1i, 495-41-0; 1j, 99-49-0; 1k, 103-36-6; 1l, 1520-50-9; 1m, 621-82-9; 2a, 2550-26-7; 2b, 1083-30-3; 2c, 5012-90-8; 2d, 956-02-5; 2e, 959-23-9; 2f, 699-17-2; 2g, 59594-93-3; 2h, 112-12-9; 2i, 495-40-9; 2j, 5948-04-9; 2k, 2021-28-5; 2l, 1520-50-9; 2m, 621-82-9.

Supplementary Material Available: Spectroscopic data (^{1}H NMR, IR, MS) for compounds (2 pages). Ordering information is given on any current masthead page.

Cytotoxic Five-Membered Cyclic Peroxides from a *Plakortis* Sponge

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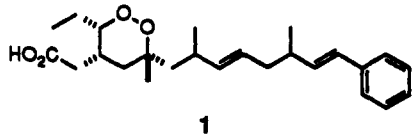
Sponges of the genus *Plakortis* have yielded a number of biosynthetically diverse natural products. For example, a *Plakortis* species was recently reported to contain the novel heteroaromatic pigments the plakinidines.[1] Other members of this genus produce interesting terpenoid[2] or polyketide[3] derived metabolites, many of which contain cyclic peroxides. Examples include plakinic acids A (1) and B (2).[3e] Although secondary metabolites containing



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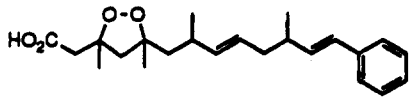

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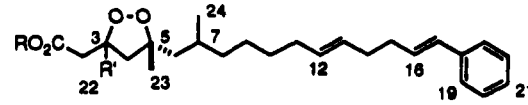
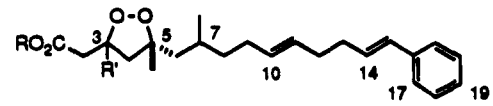
six-membered peroxide rings are not uncommon from marine sponges, compound 2 is the sole example of a naturally occurring five-membered-ring peroxide; furthermore, the relative stereochemistry of plakinic acid A (2) was never established. This paper now reports the structures of four new five-membered-ring peroxides, including assignment of the relative stereochemistry of the ring substituents. The compounds, isolated from a *Plakortis* sp. collected in the Fiji Islands,[4] have been named plakinic acids C (3) and D (5) and epiplakinic acids C (4) and D (6).

A methanolic extract (2.9 g concentrated), obtained by soaking homogenized, freeze-dried sponge tissue, exhibited cytotoxicity toward L1210 murine leukemia cells in vitro



3 R = H; R' = β-CH_3
3a R = CH_3; R' = β-CH_3
4 R = H; R' = α-CH_3
4a R = CH_3; R' = α-CH_3



5 R = H; R' = β-CH_3
5a R = CH_3; R' = β-CH_3
6 R = H; R' = α-CH_3
6a R = CH_3; R' = α-CH_3

with an ID_{50} of 0.26 μg/mL. The bioactive, hexane-soluble material, obtained by solvent partition, was subjected to silica gel flash chromatography. Treatment of an impure fraction with diazomethane yielded the methyl esters of plakinic acids C (3a, 11.8 mg) and D (5a, 9.2 mg) and the methyl esters of epiplakinic acids C (4a, 13.0 mg) and D (6a, 10.6 mg), as a mixture, which were then purified by normal-phase and reverse-phase HPLC.

The IR spectrum of plakinic acid C methyl ester (3a) showed an absorption at 1738 cm^{-1}, typical of a methyl ester, while the UV spectrum [249 nm (ε 12000), shoulders at 282 and 292 nm] was characteristic of a styrene unit.[5] A molecular formula of $C_{27}H_{40}O_4$ was establish on the basis of ^{13}C NMR and a high-resolution mass measurement of the M^+ ion. The ^{13}C NMR spectrum (Table I) displayed 25 distinct signals, of which two were assigned to the degenerate positions of a monosubstituted benzene ring. The ^{13}C NMR data, together with the results of ^{1}H NMR and HMQC[6] experiments, indicated the presence of 10 CH's, nine CH_2's, and four CH_3's, of which one was the methyl ester, two were singlets in the proton spectrum, and one was a doublet. The remaining quaternary carbons were assigned as an ipso aromatic carbon (137.80 ppm), an ester carbonyl (171.19 ppm), and two oxygenated quaternary carbons observed at δ 83.39 and 87.04.

