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Antiparasitic, Antineuroinflammatory, and Cytotoxic Polyketides from the Marine Sponge *Plakortis angulospiculatus* Collected in Brazil[#]

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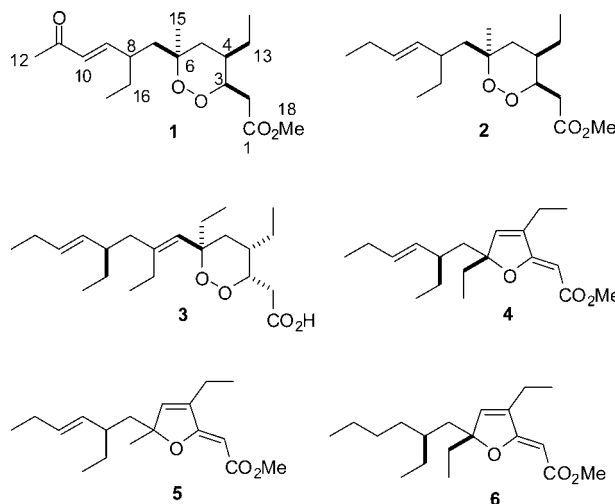
Investigation of the bioactive crude extract from the sponge *Plakortis angulospiculatus* from Brazil led to the isolation of plakorteneone (**1**) as a new polyketide, along with five known polyketides (**2–6**) previously isolated from other *Plakortis* sponges. The known polyketides were tested in antileishmanial, antitrypanosomal, antineuroinflammatory, and cytotoxicity assays. The results show that plakortide P (**3**) is a potent antiparasitic compound, against both *Leishmania chagasi* and *Trypanosoma cruzi*, and exhibited antineuroinflammatory activity. The known polyketides **2–6** were tested for cytotoxicity against four human cancer cell lines, but displayed only moderate cytotoxic activity.

Infectious diseases caused by kinetoplastid parasites, including malaria, Chagas disease, and leishmaniasis, are major public health problems in developing countries.¹ *Leishmania* parasites infect several mammalian species and are the etiologic agent of diverse clinical manifestations, such as visceral, cutaneous, and mucocutaneous leishmaniasis, affecting 12 million people worldwide.¹ In particular, visceral leishmaniasis is a progressive and fatal disease with an estimated 500 000 cases each year.² The toxicity and drug resistance to pentavalent antimonial derivatives, considered the antileishmanial drugs of first choice, severely limits presently available leishmaniasis treatment, particularly in immunocompromised patients.^{1c,d} The use of amphotericin B formulations is also limited due to high renal toxicity and other adverse effects.³

Chagas' disease caused by the protozoan *Trypanosoma cruzi* is a major cause of morbidity and mortality in many regions of South America, with approximately 16–18 million infected people worldwide and an annual incidence of 700 000–800 000 new cases.⁴ Chagas' chemotherapy is difficult, mainly due to the limited number of drugs available, which are ineffective and of high toxicity for treating chronic infections.⁵ Therefore, there is an urgent need for new drug leads active against diseases caused by kinetoplastid parasites.

Our current screening program aimed at the discovery of new marine natural products with antiparasitic activity began with the isolation and identification of new inhibitors of *Leishmania tarentolae* adenine phosphoribosyl transferase from the marine sponge *Callyspongia* sp.⁶ A continuing search of crude extracts and pure compounds active against *Leishmania* and *Trypanosoma* parasites, and inhibitors of specific kinetoplastid enzyme targets, led to a MeOH crude extract of the marine sponge *Plakortis angulospicu-*

latus as a source of active compounds. Fractionation of this crude extract afforded the isolation of one new polyketide (**1**), along with five known polyketides (**2–6**), which displayed antileishmanial, antitrypanosomal, antineuroinflammatory, and cytotoxic activity.



Results and Discussion

A freeze-dried sample of the sponge *P. angulospiculatus* was extracted with MeOH. The organic extract was concentrated and partitioned in turn with petroleum ether and EtOAc. ¹H NMR analysis of both the petroleum ether and EtOAc extracts indicated the presence of polyketides. Several separation steps by normal-phase column chromatography on silica gel followed by purification using normal-phase HPLC on either silica gel or phenyl-bonded silica columns yielded a new polyketide derivative, plakorteneone (**1**), as well as the known polyketides plakortin (**2**) {[α]_D +141 (c 1.0, CHCl₃)},⁷ plakortide P (**3**) {[α]_D –107 (c 0.56, MeOH)},^{8b} which is a stereoisomer of 3,6-epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid,^{8a,9} (2Z,6R,8R,9E)-methyl 3,6-epoxy-4,6,8-triethyl-2,4,9-dodecatrienoate (**4**) {[α]_D –258 (c 1.0, MeOH)},^{7b,c,10} spongisoritin A (**5**) {[α]_D –189 (c 0.19, MeOH)},¹¹ and (2E,6R,8S)-methyl 3,6-epoxy-4,6,8-triethyl-dodeca-2,4-dienoate (**6**) {[α]_D –135

