# Three New Peroxides from the Sponge *Plakinastrella* Species

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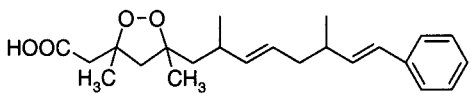
Two new five-membered-ring peroxide acids, plakinic acid F (**3**) and epiplakinic acid F (**4**), and a new peroxide−lactone, plakortolide F (**5**), were isolated from a sponge of the genus *Plakinastrella* collected from Felicite Island, Seychelles. The structures were elucidated through spectral analysis. The free acids **3** and **4** exhibit moderate antifungal activity against *Candida albicans* with minimum inhibitory concentrations of 25 $\mu$g/mL (SDB) and 3.1 $\mu$g/mL (RPMI) for **3**, and 25 $\mu$g/mL (SDB) and 6.25 $\mu$g/mL (RPMI) for **4**, respectively. Both also showed moderate in vitro inhibition of *Aspergillus fumigatus* with $IC_{90}$'s of 25 $\mu$g/mL.

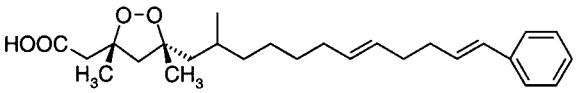
Cyclic peroxides, many of which exhibit antifungal, antibacterial, or antitumor activity, have been reported previously from a number of marine organisms, especially from sponges of the family Plakinidae.[1,2] The majority of these natural products contain six-membered peroxide rings. In addition a few, 1,2-dioxolane carboxylates with methyl substituents at the 3-, 5-positions have also been reported. Examples of the latter class include plakinic acid A (**1**),[3] plakinic acids C (**2**) and D and epiplakinic acids C and D,[4] epiplakinic acid E methyl ester,[5] (3*R**,5*S**,12*E*,-14*E*,17*Z*)-3,5-dimethyl-3,5-peroxydodeca-12,14,17-trienoic acid, its methyl ester,[6] and related saturated analogues.[7,8] Previous reports on the five-membered-ring peroxide acids suggested that these compounds are unstable, and direct $^{13}C$ NMR spectral data of the pure acids have not been reported previously. Rather, the structures of these acids were elucidated after conversion to the methyl esters, which could be more easily purified.[6]

In our continuing program to identify compounds with antifungal properties, an ethanol extract of the sponge *Plakinastrella* sp., collected by hand using scuba at a depth of 25 feet off Felicite Island, Seychelles, was found to inhibit the growth of the fungal pathogens *Candida albicans* and *Aspergillus fumigatus*. Bioassay-guided purification led to the isolation of two new 1,2-dioxolane carboxylic acids (**3** and **4**), containing a conjugated triene on the side chain, and a new peroxide−lactone (**5**), which possesses a *p*-phenol ring linked to the cyclic peroxide via an unbranched aliphatic chain. This paper reports the isolation, structure elucidation, and antifungal activity of these compounds.

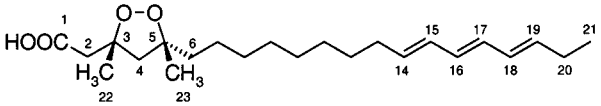
Plakinic acid F (**3**) was isolated as colorless oil. The molecular formula of $C_{23}H_{38}O_4$ was established on the basis of $^{13}C$ NMR and a high-resolution mass measurement of the $[M + Na]^+$ ion (401.2636 observed, 401.2668 calculated). The $^{13}C$ NMR spectrum (Table 1) exhibited 23 distinct resonances. When taken together with the $^1H$ NMR, the $^{13}C$ DEPT, and the proton-detected HMQC spectral data, the presence of six methine, eleven methylene, and three methyl groups could be assigned for **3**. Two of the methyl proton resonances were singlets and one was a triplet in the $^1H$ NMR spectrum. The remaining quaternary carbons were assigned as a carbonyl ($\delta$ 171.94) and



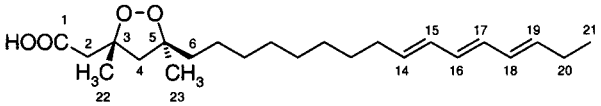
**1**



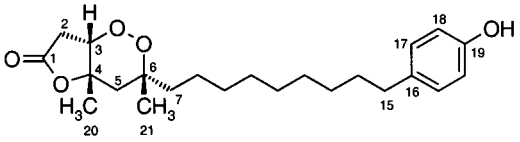
**2**



**3**



**4**



**5**

two oxygenated quaternary carbons ($\delta$ 83.78 and $\delta$ 86.96). The proton-detected HMBC experiment revealed cross-peaks between H-4$\alpha\beta$ and C-2, C-3, C-5, C-6, C-22, and C-23; H-22 displayed coupling to C-2, C-3, and C-4; and H-23 displayed coupling to C-4, C-5, and C-6. These results are consistent with a five-membered peroxide ring.[3−6] Since the IR spectrum of **3** showed a strong absorption at 1709 $cm^{-1}$ and no methyl ester signal was observed in either the $^1H$ or $^{13}C$ NMR spectra, this compound is likely to be a free 1,2-dioxolane carboxylic acid. The relative stereochemistry of the five-membered peroxide ring in **3** was assigned by interpretation of the NOESY spectrum. The

