Isolation and Biological Evaluation of Filiformin, Plakortide F, and Plakortone G from the Caribbean Sponge *Plakortis* sp.

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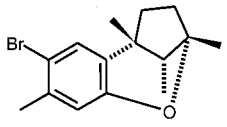
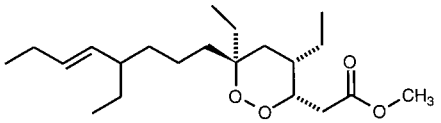
The bioassay- and spectroscopic-guided fractionation of the antimalarial extract from a Jamaican sponge, *Plakortis* sp., resulted in the isolation of three metabolites. The previously reported bromoaromatic filiformin (**1**) was obtained from our sample of *Plakortis* sp., and the potential origins of this compound are discussed. The peroxide-containing metabolite, plakortide F (**2**), is a more typical *Plakortis* metabolite and was shown to exhibit significant activity against *Plasmodium falciparum* in vitro. The isolation, structure, and bioactivity of a new lactone, plakortone G (**3**), are also reported.

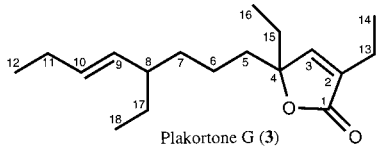
Marine sponges of the genus *Plakortis* produce a diversity of secondary metabolites, many of which are biologically active. Here we describe the isolation and structure elucidation of three compounds from the antimalarial extract of a *Plakortis* sp., one previously reported from a red alga, another previously reported from a *Plakortis* sponge, and a third unreported compound. The bromoaromatic compound, filiformin (**1**), was previously isolated from the Australian red alga *Laurencia filiformis* f. *heteroclada*,[1] and its synthesis has been reported.[2] Halogenated metabolites are commonly isolated from marine invertebrates, yet there have been no previous reports of halogenated compounds isolated from *Plakortis* spp. or from any other sponges of the order Homosclerophorida. Although the source of filiformin was not specifically addressed by this study, the fact that filiformin (**1**) was isolated in a very low yield suggests either contamination of the sponge sample by red algal filaments or a potential microbial origin for this compound. However, since *Plakortis* spp. are typically free of fouling organisms and there was no evidence of contamination of the sample by macroalgae, a microbial origin of filiformin seems plausible. In addition, filiformin shares considerable structural homology with the previously reported plakotenins from *Plakortis lita*.[3] As a result, the possibility that filiformin may be a sponge-derived metabolite cannot be ruled out. Bioactive peroxide-[4] and lactone-containing[5a] metabolites have been widely reported from sponges of the genus *Plakortis*; however, there are no previous reports of antimalarial activity from *Plakortis* metabolites. The high frequency with which *Plakortis* sponges yield new bioactive peroxide-containing metabolites coupled with the importance of the peroxide-containing antimalarial agent artemisinin clearly indicates the potential of this genus of sponges in the discovery of new antimalarials. Here we report the isolation of the known peroxide, plakortide F[6] (**2**), and provide additional biological activity. A new lactone (**3**) sharing the same carbon skeleton as **2** was also isolated, and we report its structure determination and biological activity. The primary difference between compound **3** and most previously isolated lactones from *Plakortis* spp.[5] is the absence of a tetrahydrofuran moiety in **3**. Plakortone G (**3**) is a closely related analogue of the γ-lactone isolated by Stierle and Faulkner.[7]



Filiformin (**1**)



Plakortide F (**2**)



Plakortone G (**3**)

The sponge *Plakortis* sp. was collected at a depth of −40 m at Discovery Bay, Jamaica, using closed circuit rebreathers, and frozen. The lyophilized sponge was extracted exhaustively in 1:1 IPA−EtOAc, and the crude extract exhibited cytotoxic, antimalarial, antimicrobial, and antifungal activities. The crude extract was separated by silica gel vacuum liquid chromatography (hexanes−EtOAc−MeOH), and the antimalarial activity was concentrated in the nonpolar fractions eluting with hexanes−EtOAc. The hexane and 9:1 hexane−EtOAc fractions were selected for further purification by HPLC due to their high differential activity against *Plasmodium falciparum* when compared to Vero cells. Filiformin (**1**) was eluted from the hexane fraction on normal-phase HPLC. Plakortide F (**2**) and compound **3** were isolated from the 9:1 hexane−EtOAc VLC column fraction, which was further chromatographed using normal- and reversed-phase HPLC.