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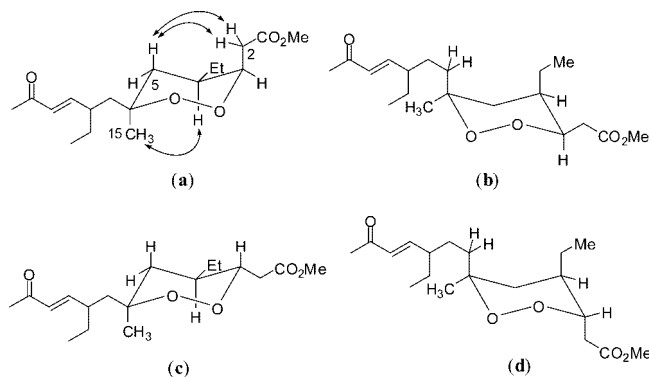
Table 1. ^1H and ^{13}C Data for Plakorteneone (**1**) in CDCl_3 and CD_3OD

position	$^{13}\text{C}^a$	$^1\text{H}^b$ (J, Hz)	$^{13}\text{C}^c$	$^1\text{H}^d$ (J, Hz)
1 (C)	172.0		174.0	
2 (CH_2)	31.5	2.87 (dd, 9.8, 15.6), 2.31 (dd, 4.0, 15.6)	32.3	2.86 (dd, 10.2, 15.6), 2.37 (dd, 3.3, 15.6)
3 (CH)	78.9	4.48 (m)	80.3	4.41 (m)
4 (CH)	34.7	2.19 (m)	36.0	2.21 (m)
5 (CH_2)	34.6	1.31 (m), 1.43 (m)	35.0	1.41 (m), 1.46 (m)
6 (C)	80.7		82.1	
7 (CH_2)	46.0	1.63 (m)	47.1	1.68 (m)
8 (CH)	39.9	2.31 (m)	41.3	2.37 (m)
9 (CH)	152.8	6.55 (dd, 9.5, 16)	155.9	6.67 (dd, 9.5, 15.9)
10 (CH)	130.7	6.03 (d, 16)	131.4	6.06 (d, 15.9)
11 (C)	198.7		n.o.	
12 (CH_3)	26.8	2.26 (s)	26.8	2.26 (s)
13 (CH_2)	25.1	1.15 (m), 1.20 (m)	26.1	1.22 (m)
14 (CH_3)	11.4	0.84 (t, 7.4)	11.8	0.86 (t, 7.4)
15 (CH_3)	22.1	1.37 (s)	22.9	1.39 (m)
16 (CH_2)	29.0	1.33 (m), 1.51 (m)	29.9	1.37 (m), 1.52 (m)
17 (CH_3)	11.0	0.89 (t, 7.4)	11.3	0.91 (t, 7.4)
18 (CH_3)	51.8	3.68 (s)	52.2	3.65 (s)

^a 100 MHz, CDCl_3 . ^b 400 MHz, CDCl_3 . ^c 100 MHz, CD_3OD . ^d 400 MHz, CD_3OD ; n.o.: not observed.

(c 0.52, MeOH)).^{9,11c} We have established the relative configurations of the known polyketides **2–6** isolated in the present investigation by comparison with ^{13}C NMR and $[\alpha]_D$ values of literature data.