A COSY experiment, along with the data presented above, allowed the construction of several partial structures, which could then be interconnected using long-range heteronuclear correlations obtained from HMBC data.[7] Key long-range correlation are as follows: H4A/H4B correlate to C2, C3, C5, C6, C22, and C23; H22 exhibits coupling to C2, C3, and C4; and H23 correlates to C4, C5, and C6. These results are consistent with a five-membered peroxide ring as reported for 2.[3e] The placement of isolated methyl group C24 at C7 was based on HMBC correlations from H6A/H6B to C24, as well as on the coupling, observed in the COSY spectrum, of H6A/H6B to H7, and H7 to H24. The terminal styrene unit was confirmed by the three-bond coupling of H16 to C18 and H17 to C19. The Δ16 double bond was assigned a trans configuration from the proton-proton coupling constant ($J_{16,17}$ = 15.5 Hz). The signals for H12 and H13 are overlapping at 200 MHz and only partially resolved at 500 MHz; however, irradiation of the H14 signal collapsed H13 to a broad

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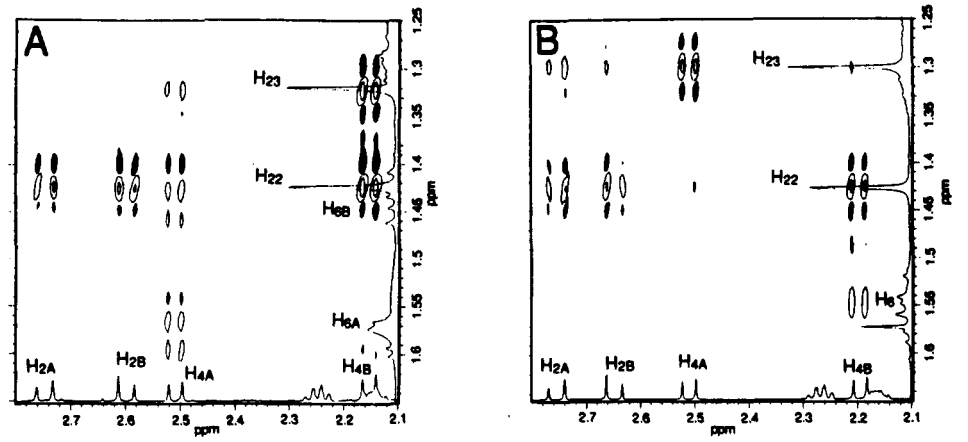

Figure 1. Phase-sensitive hypercomplex NOESY spectra of (A) plakinic acid C methyl ester (**3a**, ca. 10 mg) and (B) epiplakinic acid C methyl ester (**4a**, ca. 10 mg), in 0.6 mL of deuteriochloroform measured in a 5-mm sample tube. The experiments were recorded with a t_{mix} of 300 ms and an acquisition time in t_1 of approximately 550 ms (512 increments with a spectral width of ca. 3600 Hz; 32 scans/increment). The final data matrix was 2048 × 1024 complex points.

Table I. ^{13}C NMR Chemical Shifts for 3a, 4a, 5a, and 6a[a]

C no.	3a	4a	5a	6a
1	171.19	171.05	171.17	171.04
2	44.29	43.94	44.31	43.93
3	83.38	83.62	83.40	83.62
4	57.04	57.01	57.06	57.06
5	87.04	86.88	87.01	86.84
6	45.85	46.36	45.71	46.19
7	29.46	29.51	28.93	28.98
8	37.94	38.38	37.91	38.29
9	26.48	26.52	30.03	30.07
10	29.72	29.75	130.81	130.87
11	32.54	32.56	129.54	129.50
12	130.78	131.01	32.39	32.41
13	129.39	129.42	33.10	33.10
14	32.41	32.42	130.40	130.42
15	33.13	33.14	130.01	130.02
16	130.45	130.47	137.81	137.82
17	129.91	129.98	125.91†	125.91†
18	137.80	137.85	128.44†	128.45†
19	125.88†	125.92†	126.81	126.81
20	128.43†	128.45†	23.87	24.25
21	126.79	126.80	24.43	23.42
22	23.85	24.25	20.71	20.80
23	24.41	23.42	51.64	51.69
24	20.78	20.94	–	–
25	51.65	51.69	–	–