GCEIMS of compound **1** showed the $[M]^+$ ion at 294, consistent with the molecular formula $C_{15}H_{19}OBr$. Comparison of the molecular weight, $[\alpha]_D$, ^1H NMR, and ^{13}C NMR spectra with the literature indicated that compound **1** was filiformin.[1] Filiformin was previously found to inhibit oxygen uptake in isolated rat liver mitochondria at a concentration of 150 μM. In the present study, filiformin was assayed for NFκB activation (immunostimulation)[9] but was inactive at $\leq$50 μg/mL.

Comparison of HRESI-FTMS, $[\alpha]_D$, ^1H NMR, and ^{13}C NMR spectra with published data indicated that compound

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Table 1. Bioactivity Data for Plakortide F (**2**) and Plakortone G (**3**)

assay	plakortide F IC_{50}	plakortone G IC_{50}	controls (IC_{50})
Plasmodium falciparum (D6 clone) in vitro[12]	480 ng/mL	4200 ng/mL	artemisinin (12 ng/mL) chloroquine (18 ng/mL)
P. falciparum (chloroquine-resistant W2 clone) in vitro[12]	390 ng/mL	>4760 ng/mL	artemisinin (7 ng/mL) chloroquine (220 ng/mL)
P. berghei in vivo[10]	inactive[a]	N.T.	artemisinin (100 μM/kg)[a] chloroquine (100 μM/kg)[a]
mouse lymphoma cancer P388	1.25 μg/mL	N.T.	
human colon carcinoma HT29	1.25 μg/mL	>1 μg/mL	tamoxifen (1.86μg/mL)
human lung carcinoma A549	2.5 μg/mL	>1 μg/mL	tamoxifen (1.86μg/mL)
human melanoma MEL28	2.5 μg/mL	>10 μg/mL	tamoxifen (1.86μg/mL)
human primary tumor cells[b]	3.4−3.9 μg/mL[c]	4.7 μg/mL[c]	doxorubicin (25 nM)
HIV[13]	13−42 μM[d]	N.T.	AZT (0.004 μM)
hepatitis-B[14]	>100 μg/mL[e]	>100 μg/mL[e]	3TC (0.062−0.065 μg/mL[e])
Mycobacterium tuberculosis[15]	29% at 6.25 μg/mL[f]	4% at 6.25 μg/mL[f]	rifampin (0.25 μg/mL[g])
Toxoplasma gondii[16]	67% at 1 μM[h]	N.T.	atovaquone (100% at 1 μM[h])

[a] Compound tested at 3 × 100 μM/kg i.p.; control is i.p. dose that yields 0.1% (chloroquine) or 35.9% (artemisinin) parasitemia after 3 days. [b] Tumor tissues were obtained from patients undergoing surgical resections for therapeutic purposes. The tissues were minced and digested with enzymes. The tumor cells were isolated by size, density, and negative immunoselection. The compounds were tested against primary tumor cells maintained in short-term culture, and cytotoxicity was measured by Alamar Blue. [c] These compounds were highly cytotoxic, but were not selective for the tumor cells. [d] EC_{50}; plakortide F was cytotoxic to host cells at IC_{50} = 7.3−21.4 μM. [e] EC_{50}. [f] Percent inhibition. [g] MIC. [h] Percent inhibition after 72 h.

2 was plakortide F.[6] Plakortide F is known to activate cardiac SR-Ca^{2+} uptake,[6] but no other biological activity was previously reported. We now report the biological activity of **2** against *Plasmodium* spp., HIV-1, and several cancer cell lines (Table 1). The in vitro antimalarial activity was promising; however, this compound failed to prolong life expectancy when administered IP to *Plasmodium*-infected mice at 100 μM/kg.[10] Plakortide F also exhibits moderate activity against *Toxoplasma gondii* and HIV-1 (Table 1), although its cytotoxicity to host cells precludes further pursuit as an anti-HIV lead. Plakortide F exhibits significant activity against a number of cancer cell lines but is not selective (Table 1). Plakortide F did not exhibit antifungal or antimicrobial activity against *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Mycobacterium intracellulare*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and methicillin-resistant *S. aureus*. tested.