Plakorteneone (**1**) was isolated as an optically active glassy solid. The HRESIMS of **1** indicated a $[\text{M} + \text{H}]^+$ ion at m/z 327.2177 and a $[\text{M} + \text{Na}]^+$ ion at m/z 349.1986, corresponding to the formulas $\text{C}_{18}\text{H}_{31}\text{O}_5$ and $\text{C}_{18}\text{H}_{30}\text{O}_5\text{Na}$, respectively, with four unsaturation degrees. Since the HRMS analysis indicated the presence of five oxygens, three of which were assignable to a methyl ester [δ 172.0 (C=O) and δ 51.8 (^1H at δ 3.68, $-\text{OCH}_3$)] and a ketone (δ 198.7), we have deduced that plakorteneone is a cycloperoxide rather than a tetrahydrofuran derivative, both of which are found commonly among *Plakortis* polyketides. The presence of a conjugated methyl enone moiety in **1** was evident on the basis of the UV absorption at λ_{max} 225 nm (ϵ 8300) and upon the observation of ^{13}C NMR signals at δ 26.8 (C-12), 198.7 (C-11), 130.7 (C-10), and 152.8 (C-9). The remaining ^{13}C and ^1H NMR signals (Table 1) were very similar to those of plakortin (**2**).^{7a,f} Analysis of the ^1H – ^1H COSY and HMBC data supported structure **1** for plakorteneone. The COSY spectrum showed ^1H – ^1H correlations of a spin system including the methylene at δ 1.63 (CH_2 -7), the sp^3 methine at δ 2.31 (H-8), the sp^2 methine at δ 6.55 (H-9), and a sp^2 methine at δ 6.03 (H-10). Additionally, the COSY spectrum showed couplings between the methine at δ 2.31 (CH-8) and the methylene at δ 1.33 and 1.51 (CH_2 -16), which was attached to the methyl group at δ 0.89 (CH_3 -17). Analysis of the HMBC spectrum indicated long-range couplings from CH_3 -12 (δ 2.26) to C-11, C-10, and C-9; from H-10 to C-12, C-11, and C-8; from H-9 to C-11, C-8, and C-7; from H-8 to C-9; and from CH_2 -7 to C-9, C-8, and C-16. The connection of the side chain to C-6 was established by long-range couplings observed between the methylene at δ 1.63 (CH_2 -7) and the quaternary carbon at δ 80.7 (C-6) as well as between CH_2 -7 and the methyl signal at δ 22.1 (CH_3 -15). This methyl group showed long-range couplings with C-6 (δ 80.7), C-7 (δ 46.0), and C-5 (δ 34.6). The HMBC spectrum also showed couplings of the methylene group at δ 1.31 and 1.43 (CH_2 -5) with C-15, C-6, and the methine at δ 34.7 (CH-4). The CH-4 methine hydrogen at δ 2.19 showed a coupling with CH_2 -13 (δ 25.1), which was sequentially coupled with CH_3 -14 (δ 11.4). Although the H-4 and H-3 signals did not exhibit any mutual coupling in the COSY spectrum, H-3 (δ 4.48) showed long-range couplings with C-4 (or C-5) and with C-2 (δ 31.5) in the HMBC spectrum. The HMBC spectrum indicated that the CH_2 -2 methylene group (δ 2.31 and 2.87) was attached to the methyl ester group. Finally, the stereo-

**Figure 1.** Conformations for $(3R^*,4R^*,6S^*)$ -**1** (a and b) and for $(3S^*,4R^*,6S^*)$ -**1** (c and d), as well as NOESY correlations observed for plakorteneone **1a**.

chemistry of the conjugated enone double bond was determined as *E* ($J_{\text{H-9/H-10}}$ of 16 Hz). Therefore, the planar structure of plakorteneone (**1**) was established.¹²

A comparison of the ^{13}C NMR data of plakorteneone (**1**) with literature values for plakortin (**2**)^{7a} and 3-epi-plakortin^{7b,f} suggested that **1** has the same relative stereochemistry at the cycloperoxide ring as that of **2** (Table S1, Supporting Information). Further support for the relative stereochemistry of **1** was obtained from a ^1H NMR selective decoupling experiment in $\text{MeOH}-d_4$. Irradiation at δ 2.31 (H-2b and H-8) simplified the signals of H-3 at δ 4.41 to a doublet (4.4 and 10 Hz) and of H-9 at δ 6.67 to a doublet (16 Hz). Hence, the $^3J_{\text{H-3/H-4}}$ coupling constant is 4.4 Hz, in agreement with a $^3J_{\text{H-3/H-4}}$ equatorial/axial coupling, compatible with $(3R^*,4R^*,6S^*)$ -**1** but not with $(3S^*,4R^*,6S^*)$ -**1** (Figure 1). Analysis of the two cycloperoxide conformations for $(3R^*,4R^*,6S^*)$ -**1** (Figures 1a and 1b) indicated that conformation **1a** is less subject to steric interactions between the cycloperoxide substituents than conformation **1b**. Finally, analysis of the NOESY spectrum showed dipolar couplings between the methyl group at δ 1.37 (CH_3 -15) and the methine proton at δ 2.19 (CH-4), as well as between both protons of the methylene group at δ 2.87 and 2.31 (CH_2 -2) and the proton at δ 1.31 of CH_2 -5 (Figure 1a). These dipolar couplings confirmed the relative stereochemistry $(3R^*,4R^*,6S^*)$ for **1**, the same as plakortin (**2**).^{7a} The relative stereochemistry at C-8 could not be unambiguously established.

