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Antileishmanial Cyclic Peroxides from the Palauan Sponge *Plakortis* aff. *angulospiculatus*

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Abstract: As part of a program to discover new drugs for the treatment of tropical diseases, we have isolated and identified six new metabolites from the Palauan sponge *Plakortis* aff. *angulospiculatus*. Two cyclic peroxides show strong *in vitro* antiproliferative effects on promastigotes of *Leishmania mexicana*, a flagellate protozoan that causes leishmaniasis. The structures of the cyclic peroxides **1**, **2** and **5** and furans **3**, **4** and **6** were elucidated by interpretation of spectroscopic data, molecular modelling, and chemical interconversions.

Leishmaniasis is a tropical disease that affects an estimated 350 million people in equatorial Asia, Africa and Central and South America. It is found in many of the world's poorest countries, where it is estimated that 1.5 to 2 million people are infected each year.[1] In Central and South America, the causative agent is the protozoan *Leishmania mexicana*, which has a reservoir in the rodent population and is transmitted by flies. The most common drugs for the treatment of leishmaniasis (pentostant and glucantil) contain pentavalent antimonials that have cardiotoxic effects at the recommended doses.[2] Alternative drugs such as amphotericin B and azoles have equally unpleasant side effects. The urgent need for alternative treatments has led to a program to screen natural products for potential use in the therapy of leishmaniasis.

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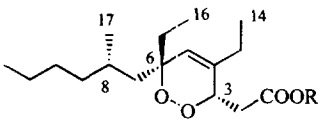
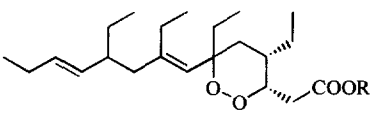
Table 1. ^{1}H and ^{13}C NMR data for peroxide **1**, iodo-lactone **7** and lactone **8**.

	1				**7**			**8**		
C#	δ_C	δ_H	mult., *J* (Hz)	HMBC	δ_C	δ_H	mult., *J* (Hz)	δ_C	δ_H	mult., *J* (Hz)
1	176.6				173.5			174.6		
2	37.1	2.91	dd, 16, 9	C1, C3, C4	34.3	2.83	dd, 18.5, 4	37.5	2.85	dd, 18.5, 6.5
		2.61	dd, 16, 3	C1, C4		2.65	dd, 18.5, 7.5		2.68	br d, 18.5
3	76.2	4.57	br dd, 9, 3	C1, C2, C4, C5, C13	80.3	4.45	dd, 7.5, 4	80.4	4.41	br d, 6.5
4	137.4				87.2			85.9		
5	125.3	5.49	br s	C3, C6, C7, C13	39.0	4.57	s	29.0	2.18	d, 15 (eq)
									1.61	d, 15 (ax)
6	83.6				85.6			83.3		
7	42.4	1.45	dd, 14, 3	C5, C6, C8, C9, C17	41.9	1.68	dd, 15, 8	41.4	1.74	dd, 15, 5
		1.39	dd, 14, 8	C5, C6, C8, C17		~1.52	m		1.35	dd, 15, 6
8	28.4	1.43	m		29.1	1.52	m	28.2	1.54	m
9	38.3	1.23	2 H		38.3	1.23	2 H	37.8	1.23	2 H
10	29.3	1.23	2 H		29.4	1.23	2 H	29.2	1.23	2 H
11	22.9	1.23	2 H		23.1	1.23	2 H	22.9	1.23	2 H
12	14.1	0.86	t, 3 H, 7	C10, C11	14.3	0.82	t, 3 H, 7	14.1	0.85	t, 3 H, 7
13	24.9	2.00	q, 2 H, 7.5	C3, C4, C5, C13	34.2	2.35	dq, 15, 7.5	34.9	1.63	q, 2 H, 7.5
									1.72	dq, 15, 7.5
14	11.7	1.06	t, 3 H, 7.5	C4, C14	6.9	0.88	t, 3 H, 7.5	7.2	0.96	t, 3 H, 7.5
15	30.9	1.73	dq, 14, 7.5	C5, C6, C7, C16	33.2	2.13	dq, 15, 7.5	32.4	~1.74	
		1.64	dq, 14, 7.5	C5, C6, C7, C16		1.69	dq, 15, 7.5		~1.60	
16	8.2	0.88	t, 3 H, 7.5	C6, C15	9.1	0.83	t, 3 H, 7.5	7.9	0.87	t, 3 H, 7.5
17	22.1	0.88	d, 3 H, 6.5	C7, C8, C9	21.6	0.89	d, 3 H, 6.5	22.6	0.89	d, 3 H, 6.5

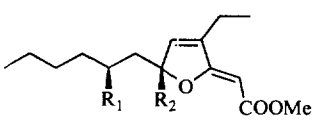
Sponges of the genus *Plakortis* are well known for their ability to produce cyclic peroxides and related metabolites.[3] Studies have shown that some cyclic peroxides from sponges are cytotoxic[4] while others activate cardiac SR Ca^{2+} pumping ATPase.[5] In this paper, we describe the isolation and identification of six new metabolites from the Palauan sponge *Plakortis* aff. *angulospiculatus* and the results of *in vitro* bioassays against *Leishmania mexicana*.



