8*S*)-**3**) was determined by ab initio optical rotation computations using a coupled Hartree–Fock (CHF) method implemented in CADPAC. Plakortolide (**1**) (*J. Org. Chem.* **1980**, *45*, 3396) and plakortolide G (**3**) exhibited potent inhibitory activity against the protozoan *Toxoplasma gondii* in HFF cells and represent the first marine natural products reported with *T. gondii* inhibitory activity.

Individuals with AIDS and other immunosuppressive disorders are susceptible to numerous protozoal, bacterial, fungal, and viral infections.[2–4] Among these is toxoplasmosis which is caused by the obligate intracellular protozoan parasite *Toxoplasma gondii*, which is a member of the phylum *Apicomplexa* and a major cause of morbidity and mortality in immunocompromised patients.[5] During a continuing investigation of marine natural products as drug leads against infectious diseases, two new cyclic peroxides incorporating a lactone ring and a terminal phenyl group were identified. Sponges of the family Plakinidae have yielded peroxylactones[1,6–8] as well as numerous other peroxide containing metabolites.[9–28] Previous reports indicate that the plakortolides are cytotoxic to tumor cell lines with plakortolides B–D exhibiting modest activity against protein kinase C isoenzymes.[9–11] We report here the isolation and structure elucidation of two new peroxylactones, as well as the inhibitory activity of these compounds against the protozoan *T. gondii.*

The sponge *Plakinastrella onkodes* was collected using closed circuit rebreathers from steep vertical slopes at a depth of −40 m near the mouth of the Rio Buenos, Discovery Bay, Jamaica. The combined extract (1:1 EtOAc/IPA and 1:1 EtOH/H_2O) was chromatographed on Si gel with a step-wise solvent gradient (hexane, ethyl acetate, methanol and finally H_2O). The fraction eluting with 1:1 hexane/ethyl acetate was further purified by reverse phase HPLC (5 μM ODS 3 100 Å, 10×250 mm) using a methanol/H_2O gradient. Final purification with a CH_3CN/H_2O solvent gradient yielded plakortolide (**1**) (162.0 mg, 1.62×10^{-2}% wet wt), plakortolide F (**2**, 5.0 mg, 5.0×10^{-4}% wet wt) and plakortolide G (**3**, 7.0 mg, 7.0×10^{-4}% wet wt).

High resolution ESI FT-ICR MS (ion cyclotron resonance) of lactone **2** provided a molecular formula $C_{24}H_{34}O_5$ requiring eight degrees of unsaturation. The infrared spectrum showed strong bands at 1782 and 3411 cm^{-1} indicating a five-membered lactone and hydroxyl functionality. The ^{13}C, ^{1}H, DEPT-135° and HMQC NMR experiments allowed for the assignment of seven methine, seven methylene, and four methyl groups (Table 1). The remaining quaternary centers consisted of aromatic (δ 142.0), carbonyl (δ 172.4), olefinic (δ 136.7) and three oxygenated (δ 107.8, 95.1, and 86.6) carbon signals. The chemical shift values of the three oxygenated carbons suggested ketal and peroxide functionality. The ^{1}H NMR spectrum (Table 1) showed resonances at δ 7.19–7.32 (m, 5H), 3.38 (d, 2H, *J*=7.3 Hz), 5.36 (br t, 1H, *J*=7.3 Hz) and 1.74 (br s, 3H assigned as a 1-methyl-3-phenylpropenyl moiety based on the HMBC correlations shown in Table 1. The AB pattern at δ 2.38 (1H, d, *J*=14.4 Hz, 5β) and 2.06 (1H, d, *J*=14.4 Hz, 5α) is homologous to the related bicyclic ring system reported earlier.[32] The peroxy hemiketal assignment was based on the ^{13}C NMR chemical shift (δ 107.8), IR hydroxy

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Table 1. ^{13}C (125 MHz, $CDCl_3$) NMR shifts for compounds **1–3**