[a] All spectra were recorded in $CDCl_3$ at 125 MHz, and chemical shifts are referenced to the solvent signal (77.0 ppm). Signals marked with the symbol (†) represent two degenerate carbon atoms.

doublet (J = 15.1 Hz), indicating a trans orientation. Decoupling of H11 provided an analogous result for H12. This assignment was supported by a computer NMR simulation in which the proton signals for H12 and H13 were reproduced when described as an ABM_2X_2 spin system with symmetrical coupling constants of J = 15.30, 6.70, and 1.60 Hz.

The relative stereochemistry of the five-membered peroxide ring in **3a** was established using a two-dimensional nuclear Overhauser effect experiment (NOESY, Figure 1A).[8] Proton H4B showed strong dipolar coupling to both methyl protons H22 and H23, while in contrast, proton H4A showed only weak correlations to H22 and

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Table II. Proton NMR Chemical Shifts for 3a, 4a, 5a, and 6a[a]

		δ ^1H (mult; J_{HH}, Hz)			
C no.		3a	4a	5a	6a
1		–	–	–	–
2		2.75 (d; 14.5)	2.75 (d; 14.4)	2.77 (d; 14.4)	2.73 (d; 14.4)
		2.60 (d; 14.5)	2.65 (d; 14.4)	2.62 (d; 14.4)	2.62 (d; 14.4)
3		–	–	–	–
4	A	2.51 (d; 12.5)	2.51 (d; 12.4)	2.53 (d; 12.4)	2.49 (d; 12.6)
	B	2.15 (d; 12.5)	2.20 (d; 12.4)	2.17 (d; 12.4)	2.17 (d; 12.6)
5		–	–	–	–
6	A	1.59 (dd; 13.7, 4.4)	1.56 (m)	1.60 (m)	1.58–1.51 (m)
	B	1.44 (dd; 13.7, 7.8)		1.47 (m)	
7		1.54 (m)	1.57 (m)	1.59 (m)	1.55 (m)
8	A	1.38 (m)	1.26 (m)	1.33 (m)	1.31 (m)
	B	1.12 (m)	1.15 (m)	1.21 (m)	1.22 (m)
9	A	1.25 (m)	1.28 (m)	2.03 (m)	1.99 (m)
	B			1.98 (m)	
10		1.28 (m)	1.32 (m)	5.44 (m; 15.3)	5.42 (m; 15.2)
11		1.97 (m)	1.99 (m)	5.45 (m; 15.3)	5.43 (m; 15.2)
12		5.44 (m; 15.3)	5.46 (m; 15.3)	2.17 (m)	2.15 (m)
13		5.46 (m; 15.3)	5.43 (m; 15.3)	2.27 (m)	2.24 (m)
14		2.14 (m)	2.17 (m)	6.22 (dt; 16.0, 6.5)	6.20 (dt; 16.0, 6.7)
15		2.24 (m)	2.27 (m)	6.39 (d; 16.0)	6.36 (d; 16.0)
16		6.20 (dt; 15.5, 6.8)	6.22 (dt; 15.8, 6.8)	–	–
17		6.36 (d; 15.5)	6.39 (d; 15.8)	7.34 (d; 7.2)	7.31 (d; 7.2)
18		–	–	7.29 (t; 7.2)	7.26 (t; 7.6)
19		7.31 (d; 7.4)	7.34 (d; 7.2)	7.19 (tt; 7.2, 1.4)	7.17 (t, 7.2)
20		7.26 (t; 7.7)	7.26 (t; 7.7)	1.45 (s)	1.41 (s)
21		7.17 (tt; 7.4, 1.3)	7.19 (tt; 7.2, 1.4)	1.34 (s)	1.28 (s)
22		1.43 (s)	1.43 (s)	0.93 (d; 6.3)	0.94 (d; 5.9)
23		1.32 (s)	1.31 (s)	3.68 (s)	3.67 (s)
24		0.89 (d; 6.4)	0.95 (d; 6.3)	–	–
25		3.66 (s)	3.69 (s)	–	–