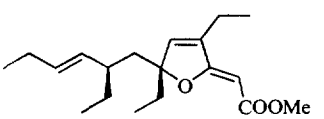
1 R = H
5 R = Me



2 R = H
11 R = Me



3 R_1 = Me, R_2 = Et
4 $R_1 = R_2$ = Et
6 $R_1 = R_2$ = Me



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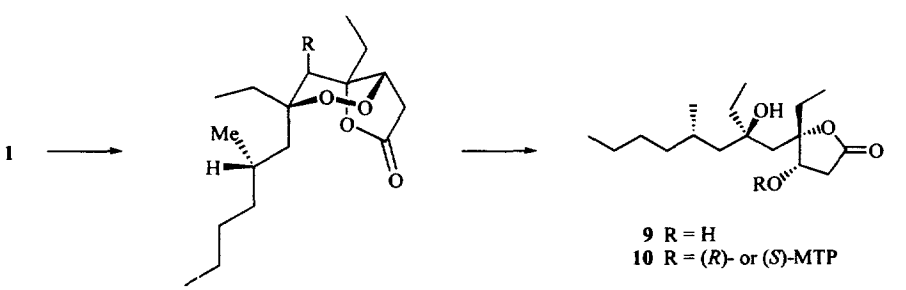
Specimens of *Plakortis* aff. *angulospiculatus* were collected in two different locations in Palau in 1993 and 1996. The 1993 specimen was extracted with methanol and, after partitioning against water, the ethyl acetate soluble material was chromatographed on silica gel to obtain (3*S*,6*R*,8*S*)-4,6-diethyl-3,6-epidioxy-8-methyldodeca-4-enoic acid (**1**), 3,6-epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid (**2**), methyl (2*Z*,6*R*,8*S*)-4,6-diethyl-3,6-epoxy-8-methyldodeca-2,4-dienoate (**3**), and methyl (2*Z*,6*R*,8*S*)-3,6-epoxy-4,6,8-triethyldodeca-2,4-dienoate (**4**). The 1996 specimen was extracted and separated in a similar manner to obtain (3*S*,6*R*,8*S*)-4,6-diethyl-3,6-epidioxy-8-methyldodeca-4-enoic acid (**1**) as the major compound, with methyl (2*Z*,6*R*,8*S*)-4,6-diethyl-3,6-epoxy-8-methyldodeca-2,4-dienoate (**3**), methyl (3*S*,6*R*,8*S*)-4,6-diethyl-3,6-epidioxy-8-methyldodeca-4-enoate (**5**), and methyl (2*Z*,6*R*,8*S*)-6,8-dimethyl-3,6-epoxy-4-ethyldodeca-2,4-dienoate (**6**) as minor metabolites.[6]

(3*S*,6*R*,8*S*)-4,6-Diethyl-3,6-epidioxy-8-methyldodeca-4-enoic acid (**1**) was isolated as a colorless oil. The molecular formula, $C_{17}H_{30}O_4$, which was deduced from a high resolution mass measurement, requires three unsaturation equivalents. The IR spectrum contained bands at 3000 (br) and 1710 cm^{-1} that were assigned to a carboxylic acid group. The ^{13}C NMR spectrum (Table 1) contained signals at δ 176.6 due to the carboxylic acid, and at 137.4 (s) and 125.3 (d) which were assigned to a trisubstituted olefin. The signals at

76.2 (d) and 83.6 (s) are the only signals for carbon-bearing oxygen and must therefore be assigned to a cyclic peroxide in order to satisfy the oxygen and unsaturation counts. In the HMBC experiment, correlations were observed from the olefinic proton signal at δ 5.49 to both the 76.2 and 83.6 signals, thereby placing the olefin in the peroxide ring, and to the C-7 signal at 42.4 (t), which in turn was correlated to the methyl doublet at 0.88, which requires the methyl group to be situated at C-8. The ^{1}H NMR spectrum contains signals for two ethyl groups, which were placed at C-4 and C-6 on the basis of HMBC correlations, and a signal at δ 4.57 (br dd, 1 H, J = 9, 3 Hz), assigned to C-3 on the peroxide ring, that was coupled to signals at 2.91 (dd, 1 H, J = 16, 9 Hz) and 2.61 (dd, 1 H, J = 16, 3 Hz), which are due to the methylene group adjacent to the carboxylic acid. The remaining NMR data are all completely compatible with the proposed structure.

In order to determine the stereochemistry of cyclic peroxide **1**, it was first converted into the corresponding iodo-lactone **7** by reaction of an aqueous solution of the sodium salt of the acid with iodine in chloroform in a two-phase reaction. The NMR data for iodo-lactone **7** was assigned as shown in Table 1. As expected, iodolactonization occurred to give a *trans* anti-planar product with H-3 axial and H-5 equatorial with respect to the peroxide ring. There are NOESY correlations between the H-5 signal and signals due to H-7 and CH_3-17, but examination of molecular models suggested that both configurations at C-6 could give rise to these correlations. However, by using PC Model (Serena Software) to generate the preferred conformartions of the four diastereoisomers at C-6 and C-8 of the lactone **8**, we concluded that the additional data afforded by having the axial proton at C-5 would permit an unambiguous assignment of the stereochemistry at both centers. Reduction of the iodo-lactone **7** with tri-*n*-butyltin hydride gave the lactone **8** in good yield. After recording the ^{1}H NMR, COSY and NOEDS spectra of lactone **8** in two solvents ($CDCl_3$ and 40% benzene-d_6 in $CDCl_3$) to clarify those regions of the spectra where signals overlapped, we were able to measure NOE correlations from the axial hydrogen at C-5 to H-3 and H-15, to establish the relative stereochemistry at C-6. The equatorial hydrogen at C-5 showed NOE correlations to H-7 and CH_3-17. Molecular modelling indicates that there is limited rotation about the 6-7 and 6-15 bonds and to a lesser extent about the 7-8 bond, a conclusion that is supported by the different chemical shifts of geminal protons at C-7 and C-15 and by the