Carbon no.	Compound				
	1 δ_C	**2** δ_C	HMBC	**3** δ_C	HMBC
1	173.9	172.4	H-2	174.4	H-2, H-3
2	34.4	41.6		34.5	
3	81.3	107.8	H-2, H-5, H-19	81.2	H-2, H-5, H-19
4	82.6	95.1	H-2, H-5, H-19	83.0	H-3, H-5, H-19
5	41.9	48.1	H-7, H-19, H-20	41.6	H-7, H-19, H-20
6	80.4	86.6	H-5, H-7, H-20	80.8	H-5, H-20
7	50.4	51.5	H-20, H-21	48.2	H-5, H-20, H-21
8	27.5	29.5	29.5 H-7, H-21	28.3	H-7, H-21
9	44.2	38.0	H-7, H-11, H-21	37.9	H-7, H-10, H-21
10	133.7	25.3	25.3 H-11	25.3	H-11
11	130.8	39.9	H-13, H-22	39.0	H-13, H-22
12	134.5	136.7	H-11, H-14, H-22	136.6	H-11, H-13, H-14, H-22
13	127.4	123.3	H-11, H-14, H-22	123.1	H-11, H-14, H-22
14	34.0	34.5	H-13, H-16	34.4	H-13, H-16
15	141.6	142.0	H-14, H-17	142.0	H-14, H-16
16	128.5 (2)	128.6 (2)	H-14, H-17	128.6 (2)	H-14, H-17
17	128.4 (2)	128.5 (2)		128.5 (2)	H-16
18	125.9	126.0	H-16	125.9	H-16
19	24.7	20.8	H-5	26.1	H-3, H-5
20	25.1	27.0	H-5	23.0	H-5
21	20.8	21.5	H-7	22.0	H-7
22	17.4	16.4	H-11, H-13	16.3	H-13
23	16.8				

absorption band and the high resolution mass analysis. The methylene chain C-9 through C-11 (δ 38.0, 25.3 and 39.9, respectively) could be assigned based on HMBC (Table 1) and COSY correlations. HMBC correlations from C-11 protons to the quaternary signal at δ 136.7 and olefinic carbon signal at δ 123.3 establish a connection to the 1-methyl-3-phenylpropenyl moiety. HMBC correlations from H_3-21 [δ 0.94 (d, 3H, J=6.6 Hz)] to C-8 methine at δ 29.5 and methylene carbon at δ 38.0 establish a connection to the lactone moiety.

The relative stereochemistry of **2** was determined by NOESY experiments and by careful comparison of NOE data with that reported for related peroxylactones.[1,6–8,32] NOESY cross-peaks between H-13 at δ 5.36 (br t, 1H, J=7.3 Hz) and H_2-11, H_3-22 at δ 1.74 (br s, 3H) and H_2-14 are consistent with *E*-configuration. The downfield shift of H_3-20 to δ 27.1 from previous assignments indicates an equatorial position.[33] Both the hydroxyl proton on C-3 and H_3-19 gave cross-peaks to H-2α at δ 2.78. In addition, H_3-19 exhibits correlations with the H-7 proton at δ 1.75