The antileishmanial, antitrypanosomal, antineuroinflammatory, and cytotoxic activities of compounds **2–6** were evaluated *in vitro*. The antileishmanial activity against *Leishmania chagasi* promastigotes showed that compounds **3–6** killed 100% of parasites at a maximal concentration of 25 $\mu\text{g/mL}$ in a dose-dependent manner, with IC_{50} values in the range 1.9–8.5 $\mu\text{g/mL}$. As shown in Table 2, the most potent antileishmanial effect was observed for plakortide P (**3**) (IC_{50} of 1.9 $\mu\text{g/mL}$). Moreover, under light microscopy it was shown that plakortide P induced a complete alteration on *L. chagasi* promastigotes, indicating a leishmanicidal effect.

Usually, antileishmanial activity is evaluated on axenic promastigotes. However, *L. chagasi* intracellular amastigotes are pathologically more relevant, because they present a distinct metabolic pattern and an increased resistance to therapeutic treatment than extracellular promastigotes.¹³ Therefore, compounds **3–6** were also submitted to an intracellular amastigote assay and demonstrated significant amastigote leishmanicidal activity (IC_{50} = 0.50–3.40 $\mu\text{g/mL}$). Interestingly, compound **3** was again the most active compound in this assay, reducing 100% intracellular amastigotes (IC_{50} = 0.5 $\mu\text{g/mL}$) while preserving the integrity of the host cell. Plakortide P (**3**) exhibited no hemolytic activity for mice erythrocytes, while polyketide **4** showed significant hemolytic activity (7.24%) at 6.25 $\mu\text{g/mL}$. Our results demonstrate that acid **3** should be further evaluated in experimental *in vivo* antileishmanial assays. In order to evaluate the selective index (SI, cytotoxicity IC_{50} on macro-

Table 2. Biological Activities of Polyketides 2–6

compound	antiparasitic ^a							antineuroinflammatory ^b			cytotoxicity ^c			
	anti-pro	anti-ama	hemolytic	cytotoxicity	SI	<i>T. cruzi</i>	SI-T.c.	O ₂ [−]	TXB ₂	LDH	MDA-MB435	HCT-8	SF295	HL60
2	6.0	n.d.	0	4.7	n.d.	n.d.		>1	>1	>10	10.4	3.0	9.8	13.3
3	1.9	0.5	0	16.6	31.7	2.3	7.1	>10	0.93	>10	n.d.	n.d.	n.d.	n.d.
4	8.5	1.6	7.24	30.2	18.6	16.5	1.8	>10	>10	>10	9.6	7.2	11.4	8.6
5	2.5	3.1	1.8	29.8	9.6	25.7	1.15	>10	>10	>10	>25	>25	>25	>25
6	3.9	3.4	0	31.6	9.2	31.6	1.0	>10	>10	>10	6.5	8	16.8	15.2
pentamidine	0.1	n.d.	n.d.	8.7	n.d.									
glucantime	n.d.	21.0	n.d.	>1000										
benznidazole						36.3								

^a Antiparasitic assays: anti-pro = anti-*L. chagasi* promastigotes and anti-ama = anti-*L. chagasi* intracellular amastigotes, both expressed as IC₅₀ in $\mu\text{g/mL}$; hemolytic activity expressed in % at 6.25 $\mu\text{g/mL}$; cytotoxicity on human macrophages expressed as IC₅₀ in $\mu\text{g/mL}$; SI = selective index; *T. cruzi* = anti-*Trypanosoma cruzi* expressed as IC₅₀ in $\mu\text{g/mL}$. n.d.: not determined. ^b Antineuroinflammatory assay: effect on rat microglia PMA [1 μM]-stimulated release of O₂⁻, TXB₂, and LDH. Data shown corresponds to two independent experiments and is expressed as IC₅₀ (μM) for O₂⁻, and TXB₂LDH (μM) is the compound's concentration causing 50% percent of the LDH release observed with 0.1% Triton X-100-treated microglia. ^c Cytotoxicity assays: MDA-MB435 (human breast), HCT-8 (colon), SF295 (human central nervous system), HL60 (leukemia), expressed as IC₅₀ in $\mu\text{g/mL}$.

phages/antileishmanial IC₅₀ on *L. chagasi* amastigotes) for compounds 2–6, the cytotoxicity against human macrophages was determined after 48 h incubation with the MTT assay. All compounds tested were cytotoxic in a dose-dependent manner (IC₅₀ = 16.6–31.6 $\mu\text{g/mL}$). The SI for compound 3 (cytotoxicity IC₅₀ on macrophages/antileishmanial IC₅₀ on *L. chagasi* amastigotes) was 31.6, which is considered excellent. A SI of 9.6 was observed for spongisoritin A (5), but with a moderate hemolytic activity (1.8%) at a concentration close to the leishmanicidal activity (IC₅₀ = 3.08 $\mu\text{g/mL}$ on amastigotes), while compound 6 demonstrated a SI of 9.2.