7 R = I
8 R = H

9 R = H
10 R = (*R*)- or (*S*)-MTP

coupling constants of the H-7 signals. Therefore, the proximity of H-5_{eq} to CH_3-17 indicated by the NOE measurement can only be accomplished in the (6*R**,8*S**) conformer. The absolute stereochemistry of this series of compounds was determined by application of Mosher's method to the diol **9**, that was formed by reduction of the lactone **8** with zinc in acetic acid. The differences in proton chemical shifts ($\Delta\delta$) of key signals in the (*R*)- and (*S*)-MTPA esters (**10*R*** and **10*S***) indicated that the absolute stereochemistry at the secondary alcohol is *S*. This requires the (3*S*,6*R*,8*S*) stereochemistry for lactone **8** which in turn establishes the stereochemistry of the starting peroxide as (3*S*,6*R*,8*S*)-4,6-diethyl-3,6-epidioxy-8-methyldodeca-4-enoic acid (**1**).

3,6-Epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid (**2**) was obtained as a pale yellow oil. The molecular formula, $C_{22}H_{38}O_4$, was determined by high resolution mass measurement. The IR spectrum contained a broad hydroxyl band at 2600-3400 cm^{-1} and a carbonyl band at 1710 cm^{-1}, which again indicated the presence of a carboxylic acid, and this assignment was supported by a ^{13}C NMR signal at δ 178.1. The ^{13}C NMR spectrum also contained signals for disubstituted and trisubstituted olefins at δ 141.9 (s), 133.0 (d), 131.7 (d) and 127.1 (d), for the carbons bearing the peroxide functionality at 84.3 (s) and 78.4 (d), and for five methyl carbons, eight methylene carbons, and two methine carbons in the aliphatic region. Comparison of these data with literature values for methyl 3,6-epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoate (**11**) strongly suggested that the peroxide **2** was the acid of a known methyl ester **11** that had been obtained by methylation of a mixture of acids from *Plakortis halichondrioides* from Belize.[7] Comparison of the ^1H NMR spectra of peroxides **2** and **11** provided support for this structural assignment and confirmed the *cis* configuration of the substituents at C-3 and C-4. Since all spectral data, especially those derived from the HMQC and HMBC experiments, were completely compatible with the proposed structure, we did not pursue the structural assignment further, other than to methylate **2** to produce a methyl ester with spectroscopic data comparable but not identical to those of **11**,[8] and thus the stereochemistry at C-6 and C-10 remain to be determined.[9]

The major furan in both the 1993 and 1996 samples was methyl (2*Z*,6*R*,8*S*)-4,6-diethyl-3,6-epoxy-8-methyldodeca-2,4-dienoate (**3**), which was isolated as a clear oil. The molecular formula was established as $C_{18}H_{30}O_3$. The IR spectrum contained bands at 1715 and 1690 cm^{-1}, which are appropriate for an unsaturated ester, and an absorption at 283 nm (ε 9,000) in the UV spectrum indicated the presence of extended conjugation. Having recently completed a study of the absolute stereochemistry of methyl (2*Z*,6*R*,8*R*,9*E*)-3,6-epoxy-4,6,8-triethyl-2,4,9-dodecatrienoate (**12**) from *P. halichondrioides*,[10] we recognized that the spectral data were diagnostic for a closely related furan ester. The ^{13}C NMR spectrum contained signals at δ 166.9 (C-1), 83.8 (C-2), 171.7 (C-3), 140.1 (C-4), 139.8 (C-5), 98.1 (C-6) and 50.5 (OMe) that were nearly identical to the corresponding signals in **12**. Both the ^1H and ^{13}C NMR spectra (Table 2) contained signals appropriate for

two ethyl groups, one secondary methyl group and a short alkyl chain. The two ethyl groups were placed at C-4 and C-6 and the secondary methyl group at C-8, resulting in the same side chain as that in peroxide **1**. If we assume the mechanism shown in Scheme 1 for the conversion of peroxide **1**, *via* the methyl ester **5**, to the corresponding unsaturated furan **3**, then the relative stereochemistry of **3** should be (6*R*,8*S*), which is the same as that determined for **12**.

The minor furan in the 1993 collection was methyl (2*Z*,6*R*,8*S*)-3,6-epoxy-4,6,8-triethyldodeca-2,4-dienoate (**4**), which was isolated as an oil. The molecular formula, $C_{19}H_{32}O_3$, derived from both ^{13}C NMR and mass spectral data, indicated that **4** is a homolog of **3**. The ^1H NMR spectrum of **4** is almost identical to that of **3** except that the signal for the methyl group at C-8 has been replaced by the signals of an ethyl group (Table 2). The 9,10-olefin in the known compound methyl (2*Z*,6*R*,8*R*,9*E*)-3,6-epoxy-4,6,8-triethyl-2,4,9-dodecatrienoate (**12**) was reduced with diimide to obtain a dihydro derivative whose ^1H and ^{13}C NMR spectra were identical to those of **4**. Based on the sign and magnitude of their optical rotations, and because the spectral data were otherwise identical, we assume that the stereochemistry of **4** is (6*R*,8*S*).

The minor furan in the 1996 collection was methyl 6,8-dimethyl-3,6-epoxy-4-ethyldodeca-2,4-dienoate (**6**), which was isolated as an oil. The molecular formula, $C_{17}H_{28}O_3$, derived from both ^{13}C NMR and mass spectral data, indicated that **6** is a lower homolog of **3**. The ^1H NMR spectrum of **6** was almost identical to that of **3** except that an ethyl group had been replaced by a methyl group at C-6 (Table 2). The stereochemistry is assumed to be the same as other compounds in this series.