Table 2. ^{1}H (500 MHz, $CDCl_3$) NMR shifts for compounds **1–3**

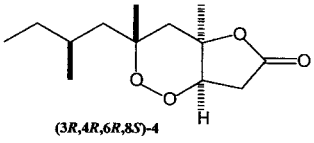
Proton no.	Compound					
	1		**2**		**3**	
	δ_H	mult, J (Hz)	δ_H	mult, J (Hz)	δ_H	mult, J (Hz)
2β	2.86	dd, 1H, 17.9, 6.0	2.87	d, 1H, 17.2	2.92	dd, 1H, 18.6, 6.1
2α	2.58	d, 1H, 17.1	2.78	d, 1H, 17.2	2.61	d, 1H, 18.5
3	4.45	d, 1H, 6.2			4.46	d, 1H, 7.3
5β	2.21	d, 1H, 15.3	2.38	d, 1H, 14.4	2.19	d, 1H, 14.8
5α	1.70	d, 1H, 15.2	2.06	d, 1H, 14.4	1.74	d, 1H, 14.7
7β	1.42	dd, 1H, 15.2, 3.0	1.44	dd, 1H, 18.5, 7.6	1.38	m, 1H
7α	1.65	dd, 1H, 14.3, 9.0	1.75	d, 1H, 18.5	1.66	m, 1H
8	1.77	m, 1H	1.61	m, 1H	1.66	m, 1H
9	2.35	d, 1H, 7.1	1.35	m, 1H	1.40	m, 1H
	2.38	d, 1H, 5.7	1.15	m, 1H	1.16	m, 1H
10			1.45	m, 2H	1.35	m, 2H
11	5.63	br s, 1H	2.04	m, 2H	2.00	m, 2H
13	5.42	br t, 1H, 7.4	5.36	br t, 1H, 7.3	5.37	br t, 1H, 6.4
14	3.44	d, 2H, 7.2	3.38	d, 2H, 7.3	3.39	d, 2H, 7.3
16	7.21	d, 2H, 7.3	7.21	d, 2H, 7.8	7.21	d, 2H, 7.4
17	7.29	d, 2H, 7.1	7.32	d, 2H, 7.6	7.29	d, 2H, 7.1
18	7.19	m, 1H	7.19	m, 1H	7.19	m, 1H
19	1.25	s, 3H	1.50	s, 3H	1.40	s, 3H
20	1.36	s, 3H	1.28	s, 3H	1.34	s, 3H
21	0.89	d, 3H, 7.2	0.94	d, 3H, 6.6	0.95	d, 3H, 8.5
22	1.72	br s, 3H	1.74	s, 3H	1.73	s, 3H
23	1.81	br s, 3H				

and H-5α proton at δ 2.06.[32] Also significant were the observed cross-peaks between H-5β at δ 2.38 and H_3-20 at δ 1.28. These data allowed the complete assignment of relative stereochemistry in the peroxylactone ring system.

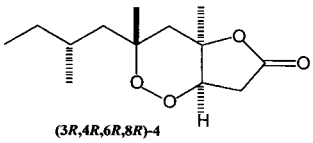
A comparison of the NMR spectra (^{13}C, ^{1}H, DEPT-135°, HMBC) for peroxylactone **3** with **2** quickly indicated that the only difference between these two structures appears at C-3 (Tables 1 and 2). The infrared spectrum indicated a γ-lactone absorption at 1792 cm^{-1}, and provided no hydroxyl band, which supported the replacement of the quaternary hemiketal at C-3 with a peroxy methine carbon. The high-resolution mass spectral data of 409.2349 $(M+Na)^+$, $C_{24}H_{34}O_4Na$ also indicated the difference of a single oxygen atom between peroxylactone **2** and **3**. The relative stereochemistry of **3** was assigned using NOE experiments and was shown to share the same configuration as structure **2**. The key difference between **2** and **3** is that the C-3 methine proton and H_3-19 both gave cross-peaks to H-2α (δ 2.61) in compound **3**. In addition, an NOE correlation between H-3 and the H_3-19 further supported a cis ring junction.

In the last two years, it has been shown that optical rotation angles can be computed reliably and accurately for a wide range of organic molecules.[34–36] A recent application to the realm of natural products is represented by the marine natural product pitiamide A.[37] Many advances were made to understand the magnitude and sign of rotation angles through atomic maps and molecular orbital contributions,[38] and coupled Hartree–Fock (CHF) analysis can be used for the assignment of the relative as well as the absolute stereochemistry of natural products.[34,37,39] The completion of the stereochemical assignment of the organic peroxide plakortolide G (**3**) by molar rotation angle ($[M]_D$) computations represented a useful extension of this methodology.