[a] All spectra were recorded in $CDCl_3$ at 500 MHz and referenced to residual solvent protons (7.24 ppm).

Table III. Cytotoxicity Data for Compounds 3–6 and 3a–6a

structure	cell line L1210[a]	KB[b]	LoVo[b]
3	0.017	0.01	0.1
3a	0.013	1.0	1.0
4	0.026	0.001	0.001
4a	0.0043	1.0	0.1
5	0.052	0.01	1.0
5a	0.29	0.1	0.1
6	0.017	0.1	1.0
6a	0.003	0.1	0.1

[a] IC_{50} in μg/mL. [b] Minimum inhibitory concentration (MIC) in μg/mL.

H23, but correlated to H6A/H6B. These results are fully consistent with a cis orientation of the methyl groups. The stereochemistry of the C7 chiral center remains to be assigned.

The isomeric relationship between epiplakinic C methyl ester (**4a**) and plakinic acid C methyl ester (**3a**) was evident from the mass spectral data, which indicated a molecular formula for $C_{27}H_{40}O_4$. The NMR data for **4a** (Tables I and II) showed slight chemical shift differences only for nuclei either contained by or attached to the peroxide ring, when compared to those of compound **3a**. While the carbon connectivity of **4a**, established using COSY, HMQC, and HMBC experiments, is identical to that of **3a**, the relative stereochemistry of substituents attached to the five-membered peroxide ring is different. A NOESY experiment (Figure 1B) showed strong dipolar coupling between protons H4A and H23 and from H4B to both H22 and H6, allowing the relative stereochemistry of structure **4a** to be unequivocally assigned as trans. An additional weak dipolar coupling between H23 and H2A/H2B further supports structure **4** for epiplakinic acid C. Assignment of C3 as the epimeric center is arbitrary, although one might speculate that, during the biosynthesis of the peroxide ring, greater stereocontrol would be realized at the reaction center nearest the C7 chiral center.

^{1}H NMR experiments (Table II) on plakinic acid D methyl ester (**5a**) and epiplakinic acid D methyl ester (**6a**) yielded results nearly identical to those obtained for **3a** and **4a**, respectively. However, the ^{13}C NMR data (Table I) and the mass spectral data, which indicated a molecular formula of $C_{25}H_{36}O_4$, revealed that **5a** and **6a** contained two fewer carbons. HMBC experiments showed long-range ^{1}H–^{13}C coupling from H8 to C6, C7, C9, and C10, indicating that the carbons assigned as C9 and C10 in **3a** and **4a** and absent in **5a** and **6a**. NOESY experiments confirmed an epimeric relationship between **5a** and **6a**, which is analogous to that demonstrated for compounds **3a** and **4a**. This data indicates a homologous relationship between plakinic acid C (**3**) and plakinic acid D (**5**) and between epiplakinic acid C (**4**) and epiplakinic acid D (**6**), as shown above.

All of the compounds reported in this paper, both methyl esters and free acids, exhibit cytotoxic activity against human epidermoid carcinoma (KB) cells (ATCC CCL 17),[9,10] human colorectal adenocarcinoma (LoVo) cells (ATCC CCL 229),[10] and L1210 murine leukemia cells (ATCC CCL 219).[11] The results of these bioassays are summarized in Table III.

Experimental Section

Carbon assignments and multiplicities were assigned using HMQC[6] and HMBC[7] experiments. HMQC and HMBC experiments were performed with proton decoupling during acquisition.