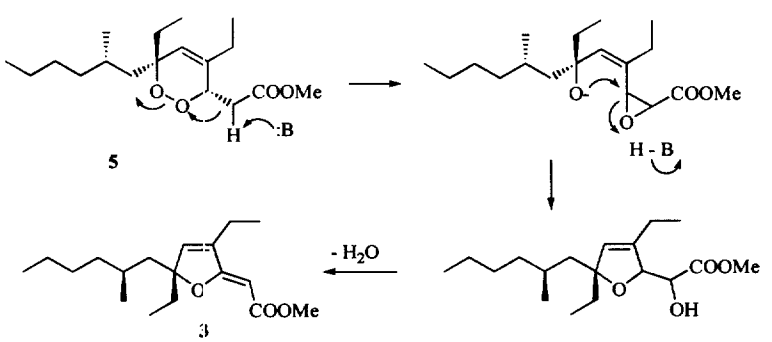
A minor peroxide from the 1996 collection was identified as methyl (3*S*,6*R*,8*S*)-4,6-diethyl-3,6-epidioxy-8-methyldodeca-4-enoate (**5**), the methyl ester of peroxide **1**. Once the molecular formula, $C_{18}H_{32}O_4$ had been established from mass spectral data, interpretation of the spectral data suggested a close relationship between the two peroxides. This proposal was confirmed by treatment of the peroxy-acid **1** with diazomethane in ether to obtain the corresponding methyl ester **5**, the spectral data for which were identical to those of the natural product.

Peroxides **1** and **2** and furans **3** and **4** were screened for their effects on the proliferation of *Leishmania mexicana* promastigotes. The parasite was most sensitive to peroxide **1** (LD_{50} 0.29 μg/mL), which caused lysis of the cell membrane after 24 h at a concentration of 1 μg/mL. A dramatic decrease in the mobility of the parasite was noted within 30 minutes after drug addition. Peroxide **2** (LD_{50} 1.00 μg/mL) was less effective while the furans **3** and **4** showed similar activity but at much higher doses (1.86 and 2.71 μg/mL respectively). These data compare well with the antileishmaniacal activity of the sponge metabolite ilimaquinone (LD_{50} 5.6 μg/mL) but the peroxides are less effective than ketoconozole (LD_{50} 0.06 μg/mL).[11]

Table 2. ^{1}H and ^{13}C NMR data for furans **3**, **4**, and **6**.

	3			**4**			**6**		
C#	δ_C	δ_H	mult., *J* (Hz)	δ_C	δ_H	mult., *J* (Hz)	δ_C	δ_H	mult., *J* (Hz)
1	166.9			166.9			166.9		
2	83.8	4.80	s	83.7	4.79	s	84.2	4.82	s
3	171.7			171.7			171.2		
4	140.1			140.0			138.9		
5	139.8	6.20	br s	139.8	6.20	s	141.4	6.26	s
6	98.1			98.0			95.4		
7	44.6	1.68	m, 2 H	41.7	1.59	m	46.1	1.67	dd, 14.5, 7
					1.78	m		1.72	dd, 14.5, 5
8	28.7	1.28	m	27.1	1.23	m	29.0	1.30	m
9	38.1	1.11	m	34.4	1.10	m, 2 H	37.9	1.22	m, 2 H
		1.23	m						
10	29.2	1.22	m, 2 H	29.0	1.17	m, 2 H	29.2	1.22	m, 2 H
11	22.9	1.22	m, 2 H	23.0	1.28	m, 2 H	22.9	1.22	m, 2 H
12	14.1	0.84	t, 3 H, 7.5	14.1	0.85	t, 3 H, 7.5	14.1	0.84	t, 3 H, 7
13	18.6	2.16	q, 2 H, 7.5	18.6	2.16	q, 2 H, 7.5	18.5	2.13	q, 2 H, 7.5
14	12.1	1.15	t, 3 H, 7.5	12.1	1.15	t, 3 H, 7.5	11.9	1.14	t, 3 H, 7.5
15	31.9	1.56	m	31.7	1.71	m	-	-	-
		1.83	m		1.83	m			
16	8.0	0.77	t, 3 H, 7.5	8.1	0.77	t, 3 H, 7.5	25.8	1.41	s, 3 H
17	21.3	0.85	d, 3 H, 6	34.3	1.18	m, 2 H	21.2	0.85	d, 3 H, 7
18	-	-	-	10.6	0.78	t, 3 H, 7.5	-	-	-
19	50.5	3.66	s, 3 H	50.5	3.66	s, 3 H	50.5	3.66	s, 3 H

Scheme 1. Proposed mechanism for the conversion of peroxide **5** to furan **3**.