The calculation of the electric dipole–magnetic dipole tensor, G' (which is proportional to the rotation angle) using the CADPAC program[40] is currently still limited to molecules slightly smaller than **3**. Accordingly, we selected segments (3*R*,4*R*,6*R*,8*S*)-**4** and (3*R*,4*R*,6*R*,8*R*)-**4** as appropriate models for **3**. Both segments contain all the stereocenters present in both **1** and **3**.



(3*R*,4*R*,6*R*,8*S*)-**4**



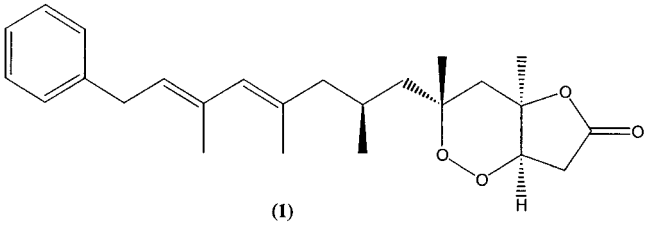
(3*R*,4*R*,6*R*,8*R*)-**4**

The experimental measurements of $[M]_D$ (the molar rotation angle are proportional to the specific rotation $[\alpha]_D$; $[M]_D=[\alpha]_D \cdot MW/100$) arise from a distribution of thermally accessible conformers in solution. Hence, our calculations of the specific rotational angles for (3*R*,4*R*,6*R*,8*S*)-**4** and (3*R*,4*R*,6*R*,8*R*)-**4** use conformations that are within ~9–10 kJ/mol of the lowest energy conformer, which ensures that all relevant thermally accessible structures are accounted for in the rotation angle computations. For each segment, we obtained 40 unique low-energy conformations from Monte Carlo conformational searches with Macromodel using the MM2* force field and the chloroform solvent continuum model.[41] The molar rotations were computed using the CADPAC program with a Gaussian 6-31G basis set.[42,43] These rotations were Boltzmann-weighted and summed to obtain the molar rotation angle. More detailed discussions of this methodology have been published elsewhere.[34,38] The Boltzmann-averaged molar rotations ($[M]_D$) for (3*R*,4*R*,6*R*,8*S*)-**4** and (3*R*,4*R*,6*R*,8*R*)-**4** were +18.9 and +91.1°, respectively. The experimentally measured molar rotation angle for plakortolide G (**3**) is +23.2. Accordingly, the computed $[M]_D$ of (3*R*,4*R*,6*R*,8*S*)-**4** is in excellent agreement with the experimental value and allows the assignment of the absolute configuration of (3*R*,4*R*,6*R*,8*S*) to the natural product. Therefore, the absolute configuration of plakortolide (**1**) was assigned because its $[M]_D$ value (+22.3°) was comparable to plakortolide G (**3**).

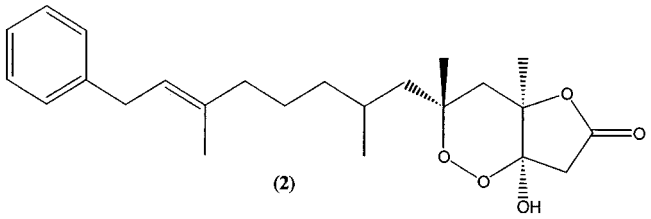
1. Conclusions

In this article, we report the structure of two new cyclic peroxylactones and the assignment of their stereochemistry by a combination of spectroscopic and ab initio quantum chemical tools. The optical rotation values for compounds (**1**–**3**) were measured carefully and are reported in $CHCl_3$. Compounds (**1**) and (**3**) both share an identical chiral moiety and provide optical rotation values that are comparable both in sign and magnitude. Thus, one can conclude that the optical rotation computations allow the assignment of the absolute configuration of plakortolide (**1**) and plakortolide G (**3**) as (3*R*,4*R*,6*R*,8*S*). The NOESY NMR spectrum of (**2**) allowed the unambiguous assignment of relative configuration at C-3, C-4 and C-6. Extensive computation times and lack of significant bioactivity precluded the complete assignment of (**2**) by modeling. However, the NOE data would suggest that it is comparable to structures (**1**) and (**3**).