With the purpose of determining the mode of action of plakortide P (3), peritoneal macrophages were incubated for 24 h and the production of nitric oxide (NO) was determined by the Griess reaction.¹⁴ Bacterial lipopolysaccharide (LPS) was used as an internal control for macrophage activation. Immunologically activated macrophages eliminate intracellular amastigotes with NO production induced by INF- γ from CD4⁺ T-cells type 1.¹⁵ In contrast to LPS, acid 3 did not up-regulate NO production by macrophages. Pentavalent antimony has been shown to induce extracellular signal-regulated kinase 1 (ERK-1) and ERK-2 phosphorylation through phosphoinositide 3-kinase (PI3K), resulting in the up-regulation of NO by macrophages.¹⁶ The antileishmanial effects of pentavalent antimony appear to suggest host cell activation through the up-regulation of reactive oxygen species (ROS), indicating the requirement of a functional T-cell action for pentavalent antimony activity.¹⁶ Thus, AIDS immunocompromised *Leishmania* spp.-infected patients cannot be efficiently treated with pentavalent antimonials. Our data appear to suggest that plakortide P (3) eliminated efficiently intracellular amastigotes by other than NO up-regulation, probably involved in the specific leishmanicidal activity.

Aiming to further evaluate the ultrastructural changes on *L. chagasi* in the presence of acid 3, promastigotes were incubated with compound 3 and analyzed under transmission electron microscopy. Figure 2A shows promastigotes in the absence of 3. After a 3 h incubation with 3, both a mitochondrial swelling in promastigotes (Figure 2B, C (white arrow)) and a concomitant increase in the number of vacuoles were observed. Furthermore, abnormal parasite chromatin was also observed (Figure 2B, black arrow), while in the flagellar pocket blebs were present in the flagellar membrane, demonstrating initial damage induced by compound 3. In spite of these significant modifications, no pore-forming alterations were noted within the cellular membrane, whose integrity was preserved (Figure 2F, white arrow). After 5 h incubation, portions of the cellular membrane detached, forming blebs (Figure 2E, black arrow), and the nucleus was completely destroyed along with complete chromatin decondensation and formation of blebs in the nuclear membrane (Figure 2D, white

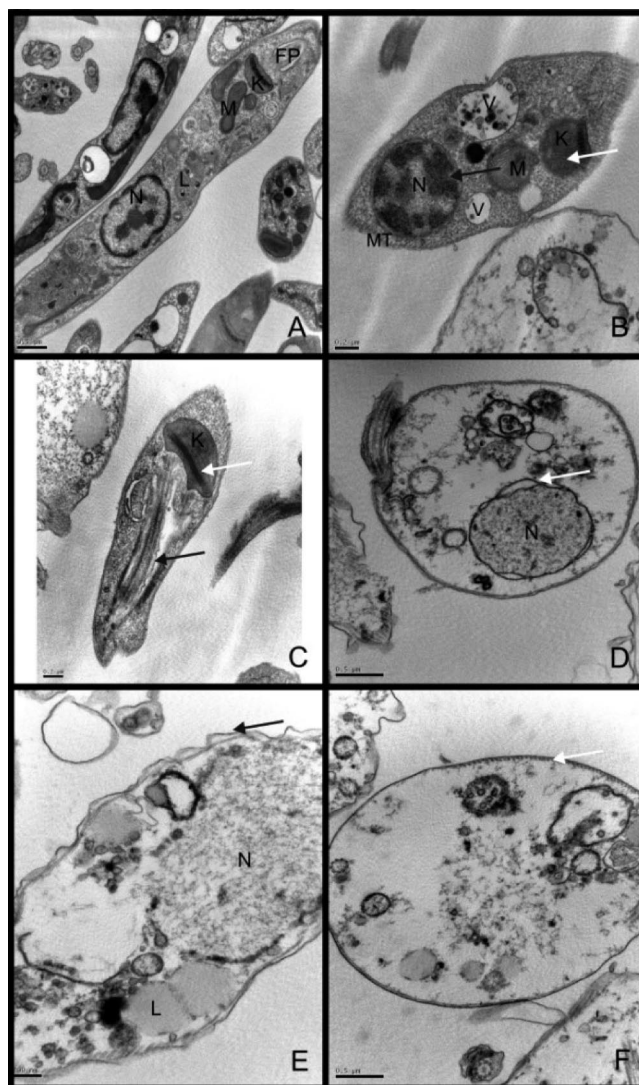


Figure 2. Transmission electron microscopy of *Leishmania chagasi* incubated with acid 3. Promastigotes were incubated for different periods at 24 °C. (A) control group. (B, C) 3 h incubation. (D, E, F) 5 h incubation. K, kinetoplast; M, mitochondria; N, nucleus; FP, flagellar pocket; L, lipid inclusion; V, vacuoles; MT, microtubules.

arrow). These observations indicated an extensive morphology alteration of *L. chagasi* promastigotes in the presence of acid 3.