Experimental Section

1993 Collection and Extraction: The sponge *Plakortis* aff. *angulospiculatus* (Collection no. 93-072) was collected by hand using SCUBA from a reef (-15 m) at the south-western side of Palau and was immediately frozen. After 10 months at -20°C, the sponge (650 g wet wt.) was extracted with MeOH (3 x 1L) and the combined extracts were concentrated to an aqueous suspension. Water (200 mL) was added and the aqueous phase was extracted with EtOAc (2 x 200 mL) to obtain, after drying over Na_2SO_4 and evaporation of the solvent, a crude organic extract (21 g) as an oil. A portion (2.1 g) of the oil was chromatographed on silica gel 60 (200 g) using 19:1 CH_2Cl_2/MeOH as eluant to obtain three fractions. The least polar fraction was purified by HPLC on silica using 9:1 hexane/EtOAc as eluant to obtain methyl (2*Z*,6*R*,8*S*)-4,6-diethyl-3,6-epoxy-8-methyldodeca-2,4-dienoate (**3**, 15 mg, 0.08% dry wt.), and methyl (2*Z*,6*R*,8*S*)-3,6-epoxy-4,6,8-triethyldodeca-2,4-dienoate (**4**, 49 mg, 0.27% dry wt.). The second and third fractions from the silica column were combined and further purified by HPLC on silica using 7:3 hexane/EtOAc as eluant to obtain (3*S*,6*R*,8*S*)-4,6-diethyl-3,6-epidioxy-8-methyldodeca-4-enoic acid (**1**, 164 mg, 0.9% dry wt.) and 3,6-epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid (**2**, 50 mg, 0.27% dry wt.).

1996 Collection and Extraction: The sponge *Plakortis* aff. *angulospiculatus* (Collection no. 96-233) was collected by hand using SCUBA at Siaes Tunnel (-25 m) in Palau and was immediately frozen. Half of the frozen sponge (500 g) was extracted with methanol (1 L) at room temperature. The crude extract, which showed modest levels of activity in a brine shrimp lethality assay, was partitioned between CH_2Cl_2 and 15% aqueous MeOH and the organic extract was dried over Na_2SO_4 and the solvent evaporated to obtain a crude organic extract (440 mg). A portion of the organic extract (75 mg) was chromatographed on silica gel using a hexane/EtOAc gradient to obtain (3*S*,6*R*,8*S*)-4,6-diethyl-3,6-epidioxy-8-methyldodeca-4-enoic acid (**1**, 42 mg, 0.049% wet wt.) as the major compound, with methyl (2*Z*,6*R*,8*S*)-4,6-diethyl-3,6-epoxy-8-methyldodeca-2,4-dienoate (**3**, 8.6 mg, 0.01% wet wt.), methyl (3*S*,6*R*,8*S*)-4,6-diethyl-3,6-epidioxy-8-methyldodeca-4-enoate (**5**, 7.4 mg, 0.0084% wet wt.), and methyl 6,8-dimethyl-3,6-epoxy-4-ethyldodeca-2,4-dienoate (**6**, 2 mg, 0.0023% dry wt.) as minor constituents.

(3*S*,6*R*,8*S*)-4,6-Diethyl-3,6-epidioxy-8-methyldodeca-4-enoic acid (1): colorless oil; $[\alpha]_D$ -19.8 (*c* 0.89, $CHCl_3$); IR (film) 2500-3400 (br), 1715, 1440, 1280 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100MHz) see table 1; EIMS *m/z* (int., %) 283 (1, M - Me)$^+$, 269 (7, M - Et)$^+$, 207 (7), 181 (13), 167 (11), 139 (30), 125 (100%); HRFABMS *m/z* 316.2477, calcd. for $C_{17}H_{34}NO_4$ (M + NH_4)$^+$ 316.2487.

3,6-Epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid (2): colorless oil; $[\alpha]_D$ +164 (*c* 2.4, $CHCl_3$); IR (film) 2500-3400 (br), 1710, 1460, 1290 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.82 (t, 3 H, *J* = 7 Hz), 0.83 (t, 3 H, *J* = 7.5 Hz), 0.89 (t, 3 H, *J* = 7.5 Hz), 0.93 (t, 3 H, *J* = 7 Hz), 0.94 (t, 3 H, *J* = 7.5 Hz), 1.10-1.30 (m, 4 H), 1.38 (m, 1 H), 1.52 (q, 2 H, *J* = 7 Hz), 1.70 (m, 1 H), 1.87-2.01 (m, 4 H), 2.04-2.21 (m, 4 H), 2.40 (dd, 1 H, *J* =

16, 3 Hz), 3.04 (dd, 1 H, J = 16, 9.5 Hz), 4.41 (m, 1 H), 5.09 (dt, 1 H, J = 15.5, 9 Hz), 5.15 (s, 1 H), 5.35 (dt, 1 H, J = 15.5, 6) ; ^{13}C NMR ($CDCl_3$, 50 MHz) δ 178.2 (C-1), 141.9 (C-8), 133.0 (C-11), 131.7 (C-12), 127.1 (C-7), 84.3 (C-6), 78.4 (C-3), 42.6 (C-10), 42.4 (C-9), 35.7 (C-5), 35.3 (C-4), 32.8 (C-17), 31.4 (C-2), 27.9 (C-21), 25.6 (C-13), 24.9 (C-15), 22.8 (C-19), 13.9 (C-14), 12.1 (C-20), 11.6 (C-22), 10.9 (C-16), 7.6 (C-18); CIMS *m/z* (int., %) 367 (5, M + H)$^+$, 349 (35), 321 (42), 305 (84), 223 (100), 185 (96); HRFABMS *m/z* 367.2859, calcd. for $C_{22}H_{39}O_4$ (M + H)$^+$ 367.2848.

Methyl (2*Z*,6*R*,8*S*)-4,6-diethyl-3,6-epoxy-8-methyldodeca-2,4-dienoate (3): colorless oil; $[\alpha]_D$ -92 (*c* 1.1, $CHCl_3$); UV (MeOH) 285 nm (ε 11100); IR (film) 2960, 2930, 1710, 1690, 1630, 1160 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) see table 2; CIMS *m/z* (int., %) 295 (100, M + H)$^+$, 263 (55), 195 (11); HRFABMS *m/z* 295.2266, calcd. for $C_{18}H_{31}O_3$ (M + H)$^+$ 295.2273.