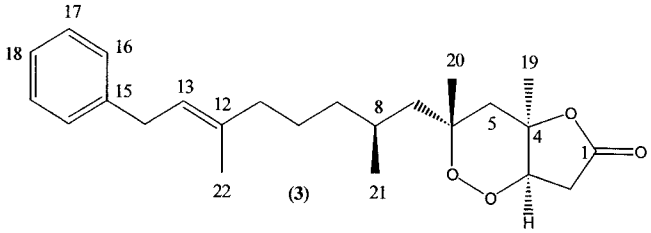
We have further evaluated the in vitro anti-*T. gondii* activity of the previously isolated cyclic peroxylactone plakortolide (**1**). An IC_{50} value of 64 nM was obtained showing no toxicity for the host cells up to 0.3 μM concentration. However, toxicity increased to 83% at 1.0 μM. Plakortolide F (**2**) displayed no in vitro activity and low toxicity, while plakortolide G (**3**) displayed at 10.0 μM concentration an increase from 72% inhibition after 24 h to 100% inhibition of the parasite at 72 h with no toxicity for the host cells. The biological data obtained indicate that a decrease in oxidation and an increase in conjugation in the aliphatic side chain increases anti-*T. gondii* activity in vitro. This is the first reported anti-*T. gondii* activity from this class of compounds. In vivo biological evaluations are currently under investigation, and the results will be reported in due course.



(**1**)



(2)



(3)

2. Experimental

2.1. 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 with a JASCO DIP-370 digital Polarimeter. NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer using the solvent peak as the internal standard. ESI-FT-ICR MS spectra were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution HPLC FT-ICR MS spectrometer by direct injection into an electrospray interface. Semi-preparative HPLC was carried out on a Waters 510 system with a gradient programmer.

2.1.1. Animal material. The sponge forms an erect tapering tube with a large apical oscule, and was collected using closed circuit underwater breathing apparatus (CCUBA) from inside caverns at a depth of −40 m near the mouth of the Rio Buenos, Discovery Bay, Jamaica, July 1998. The texture is tough and slightly elastic, the sponge surface is rough to touch. The color in life is chocolate brown. The skeleton consists of a confusion of mesoscleres similar to those of Plakortis (small diods and triods) to which large oxea-like diods and calthrops similar to those of astrophorids, such a Pachastrella, are added. The sponge is similar to *Plakinastrella onkodes*, Uliczka 1929 (Order Homosclerophorida, Family Plakinidae), but the large diods and calthrops are smaller than in the described species. A voucher specimen has been deposited at the Natural History Museum, London, UK (BMNH 2000.7.17.1).

2.1.2. Extraction and isolation. The sponge (1.0 kg) was initially preserved frozen. The crude extract (2.3 g) was obtained by extracting homogenized, freeze-dried sponge with 1:1 EtOAc/IPA (4×250 mL) and followed by 1:1 EtOH/H_2O (4×250 mL) were combined and concentrated under vacuum. The extract was subjected to silica flash chromatography using a gradient of hexane to ethyl acetate and finally methanol. Further purification was carried out on an IB-SIL 5 μM C18 column (10×250 mm, Phenomenex) using a mobile phase of MeOH–H_2O on a linear gradient followed by reverse-phase HPLC (Prodigy 5 μM ODS 3 100 Å, 10×250 mm Phenomenex) using CH_3CN–H_2O as an eluent (flow rate of 10 mL/min and UV detection at 256 nm).