Compounds 2–6 were also tested against the etiologic agent of Chagas' disease, *Trypanosoma cruzi*. The antitrypanosomal activity

was determined using LLC-MK2-derived trypomastigotes of *T. cruzi* (Y strain) using the MTT assay. After a 24 h incubation, all *T. cruzi* parasites were killed by compounds **3–6**, with acid **3** showing the highest antitrypanosomal activity (IC_{50} value of 2.3 $\mu\text{g/mL}$), 15-fold more potent activity than benznidazole, the only clinically available antitrypanosomal agent, but this is ineffective on chronic Chagas' disease.⁴ The cytotoxicity assay with plakortide P (**3**) against mammalian cells resulted in a SI value of 7.15 for *T. cruzi* (Table 2).

Table 2 also presents the antineuroinflammatory effect of compounds **3–6**, determined as the effect on the release of thromboxane B_2 (TXB₂) or superoxide anion (O_2^-) from activated rat brain microglia,¹⁷ both mediators involved in neurodegenerative disorders and neuroinflammation.^{18,19} Compound **3** potently inhibited TXB₂ generation (IC_{50} = 0.93 μM) with low lactate dehydrogenase release (LDH_{50} > 10 μM), an enzyme considered a marker for cell toxicity.^{17,19} In contrast, while compounds **4–6** were relatively nontoxic to the microglia cells (LDH_{50} > 10 μM), they were minimally effective as inhibitors of TXB₂ and O_2^- release (IC_{50} > 10 μM). Thus, the current data suggest that the effect of compound **3** on microglia TXB₂ release was of a pharmacological rather than of a toxicological nature, indicating that compound **3** may be considered as a lead compound for the development of a potentially novel antineuroinflammatory agent.²⁰

Polyketides **2** and **4–6** displayed only weak cytotoxic activity against four human cancer cell lines (Table 2).

In conclusion, the present investigation reports the isolation of plakorteneone (**1**), a new polyketide from the sponge *P. angulospiculatus*, along with five known polyketides (**2–6**). Unfortunately, due to the small amount of **1** available, it could not be tested in the antiparasitic, antineuroinflammatory, and cytotoxic bioassays used. Polyketides **2** and **4–6** displayed only moderate cytotoxicity for a small panel of cancer cell lines, and only acid **3** displayed antineuroinflammatory, leishmanicidal, and trypanocidal activity, as well as low hemolytic and cytotoxic activity in human macrophages. The highly selective antileishmanial activity to *L. chagasi* of plakortide P (**3**), isolated from *P. angulospiculatus*, is described for the first time.

Related polyketides isolated from *Plakortis* spp. are known to display antiparasitic activity. The carboxylic acid **3** and a related polyketide isolated from *Plakortis* aff. *angulospiculatus* exhibited cytotoxic activity against *L. mexicana*.^{9a} The acid **3** and its enantiomer have recently been reisolated from *Plakortis* sp., and the antileishmanial activity against *L. mexicana* was confirmed.^{9b} Polyketides from *Plakortis* sp. and from *P. halichondrioides* exhibited significant antimalarial activity *in vitro* against *Plasmodium falciparum*.²¹ Related compounds previously isolated from *Plakortis* sponges were shown to exhibit antimalarial^{7f} and cytotoxic activities^{7g,h} and inspired the preparation of several semisynthetic derivatives to obtain insights on the structural requirements for the antimalarial activity.⁷ⁱ To the best of our knowledge, only sesterterpenes from the sponge *Hyrtios erecta* have been previously described as marine natural products active against *T. cruzi*.²² Finally, the conjugated enone moiety in **1** is not without precedent among *Plakortis* polyketides, as the same chromophore is present in plakortide I, a lower homologue of **1**, which was isolated from *Plakortis* sp.,²³ as well as in a higher homologue of **1** reported from another *Plakortis* sp.²⁴

Experimental Section

General Experimental Procedures. The general experimental procedures used are the same as previously reported.²⁵ Sodium dodecyl sulfate (SDS), lipopolysaccharide (LPS), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (Thiazol blue; MTT), M-199, and RPMI-PR 1640 media (without phenol red), pentavalent antimony, and pentamidine were purchased from commercial suppliers and used without further purification.