Methyl (2*Z*,6*R*,8*S*)-3,6-epoxy-4,6,8-triethyldodeca-2,4-dienoate (4): colorless oil; $[\alpha]_D$ -78 (*c* 0.19, $CHCl_3$); UV (MeOH) 285 nm (ε 8900); IR (film) 2955, 2920, 1715, 1690, 1630, 1165 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 50 MHz) see table 2; CIMS *m/z* (int., %) 309 (100, M + H)$^+$, 277 (41), 195 (8); HRFABMS *m/z* 309.2435, calcd. for $C_{18}H_{31}O_3$ (M + H)$^+$ 309.2430.

Methyl (3*S*,6*R*,8*S*)-4,6-diethyl-3,6-epidioxy-8-methyldodeca-4-enoate (5): colorless oil; $[\alpha]_D$ -21.1 (*c* 0.36, $CHCl_3$); IR (film) 2955, 2920, 1745, 1460, 1255 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.86 (t, 3 H, J = 7 Hz, H-12), 0.87 (t, 3 H, J = 7.5 Hz, H-16), 0.88 (d, 3 H, J = 6.5 Hz, H-17), 1.06 (t, 3 H, J = 7.5 Hz, H-14), 1.22 (m, 6 H, H-9, H-10, H-11), 1.36 (dd, 1 H, J = 14.5, 8, H-7), 1.43 (m, 1 H, H-8), 1.45 (dd, 1 H, J = 14.5, 3 Hz, H-7), 1.63 (dd, 1 H, J = 14.5, 7.5 Hz, H-15), 1.73 (dd, 1 H, J = 14.5, 7.5 Hz, H-15), 1.99 (m, 2 H, H-13), 2.55 (dd, 1 H, J = 16, 3 Hz, H-2), 2.88 (dd, 1 H, J = 16, 9 Hz, H-2), 3.70 (s, 3 H, OMe), 4.59 (dd, 1 H, J = 9, 3 Hz, H-3), 5.48 (br d, 1 H, J = 1.5, H-5) ; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.6 (C-1), 137.6 (C-4), 125.2 (C-5), 83.5 (C-6), 76.6 (C-3), 51.9 (OMe), 42.4 (C-7), 38.3 (C-9), 37.2 (C-2), 30.9 (C-15), 29.3 (C-10), 28.4 (C-8), 24.9 (C-13), 22.9 (C-11), 22.2 (C-17), 14.1 (C-12), 11.8 (C-14), 8.2 (C-16); EIMS *m/z* (int., %) 297 (1, M - Me)$^+$, 283 (5), 209 (6), 181 (19), 139 (30), 125 (100); HRCIMS *m/z* 330.2630, calcd. for $C_{18}H_{36}NO_4$ (M + NH_4)$^+$ 330.2644.

Methyl (2*Z*,6*R*,8*S*)-6,8-dimethyl-3,6-epoxy-4-ethyldodeca-2,4-dienoate (6): colorless oil; $[\alpha]_D$ - 129 (*c* 0.5, $CHCl_3$); UV (MeOH) 283 nm (ε 9400); IR (film) 2960, 2930, 1715, 1690, 1630, 1165 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) see table 2; EIMS *m/z* (int., %) 280 (6, M$^+$), 256 (1), 249 (3), 195 (2), 181 (100); HRFABMS *m/z* 281.2106, calcd. for $C_{16}H_{27}O_3$ (M + H)$^+$ 281.2117.

Methylation of (3*S*,6*R*,8*S*)-4,6-diethyl-3,6-epidioxy-8-methyldodeca-4-enoic acid (1): Excess diazomethane was distilled into a solution of the peroxy-acid **1** (4 mg) in Et_2O (1 mL) at 10 °C. After 30 min at 10 °C, any excess diazomethane was destroyed by addition of acetic acid (1 drop) and the solution was filtered through a plug of silica gel to obtain the methyl ester **5** (4 mg, 96% yield), that was identical in all respects to the natural product.

Methylation of 3,6-epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid (2): Diazomethane (0.24 mmol), generated from nitrosomethylurea in 1:1 Et_2O/ 40% aqueous KOH (5 mL), was collected in the organic layer, which was added to a solution of the peroxy-acid **2** (18.4 mg, 0.05 mmol) in Et_2O (5 mL) at 10 °C. After stirring for 30 min at 10 °C, 5% acetic acid (5 mL) was added and the organic layer was washed with 5% $NaHCO_3$ (5 mL) and H_2O (5 mL). The organic layer was dried and evaporated to give an oil which was chromatographed on silica (7:3 $CHCl_3$/hexane) to obtain methyl 3,6-epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoate (**11**, 18 mg, 94% yield): ^1H NMR ($CDCl_3$) δ 0.82 (t, 3 H, J = 7 Hz), 0.83 (t, 3 H, J = 7.5 Hz), 0.88 (t, 3 H, J = 7.5 Hz), 0.92 (t, 3 H, J = 7 Hz), 0.94 (t, 3 H, J = 7.5 Hz), 1.14 (d, 2 H), 1.25 (m, 1 H), 1.38 (d, 2 H), 1.56 (m, 2 H), 1.70 (m, 1 H), 1.95 (m, 2 H), 1.97 (d, 2 H), 2.10 (m, 1 H), 2.11 (m, 3 H), 2.13 (m, 1 H), 2.39 (dd, 1 H, J = 16, 3 Hz), 3.00 (dd, 1 H, J = 16, 9.5 Hz), 3.69 (s, 3 H), 4.43 (m, 1 H), 5.12 (m, 1 H) 5.17 (s, 1 H), 5.35 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 7.8, 11.1, 11.8, 12.2, 14.0, 23.0, 25.1, 25.7, 27.9, 31.4, 32.9, 35.5, 36.0, 42.5, 42.8, 51.9, 78.8, 84.4, 127.4, 131.8, 133.1, 142.5, 172.4.