2.2. Assay material and methods

2.2.1. *Toxoplasma gondii*. Tachyzoites of the RH strain were obtained from the peritoneal cavities of mice infected for 2 days with the parasite, and tissue cysts of the strain were obtained from the brain of chronically-infected mice.[29]

2.2.2. In vitro studies. In vitro activity was defined as the capacity of the drug to inhibit intracellular replication of *T. gondii* and was determined by using [^{3}H]-uracil incorporation.[30] Briefly, confluent monolayers of human foreskin fibroblast (HFF, ATCC CRL1635) grown in Dulbecco's modified Eagle medium (DMEM, Gibco BRL, Grand Island, NY) containing 100 units of penicillin, 1 μg of streptomycin per mL, and 10% heat inactivated fetal bovine serum (HyClone, Logan, Utah) in 96-well flat bottom tissue culture microtiter plates (Costar Corporation Cambridge, MA) were infected with 4×10^4 tachyzoites/well. Each drug was dissolved in a small volume of dimethyl sulfoxide (DMSO) and diluted with DMEM to desired concentrations with the final DMSO concentration in solution being less than 1%. Four hours following infection, monolayers were washed to remove extracellular parasites and different concentrations of the test drugs were added in triplicate. Addition of the drugs to the wells marked the starting time point. [^{3}H]-uracil (1 Ci/well) was added to each well 4 h prior to harvesting the cells at 24, 48, and 72 h following addition of the test drugs and the plates were incubated at 37°C till the end point. At each end point, cells were harvested and radioactivity incorporation was counted in a scintillation counter. Infected monolayers treated with medium that contained the respective drug diluent alone served as negative control. Toxicity of the drugs for the HFF cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) cell proliferation assay using Cell Titer 96 Kit (Promega, Madison, WI).[31]

2.2.3. Plakortolide [1]. Clear, colorless oil; $[\alpha]_D=+5.6$ (c=0.014, $CHCl_3$); spectral data (^{1}H NMR, ^{13}C NMR, IR, MS) were identical to those described by Stierle and Faulkner.[1]

2.2.4. Plakortolide F [2]. Clear, colorless oil; $[\alpha]_D=-59.2$ (c=0.025, $CHCl_3$); UV (EtOH) 202 nm (ϵ 50, 359), 246 nm (ϵ 20, 512), 360 nm (ϵ 1, 665); IR ν_{max} (neat) 3411, 2928, 2853, 1782, 1452, 1379, 1265, 1162, 1060, 941 cm^{-1}; hresims m/z 425.2351 ({M+Na}$^+$, $C_{24}H_{34}O_5Na$, Δ 4.7 mmu of calcd); ^{1}H NMR ($CDCl_3$) Table 2 and ^{13}C NMR ($CDCl_3$) Table 1.

2.2.5. Plakortolide G [3]. Clear, colorless oil; $[\alpha]_D=+6.0$ (c=0.015, $CHCl_3$); UV (EtOH) 202 nm (ϵ 92, 224), 250 nm (ϵ 36, 392), 368 nm (ϵ 2774); IR ν_{max} (neat) 2938, 2863, 1781, 1453, 1382, 1271, 1172, 1078, 950, 701 cm^{-1}; hresims m/z 409.2349 ({M+Na}$^+$, $C_{24}H_{34}O_4Na$, Δ 0.6 mmu of calcd); ^{1}H NMR ($CDCl_3$) Table 2 and ^{13}C NMR ($CDCl_3$) Table 1.

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42. Simple basis sets like 6-31G, 6-31G* and DZP are shown to be accurate in predicting the correct stereochemistry, provided all the lower energy conformations are included in the Boltzmann sum of molar rotation angles (see Refs. 34,35,37,38).
43. Taking the origin as the center of mass in the CADPAC calculations is expected to avoid substantive gauge origin dependent results for structures of this size (see Refs. 34,35,37,38).