Collection, Extraction, and Purification. The dark brown/black *Plakortis* sponge was collected by hand using SCUBA at depths of 15–20 m in the Yasawa Island chain, Fiji. The collection was kept frozen until workup. The homogenized, freeze-dried sponge tissue was repeatedly extracted with methanol, and the combined extracts were concentrated under reduced pressure to give 2.9 g of an oily residue. The crude extract was partitioned between hexane and 10% aqueous methanol. The cytotoxic hexane fraction was concentrated to give 560 mg of a dark orange, oil, which was subjected to silica gel flash chromatography using a stepwise solvent gradient (hexane/$CHCl_3$/MeOH, 85:10:5 to 60:35:5). The IR spectrum of the most polar fractions indicated the presence of carboxylic acids, so the largest fraction (204 mg) was treated with diazomethane, prepared fresh from Diazald, resulting in the methylation of all of the fraction components. Normal-phase HPLC (Rainin Dynamax 60A silica, 8 μm, 250 × 10 mm) with 98:2 isooctane/ethyl acetate, gave pure **3a** (9.2 mg) and **6a** (13.0 mg) as colorless oils. A mixed fraction was further purified by reverse-phase HPLC (Rainin Dynamax 60A C18, 8 μm, 250 × 10 mm) with 95:5 CH_3CN/H_2O, to give **5a** (10.6 mg) and **4a** (11.8 mg), also as colorless oils.

Pure samples of free acids **3–6** for biological testing were isolated by silica gel flash chromatography followed by both reverse-phase (95:5, CH_3CN/H_2O) and normal-phase (98:2, dichloroethane/MeOH) HPLC.

Plakinic acid C methyl ester (3a): $[\alpha]^{27}_D$ +31.71° (*c* 0.098, $CHCL_3$); UV (MeOH) λ_{max} 204, 249 nm; IR (film) 2926, 2852, 1738, 1436, 1206, 965 cm^{-1}; ^{1}H NMR ($CDCl_3$) see Table II; ^{13}C NMR ($CDCl_3$) see Table I; EIMS *m/z* (rel intensity) 428 (M^+, <1), 410 (<1), 336 (1), 298 (5), 117 (100), 91 (20); HREIMS calcd for $C_{27}H_{40}O_4$ 428.2927, found 428.2977.

Epiplakinic acid C methyl ester (4a): $[\alpha]^{26}_D$ −22.66° (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} 205, 249 nm; IR (film) 2926, 2851, 1738, 1436, 1208, 965 cm^{-1}; ^{1}H NMR ($CDCl_3$) see Table II; ^{13}C NMR ($CDCl_3$) see Table I; EIMS *m/z* (rel intensity) 428 (M^+, <1), 410 (<1), 336 (1), 298 (5), 117 (100), 91 (20); HREIMS calcd for $C_{27}H_{40}O_4$ 428.2927, found 428.2965.

Plakinic acid D methyl ester (5a): $[\alpha]^{27}_D$ +40.53° (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} 204, 250 nm; IR (film) 2927, 2846, 1738, 1436, 1208, 966 cm^{-1}; ^{1}H NMR ($CDCl_3$) see Table II; ^{13}C NMR ($CDCl_3$) see Table I; EIMS *m/z* (rel intensity) 400 (M^+, <1), 382 (<1), 352 (<1), 270 (10), 117 (100), 91 (58); HREIMS calcd for $C_{25}H_{36}O_4$ 400.2614, found 400.2615.

Epiplakinic acid D methyl ester (6a): $[\alpha]^{26}_D$ −16.73° (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} 204, 250 nm; IR (film) 2925, 2847, 1738, 1436, 1208, 966 cm^{-1}; ^{1}H NMR ($CDCl_3$) see Table II; ^{13}C NMR ($CDCl_3$) see Table I; EIMS *m/z* (rel intensity) 400 (M^+, <1), 382 (<1), 352 (<1), 270 (2), 117 (100), 91 (20); HREIMS calcd for $C_{25}H_{36}O_4$ 400.2614, found 400.2648.

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Supplementary Material Available: ^{13}C NMR spectra (^{1}H decoupled) for compounds **3a**, **4a**, **5a**, and **6a** (4 pages). Ordering information is given on any current masthead page.

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