Iodolactonization of (3*S*,6*R*,8*S*)-4,6-diethyl-3,6-epidioxy-8-methyldodeca-4-enoic acid (1): Iodine (50 mg, 0.32 mmol) was added to a cooled two-phase reaction mixture consisting of chloroform (1.5 mL) and a homogeneous solution of the acid **1** (5 mg, 0.017 mmol) and $NaHCO_3$ (10 mg, 0.119 mmol) in water (1.5 mL) at 0-5°C. The mixture was stirred at 25°C for 3 days, when the phases were separated. The $CHCl_3$ layer was washed with 10% aqueous $Na_2S_2O_3$ solution until it was colorless and then with water (1 mL) and brine (1 mL). The solution was dried over anhydrous Na_2SO_4 and the solvent removed. The residue was purified by chromatography on silica gel using 5% EtOAc in hexane as eluant to obtain the iodo-lactone **7** (4 mg, 55% yield) as an oil: $[\alpha]_D$ - 34 (*c* 0.22, $CHCl_3$); IR (film) 2950, 2930, 1790, 1460, 1435, 1215 cm^{-1}; ^1H NMR (5:1 $CDCl_3/C_6D_6$, 300 MHz) see Table 1; ^{13}C NMR (5:1 $CDCl_3/C_6D_6$, 100 MHz) see table 1; EIMS *m/z* (int., %) 395 (1, M - Et), 297 (1), 225 (6), 198 (100); HRFABMS *m/z* 447.0999, calcd. for $C_{17}H_{29}IO_4Na$ $(M + Na)^+$ 447.1008.

Deiodination of iodo-lactone 7: Tri-*n*-butyltin hydride (8 mg, 3 eq.) and AIBN (4.5 mg, 2 eq.) were added to a stirred solution of iodo-lactone **7** (3.5 mg, 0.0082 mmol) in benzene (3 mL) and the mixture was stirred for 15 min. at 25°C. The reaction mixture was filtered through a small column of silica gel and purified by HPLC on silica using 10% EtOAc in hexane to obtain the lactone **8** (2.4 mg, 98% yield) as a colorless oil: IR (film) 2955, 2930, 1780, 1460, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS *m/z* (int., %) 269 (16, M - Et), 256 (1), 199 (11), 127 (100); HRFABMS *m/z* 316.2493, calcd. for $C_{17}H_{34}NO_4$ $(M + NH_4)^+$ 316.2488.

Reduction of lactone 8 to diol 9: Zinc (60 mg) was added to a solution of the lactone (**8**, 10 mg, 0.034 mmol) in Et_2O (1.5 mL) containing acetic acid (100 μL) and the reaction mixture was stirred overnight at 25°C. The solution was filtered to remove the excess zinc and zinc acetate and the solvent was removed to obtain the diol **9** (9 mg, 90% yield) as a colorless oil: IR (film) 2960, 2930, 1760, 1460, 1440, 1250, 1220,

1080, 950 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.87 (t, 3 H, J = 7 Hz), 0.90 (t, 3 H, J = 7.5 Hz), 0.95 (d, 3 H, J = 6.5 Hz), 0.96 (t, 3 H, J = 7.5 Hz), 1.23 (m, 6 H), 1.46 (dd, 1 H, J = 14, 6.5 Hz), 1.53 (m, 1 H), 1.62 (dd, 1 H, J = 14, 4 Hz), 1.63 (q, 2 H), 1.65 (dq, 1 H, J = 15, 7.5 Hz), 1.71 (dq, 1 H, J = 15, 7.5 Hz), 2.04 (d, 1 H, J = 15 Hz), 2.10 (d, 1 H, J = 15 Hz), 2.54 (dd, 1 H, J = 18.5, 2 Hz), 2.90 (dd, 1 H, J = 18.5, 8 Hz), 4.16 (dd, 1 H, J = 8, 2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 7.6, 8.3, 14.1, 21.9, 22.9, 28.7, 29.2, 32.4, 33.4, 38.4, 38.5, 38.6, 47.8, 73.1, 75.7, 92.8, 175.4; EIMS *m/z* (int., %) 285 (1, M - Me), 271 (2, M - Et), 257 (27), 183 (100), 127 (72); HRFABMS *m/z* 323.2208, calcd. for $C_{17}H_{32}O_4Na$ $(M + Na)^+$ 323.2198.

Preparation of (*R*)- and (*S*)-MTPA esters 10*R* and 10*S*: (*R*)- or (*S*)-Methoxytrifluoromethylphenylacetic acid (4 mg, 17 μmol) was added to two solutions of the diol **9** (1 mg, 3.3 μmol), DCC (4 mg, 19 μmol) and DMAP (0.5 mg, 4 μmol) in dichloromethane (0.5 mL, distilled from P_2O_5) and the reaction mixture was stirred under dry nitrogen at room temperature for 12 h. The solvent was evaporated and the residue was chromatographed on silica gel using a disposable pipet column with 10% EtOAc in hexane as eluant to obtain either the (*R*)-MTPA ester (**10*R***, 1.2 mg, 69% yield) or the (*S*)-MTPA ester (**10*S***, 1.3 mg, 74% yield). The Δδ values (ppm, $\delta_S - \delta_R$) were measured: H-2α, -0.013; H-2β, -0.108; H-3, -0.027; H-5α, +0.135; H-5β, +0.081; H-13, +0.042; H-13', 0.025; Me-14, +0.026; Me-16, +0.065; Me-17, +0.030.