Compound **3** was assigned the molecular formula $C_{18}H_{30}O_2$, by HRESI-FTMS (279.2353 $[M + H]^+$). The strong IR absorption at 1754 cm^{-1} indicated the presence of a five-membered α,β-unsaturated lactone. The ^{13}C and DEPT spectra indicated four methyl, seven methylene, four methine, and three quaternary carbons. The carbonyl carbon signal at δ 174.0 (C-1), along with the oxygenated carbon resonance at δ 89.8 (C-4), further confirmed the presence of a lactone. An olefinic methine carbon peak at δ 150.7 (C-3) and a quaternary carbon at δ 136.3 (C-2) complete the unsaturated lactone ring. HMBC correlations supported the presence of an ethyl side chain attached to C-2, consisting of a methylene carbon [δ 18.9 (C-13)] and a methyl group [δ 12.5 (C-14)]. The long-range allylic coupling between protons H-3 and H-13 further supports the location of this side chain, as does the NOESY correlation between protons H-3 and H-14. An additional ethyl side chain at the quaternary C-4 (δ 89.8) was supported by the presence of HMBC correlations between C-4 and the methylene protons H-15 and methyl protons H-16, as well as a NOESY correlation between protons H-3 and H-16. HMBC correlations between C-5 and protons H-3, H-4, H-15, H-6, and H-7 indicated that a chain of three methylene carbons (δ 37.3, 21.6, 35.5) connected the quaternary C-4 to a methine carbon [δ 44.8 (C-8)]. In addition, two carbon chains originate from C-8, one an ethyl consisting of a methylene carbon [δ 28.6 (C-17)] and a methyl group [δ 12.1 (C-18)] and the other a four-carbon chain with olefinic carbons [δ 133.4 (C-9) and δ 132.8 (C-10)]. The placement of these carbon chains was confirmed by HMBC correlations between C-18 and protons H-17 and H-8, between C-12 and H-11 and H-10, and between the olefinic carbon C-9 and protons H-7, H-8, H-10, H-11, and H-17. The C-9,10 double bond was assigned the *E* configuration on the basis of the allylic carbon chemical shifts of C-8 (δ 44.8) and C-11 (δ 26.1), combined with the olefinic proton coupling of 15 Hz. Also supporting the *E* configuration is the NOESY correlation between protons H-10 and H-8.

The previously reported plakortones A−D were found to activate cardiac SR-Ca^{2+}-pumping ATPase,[5a] and plakortones B−F exhibited cytotoxicity against a murine fibrosarcoma cell line.[5b] We have found that plakortone G (**3**) exhibits reduced antimalarial activity against both the D6 and W2 clones of *Plasmodium falciparum*, when compared with the peroxide plakortide F (Table 1). Plakortone G is highly cytotoxic, but does not exhibit selectivity toward a particular tumor cell line (Table 1). Plakortone G also did not exhibit antifungal or antimicrobial activity against *C. albicans*, *A. fumigatus*, *C. neoformans*, *M. intracellulare*, *P. aeruginosa*, *S. aureus*, and methicillin-resistant *S. aureus*, and was generally less active that plakortide F.

Compounds **2** and **3** share a common carbon backbone and differ primarily in that compound **2** is a peroxide and **3** is a lactone. This suggests that compound **3** may indeed be derived from the peroxide **2**. Compound **3** could clearly result from reduction of the peroxide in **2**, followed by oxidation at C-1, -2, and -3, with subsequent lactonization. This modification in structure results in a significant difference in bioactivity, as **2** is an order of magnitude more active against *Plasmodium falciparum* than is **3**. This suggests that the peroxide is necessary for antimalarial activity, and reduction of the peroxide may explain the loss of activity in vivo. Derivatives of the antimalarial endoperoxide artemisinin that lack the peroxy group are inactive against *Plasmodium* spp.[11] The diversity of peroxide-

containing compounds produced by sponges of the genus *Plakortis* makes this group of particular interest in the search for novel antimalarials.

Experimental Section

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 Bruker DRX 500 and DRX 400 spectrometers using the solvent peak as the internal standard. ESI-FTMS analyses were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron HR HPLC-FT spectrometer by direct injection into an electrospray interface. GC−MS spectra were obtained on a Thermo Quest CE Instruments Finnegan Trace 2000 Series gas chromatograph that interfaced with a Finnegan Trace mass spectrometer. For gas chromatographic separations a 0.25 mm × 15 m, 0.25 μm film thickness, DB-5 column (J & W Scientific, Inc.) was used, and a similar column was used for GC/FID under the same conditions. Oven temperature was programmed: 150 °C (1 min)−250 °C at 10 °C/min, with helium as a carrier gas at a linear velocity of 32 cm/s. HPLC was performed on a Waters 510 model system.

Animal Material. The sponge *Plakortis* sp. was collected in June 1998 using a closed circuit rebreather at a depth of −40 m off of Discovery Bay, Jamaica. A portion of the frozen material was identified by Michelle Kelly as an undescribed species of *Plakortis* (order Homosclerophorida, family Plakinidae). The sponge is brown throughout in life and forms a thickly encrusting pad, very dense and uniformly compressible, with a velvety soft surface. The sponge has diod spicules in one to two size categories and occasional triods. A voucher specimen has been deposited in the Natural History Museum, London (BMNH 2000.7.17.18).