Animal Material. The sponge *Plakortis angulospiculatus* was collected in August/September 1999, at the Baía de Todos os Santos,

Salvador, Bahia state, Brazil, and immediately frozen. A voucher specimen was deposited in the Porifera collection of the Museu Nacional, UFRJ, RJ, Brazil (MNRJ 2515).

Extraction and Isolation. The frozen sponge (134.0 g, wet) was freeze-dried, extracted with MeOH, and filtered. The solvent was evaporated down to a volume of 250 mL of MeOH/H₂O. The MeOH/H₂O phase was partitioned against petroleum ether, evaporated, suspended in 1:1 EtOAc/H₂O, and partitioned again. ¹H NMR analysis of the petroleum ether (2.34 g) and EtOAc (2.24 g) extracts indicated the presence of *Plakortis* polyketides.

The EtOAc extract was subjected to a separation by silica gel CC, with a gradient of MeOH in CH₂Cl₂, to give eight fractions (PaAE-1 to PaAE-8). Only fraction PaAE-1 (260.0 mg) indicated the presence of polyketides by ¹H NMR spectroscopy and was separated by silica gel CC with a gradient of CH₂Cl₂ in *n*-hexane. Six fractions were obtained (PaAE-1A to PaAE-1F), of which only fraction PaAE-1B (39.0 mg) contained polyketides. This fraction was purified by HPLC using a Waters μ -Porasil silica gel column (125 Å, 10 μm , 7.8 \times 300 mm) with 9:1 *n*-hexane/*i*-PrOH, to give 4.3 mg of plakortin (**2**).

The petroleum ether extract (2.34 g) was subjected to CC on silica gel with a gradient of CH₂Cl₂ in *n*-hexane, to give five fractions (PaE-1 to PaE-5). Only fraction PaE-2 (1.36 g) presented polyketides and was further separated by silica gel CC with a gradient of CH₂Cl₂ in *n*-hexane, to give four fractions (PaE-2A to PaE-2D). Fraction PaE-2A was subjected to two subsequent purifications by HPLC, the first on a phenyl-bonded silica column (Phenomenex Prodigy 5 μm phenyl-3 100 Å, 4.6 \times 250 mm) with 95:5 *n*-hexane/EtOAc and the second on a μ -Porasil silica gel column with 98:2 *n*-hexane/*i*-PrOH, to give 2.0 mg of plakorteneone (**1**). Fractions PaE-2B and PaE-2C were both separated by HPLC using a Waters Nova Pak silica gel column (6 μm , 19 \times 300 mm) with 9:1 *n*-hexane/EtOAc, to give 122.0 mg of methyl 3,6-epoxy-4,6,8-triethyl-2,4,9-dodecatrionoate (**4**), 21.0 mg of spongisoritin A (**5**), and 30.0 mg of methyl 3,6-epoxy-4,6,8-triethyldodeca-2,4-dienoate (**6**). Fraction PaE-1D was purified using a Nova Pak Si gel column with 85:15 *n*-hexane/EtOAc, to give 26.0 mg of 3,6-epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid (**3**).

Plakorteneone (1): colorless, glassy solid, $[\alpha]_D^{25} +45.5$ (c 0.2, MeOH); UV (MeOH) λ_{max} 225 (ε 8300) nm; ¹H NMR (CDCl₃ and CD₃OD, 400 MHz), see Table 1; ¹³C NMR (CDCl₃ and CD₃OD, 100 MHz), see Table 1; positive APCIMS m/z 327.4 [M + H]⁺ (91%), 277.3 (10%), 241.3 (100%), 213.4 (35%); HRESIMS m/z found 327.2177 [M + H]⁺, calcd for C₁₈H₃₁O₅, 327.21660; m/z found 349.1986 [M + Na]⁺, calcd for C₁₈H₃₀O₅Na, 349.1990.

Bioassay Procedures. BALB/c mice and golden hamsters were supplied by the animal breeding facility at the Instituto Adolfo Lutz of São Paulo and maintained in sterilized cages under a controlled environment, receiving water and food *ad libitum*. Animal procedures were performed with the approval of the Research Ethics Commission, in agreement with the Guide for the Care and Use of Laboratory Animals from the National Academy of Sciences (<http://www.nas.edu>).

Parasite Maintenance. *Leishmania chagasi* (MHOM/BR/1972/LD) was maintained in infected golden hamsters. Approximately 60–70 days postinfection, amastigotes were obtained from hamster spleens by differential centrifugation, and the parasite burden was determined using the Stauber method.²⁶ Isolated promastigotes were maintained in M-199 medium supplemented with 10% (v/v) calf serum and 0.25% (v/v) hemin at 24 °C.