Diimide reduction of methyl (2*Z*,6*R*,8*R*,9*E*)-3,6-epoxy-4,6,8-triethyl-2,4,9-dodecatriene (12): A solution of sodium acetate (15 mg) in water (1 mL) was added over the course of 3 hr to a refluxing solution of the furan **11** (4 mg, 0.013 mmol) and *p*-toluenesulfonhydrazide (20 mg) in 1:1 THF/H_2O (2 mL). Heating was continued for an additional 30 min. The cooled solution was concentrated under reduced pressure, saturated aqueous ammonium chloride (1 mL) and water (0.5 mL) were added, and the product extracted with CH_2Cl_2 (4 x 5 mL). The combined CH_2Cl_2 extracts were washed with 2N NaOH solution (2 x 5 mL) and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by HPLC on Partisil using 8% EtOAc in hexane as eluant to obtain methyl (2*Z*,6*R*,8*S*)-3,6-epoxy-4,6,8-triethyl-2,4-dodecadienoate (**4**, 3 mg, 75% yield), $[\alpha]_D$ -111 (*c* 0.2, $CHCl_3$). The ^1H and ^{13}C NMR spectra are identical to those of the natural product.

Biological Assay: The bioassay employed parasites of the trypanosomatide family characterized by Ramirez and Guevara[12] as *Leishmania mexicana* (NR strain). The cells were cultured in Liver Infusion Tryptose medium (LIT),[13] supplemented with 10% Fetal Calf Serum (GIBCO) and transferred to fresh medium every 5 days. The bioassays were carried out in triplicate. As described by Rangel *et al.*,[14] different concentrations of the drugs in DMSO solution were added to a suspension of the cells (2 x 10^6) in PBS (10 mL) at pH 7.2. DMSO alone was used as control. The cultures were placed in a New Bauer chamber and monitored daily for 96 h using light dispersion at 560 nm to determine cell density. The cells were also monitored using a Nikon-diaphot microscope to determine mobility of the parasite and the integrity of the membrane.

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References and Notes

1. Ashford, R. W.; Desjeux, P.; De Raedt, P. *Parasitology Today, Sect. B.*, **1992**, *8*, 104 - 105.
2. Jackson, J. E.; Tally, J. D.; Tang, D.B. *Am. J. Trop. Med. Hyg.*, **1989**, *41*, 318-330.
3. Faulkner, D. J. *Nat. Prod. Rep.*, **1997**, *14*, 259-302 and previous reports in this series.
4. a) Sakemi, S.; Higa, T.; Anthoni, U.; Christophersen, C. *Tetrahedron*, **1987**, *43*, 263-268. b) Gunasekera, S. P.; Gunasekera, M.; Gunawardana, G. P.; McCarthy, P.; Burres, N. *J. Nat. Prod.*, **1990**, *53*, 669-674. c) Rudi, A.; Kashman, Y. *J. Nat. Prod.*, **1993**, *56*, 1827-1830. d) Ichiba, T.; Scheuer, P. J.; Kelly-Borges, M. *Tetrahedron*, **1995**, *51*, 12195-12202 (erratum: *ibid.*, **1996**, *52*, 14079).
5. a) Patil, A.D.; Freyer, A.J.; Bean, M.F.; Carté, B. K., Westley, J. W.; Johnson, R. K.; Laharoute, P. *Tetrahedron*, **1996**, *52*, 337-394. b) Patil, A.D.; Freyer, A.J.; Carté, B. K.; Johnson, R. K.; Laharoute, P. *J. Nat. Prod.*, **1996**, *59*, 219-223.
6. Extraction of a small portion of this sponge with ethanol gave the same array of methyl esters **3**, **5**, and **6**, indicating that they are not artifacts of the isolation procedure.
7. Stierle, D.B.; Faulkner, D.J. *J. Org. Chem.*, 1980, 45, 3396-3401.
8. The NMR spectrometers used in the original research were vastly different to those used in the current research and made direct comparison of the spectra almost useless.
9. By the time we had made the observation in reference 8, the small quantity of peroxide **2** remaining after biological evaluation was insufficient to allow us to pursue the absolute stereochemistry. Since the second specimen of *P.* aff. *angulospiculatus* did not contain peroxide **2**, we will not be able to determine the absolute stereochemistry until we locate the correct sponge.
9. Schmidt, E.W.; Faulkner, D.J. *Tetrahedron Lett.*, **1996**, *37*, 6681-6684.
10. Rangel, H.R.; Daggar, F; Compagnone, R.S. *Cell Biol. Int.*, **1997**, *21*, 337-339.
11. Ramirez, J.L.; Guevara, P. *Mol. Biochem. Parasitol.*, **1987**, *22*, 177-183.
12. Hernandez, A.G.; Rascon, A.; Kutner, S.; Roman, H.; Campos, H. *Mol. Biol. Rep.*, **1993**, *18*, 189-195.
13. Rangel, H.R.; Dagger, F.; Hernandez, A.G.; Liendo, A.; Urbina, J.A. *Antimicrob. Agents Chemother.*, **1996**, *40*, 2785-2791.