Extraction and Purification. The remainder of the frozen material was lyophilized (149 g dry wt) and extracted with 1:1 IPA−EtOAc (4 × 2 L). The crude extract (15.3 g) was purified by Si gel vacuum liquid chromatography using a step gradient from hexane to EtOAc to MeOH. The hexane fraction was purified by normal-phase HPLC (YMC Si 5 μm column; 22 × 500 mm; hexane) to yield filiformin (**1**) (1.0 mg; 0.00067% dry wt). The 9:1 hexane−EtOAc fraction was further purified by normal-phase HPLC (YMC Si 5 μm column; 22 × 500 mm; hexane to EtOAc using a linear gradient). The antimalarial activity was restricted to the 33% EtOAc fraction, which was further purified by normal-phase HPLC (YMC Si 5 μm column; 22 × 500 mm; hexane to 25% ether using a linear gradient). Antimalarial fractions were eluted with 16−25% ether. Two of these fractions were further purified by normal phase HPLC (Phenomenex Ultracarb 5 μm ODS-30 column; 21.2 × 250 mm; 1:5 H_2O−CH_3CN to CH_3CN). Plakortide F (**2**) (138.8 mg; 0.093% dry wt) eluted with 97−100% CH_3CN, while plakortone G (**3**) (20.1 mg; 0.013% dry wt) eluted with 80% CH_3CN.

The GCEIMS of **1** gave the molecular $[M]^+$ ion at 294 (calcd for $C_{15}H_{19}OBr$, 294.0619). The $[\alpha]_D$ of **1** was −20° (*c* 0.0002, $CHCl_3$). The $[\alpha]_D$ and ^{13}C NMR spectra of compound **1** were identical to the published data for filiformin.[1] The 500 MHz ^1H NMR spectrum was resolved with greater definition than previously published[1] and is reported here: ($CDCl_3$, 500 MHz) δ 0.65 (3H, d, J = 6.8 Hz, C-7′), 1.23 (3H, s, C-6′), 1.28 (3H, s, C-8′), 1.44 (1H, m, C-2′), 1.58 (1H, m, C-5′), 1.68 (1H, m, C-4′), 1.77 (1H, m, C-5′), 1.91 (1H, m, C-4′), 2.17 (3H, s, C-7), 6.47 (1H, s, C-6), 7.11 (1H, s, C-3).

The HRESI-FTMS of **2** gave the molecular ion at *m/z* 355.2827 $[M + H]^+$ (calcd for $C_{21}H_{38}O_4$, 354.2770). The $[\alpha]_D$ of **2** was −161.6° (*c* 0.0365, $CHCl_3$). The ^1H and ^{13}C NMR spectra of compound **2** were identical to the published data for plakortide F.[6]

Plakortone G (**3**): colorless oil; $[\alpha]_D$ − 25.9° (*c* 0.0083, $CHCl_3$); UV ($CHCl_3$) λ_{max} 246 nm (ϵ 409); IR ($CHCl_3$) ν_{max} 2965 (m), 2933 (m), 2874 (m), 1754 (s, lactone), 1459 (w), 968 (w) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.79 (3H, t, J = 6.8 Hz, H-18), 0.80 (3H, t, J = 6.9 Hz, H-16), 0.93 (3H, t, J = 7.4 Hz, H-12), 1.13 (3H, t, J = 7.5 Hz, H-14), 1.18 (4H, m, H-6 (2H), H-7a, H-17a), 1.28 (1H, m, H-7b), 1.33 (1H, m, H-17b), 1.61 (1H, m, H-5a), 1.71 (1H, m, H-15a), 1.74 (1H, m, H-8), 1.76 (1H, m, H-5b), 1.79 (1H, m, H-15b), 1.96 (2H, p, J = 7.4 Hz, H-11), 2.27 (2H, dq, J = 1.3, 7.4 Hz, H-13), 5.0 (1H, dd, J = 8.9, 15.2 Hz, H-9), 5.3 (1H, dt, J = 6.4, 15.3 Hz, H-10), 6.82 (1H, br. s, H-3); ^{13}C NMR ($CDCl_3$, 100 MHz) 8.2 (C-16), 12.1 (C-18), 12.5 (C-14), 14.7 (C-12), 18.9 (C-13), 21.6 (C-6), 26.1 (C-11), 28.6 (C-17), 30.4 (C-15), 35.5 (C-7), 37.3 (C-5), 44.8 (C-8), 89.8 (C-4), 132.8 (C-10), 133.4 (C-9), 136.3 (C-2), 150.7 (C-3), 174.0 (C-1); HRESI-FTMS *m/z* 279.2353 $[M + H]^+$ (calcd for $C_{18}H_{30}O_2$, 278.2246).

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Supporting Information Available: ^1H, ^{13}C, DEPT 135, HMQC, HMBC, COSY, and NOESY NMR spectra for compound **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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