Antileishmanial Activity. The antileishmanial activity against *L. chagasi* promastigotes was determined as described elsewhere,²⁷ using pentamidine as standard. Promastigotes were counted in a Neubauer hemocytometer and seeded at 1×10^6 cells/well in 96-well microplates. Test secretions were incubated in concentrations ranging from 0.488 to 500 $\mu\text{g/mL}$ (based on dry weight) for 48 h at 24 °C. Parasite viability was determined using the MTT assay at 570 nm.²⁸ The antileishmanial activity against intracellular amastigotes was determined with infected macrophages, using pentavalent antimony as a standard. The parasite burden was defined as the mean number of amastigotes per macrophage out of 500 cells.

Antitrypanosomal Activity. Free trypomastigotes obtained from LLC-MK2 cultures were counted in a Neubauer hemocytometer and seeded at 1×10^6 cells/well in 96-well microplates. The test secretions were incubated to the highest concentration (100 $\mu\text{g/mL}$) for 24 h at 37 °C in a 5% CO₂ humidified incubator with benznidazole as standard. The trypomastigote viability was based on the cellular conversion of the soluble tetrazolium salt MTT into the insoluble formazan by

mitochondrial enzymes.²⁹ The formazan extraction was carried out with 10% (v/v) SDS for 18 h (100 μ L/well) at 24 °C.

Cytotoxicity Assays on Macrophages. Macrophages were obtained from the peritoneal cavity of BALB/c mice in RPMI-PR-1640 medium (without phenol red) and transferred at 4×10^5 cells to 96-well microplates for 2 h for attachment at 37 °C in a 5% CO₂ incubator. Cells were washed twice with medium at 37 °C and further incubated with pure compounds in the range 3.9 to 500 μ g/mL for 48 h at 37 °C. The viability of macrophages was determined using the MTT assay.²⁸ Briefly, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was dissolved in phosphate-buffered saline (PBS) at 5 mg/mL and incubated with cells (20 μ L/well) for 4 h under the same conditions. The extraction of the mitochondrial formazan was performed with 100 μ L of 10% SDS with a further 24 h incubation. The optical density was read at 570 nm using control wells without drugs (100% viability) and without cells (blank). Pentamidine was used as standard.

Hemolytic Activity. The hemolytic activity of isolated compounds was evaluated on BALB/c erythrocytes. A 3% suspension of mice erythrocytes was incubated for 2 h with isolated compounds in 96-well U-shaped microplates at 25 °C, and the supernatant was read at 550 nm in a Multiskan reader.³⁰

Macrophage Nitric Oxide Production. The production of nitric oxide (NO) by macrophages was measured in the presence of acid 3 using the Griess reaction.¹⁴ Peritoneal macrophages were incubated for 24 h at 37 °C with compound 3 (4 μ g/mL). Lipopolysaccharide (50 μ g/mL) was used to induce NO up-regulation. The absorbance was determined at 540 nm using a microplate reader.

Transmission Electron Microscopy Analysis. *L. chagasi* promastigotes were incubated with compound 3 at 17 μ g/mL for different periods (0, 1, 3, 5, 7 h) at 24 °C in 24-well plates. Subsequently, promastigotes were processed³¹ and observed in a JEOL transmission electron microscope.

Antineuroinflammatory Assay. Rat neonatal brain microglia (2×10^5 cells) were seeded into each well of 24-well flat-bottom culture clusters and stimulated with *Escherichia coli* lipopolysaccharide (LPS) (0.3 ng/mL) in Dulbecco's modified Eagle medium + 10% fetal bovine serum + penicillin + streptomycin for 17 h in a humidified 5% CO₂ incubator at 35.9 °C as described.¹⁷ Medium was then removed, and the microglia were washed with warm (37 °C) Hanks' balanced salt solution (HBSS) and then incubated with compounds (0.1–10 μ M) or vehicle (DMSO) for 15 min prior to stimulation with phorbol 12-myristate 13-acetate (PMA) (1 μ M). All experimental treatments were run in duplicate and in a final volume of 1 mL. Seventy minutes after PMA stimulation, HBSS was aspirated from each well and superoxide anion (O₂^{•−}), thromboxane B₂ (TXB₂), and lactate dehydrogenase (LDH) release were determined as described.¹⁷ Table 2 shows data from two independent experiments, expressed as the compound's 50% inhibitory concentration (IC₅₀) for either O₂^{•−} or TXB₂. LDH release from microglia was determined spectrophotometrically as described.¹⁷ LDH release was expressed as the compound concentration (μ M) that yielded 50% of the LDH release observed with 0.1% Triton X-100-treated control microglia.

Cytotoxicity Assay on Cancer Cell Lines. Cell lines and procedures were used as recently described.³²

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Supporting Information Available: This material is available free of charge via the Internet at <http://pubs.acs.org>.

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