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**Table 1.** $^{13}C$ NMR Data (125 MHz, $CDCl_3$) for **3**−**5**

| C | **3** $\delta$ (DEPT) | 4 $\delta$ (DEPT) | **5** $\delta$ (DEPT) |
|---|---|---|---|
| 1 | 171.94 (s) | 171.80 (s) | 174.27 (s) |
| 2 | 43.62 (t) | 43.94 (t) | 34.13 (t) |
| 3 | 83.78 (s) | 83.68 (s) | 80.85 (d) |
| 4 | 55.56 (t) | 55.51 (t) | 82.64 (s) |
| 5 | 86.96 (s) | 86.86 (s) | 40.24 (t) |
| 6 | 38.72 (t) | 38.77 (t) | 80.23 (s) |
| 7 | 24.89 (t) | 24.86 (t) | 36.97 (t) |
| 8 | 29.70 (t)[a] | 29.70 (t)[c] | 23.76 (t) |
| 9 | 29.38 (t)[a] | 29.42 (t)[c] | 29.39 (t)[e] |
| 10 | 29.14 (t)[a] | 29.14 (t)[c] | 29.50 (t)[e] |
| 11 | 30.02 (t)[a] | 30.02 (t)[c] | 29.99 (t)[e] |
| 12 | 29.39 (t) | 29.70 (t) | 29.26 (t)[e] |
| 13 | 32.79 (t) | 32.78 (t) | 28.90 (t)[e] |
| 14 | 134.42 (d) | 134.41 (d) | 31.53 (t) |
| 15 | 130.49 (d)[b] | 130.48 (d)[d] | 34.93 (t) |
| 16 | 130.88 (d)[b] | 130.83 (d)[d] | 135.09 (s) |
| 17 | 130.88 (d)[b] | 130.87 (d)[d] | 129.45 (d) |
| 18 | 129.52 (d) | 129.51 (d) | 115.02 (d) |
| 19 | 135.93 (d) | 135.90 (d) | 153.50 (s) |
| 20 | 25.81 (t) | 25.81 (t) | 25.90 (q) |
| 21 | 13.61 (q) | 14.11 (q) | 24.85 (q) |
| 22 | 23.61 (q) | 24.75 (q) | |
| 23 | 24.83 (q) | 23.69 (q) | |

[a−e] These assignments can be interchanged.

H-4$\beta$ signal observed at $\delta$ 2.14 showed strong coupling to both methyl resonances C$H_3$-22 and C$H_3$-23, while in contrast, the H-4α signal observed at $\delta$ 2.48 did not display any correlation to either C$H_3$-22 or C$H_3$-23, but strong coupling to $H_2$-6 was observed. This confirms a cis relationship between the two methyl groups.

The remaining part of the molecule was established to be a conjugated triene along the unbranched alkyl chain on the basis of interpretation of the 1D $^1H$ NMR and the 2D $^1H-^1H$ COSY spectra. The position of the triene at C-14, C-16, and C-18 was determined from the COSY, HMQC, and HMBC data. Obvious long-range correlations were observed in the HMBC experiment as follows: the terminal methyl protons observed at $\delta$ 0.99 showed coupling to C-20 ($\delta$ 25.81) and C-19 ($\delta$ 135.93); the allylic proton H-20 ($\delta$ 2.10) showed coupling to C-18 ($\delta$ 129.52), C-19 ($\delta$ 135.93), and C-21 ($\delta$ 13.61). 2D COSYDFTP and 2D *J*-resolved $^1H$ NMR experiments indicated large *J* values ($J \approx 14$ Hz) for the scalar coupling between H-19 and H-18 on one side of the triene unit and between H-14 and H-15 on the other side, allowing for the assignment of *E* configuration to the "external" double bonds of the triene moiety. Assignment of the geometry of the C-16−C-17 double bond was less straightforward. The resonances of the four "inner" protons largely overlap, giving rise to a complex pattern observed between 6.1 and 6.2 ppm in $C_6D_6$ which is not resolved on solvent change ($CDCl_3$ or $CD_3OCD_3$). However, the configuration at C-16 was deduced to be *E*, because both C-15 and C-18 would be predicted to be observed at higher field (ca. 125 ppm) if the double bond at C-16 were the *Z* configuration.[9,10]

The isomeric relationship between **3** and **4** was evident from the mass spectral data, which indicated an identical molecular formula of $C_{23}H_{38}O_4$. The NMR data for **4**, when compared to those of compound **3** (Tables 1 and 2), showed slight chemical shift differences only for the nuclei contained or attached to the peroxide ring. While the carbon connectivity of **4**, established by COSY, HMQC, and HMBC experiments, is found to be identical to that of **3**, only the relative stereochemistry of the peroxide ring is different. A NOESY spectrum exhibited strong correlation between the H-4α signal observed at $\delta$ 2.40 and C$H_3$-23 and between the H-4$\beta$ signal observed at $\delta$ 2.25 and both C$H_3$-22 and H-6, suggesting that the relative stereochemistry of **4** is trans.

The molecular formula of plakortolide F (**5**) is $C_{23}H_{34}O_5$, based upon the high-resolution mass measurement of the $[M + H]^+$ ion (*m*/*z* 391.2439 observed, 391.2486 calcd.). This was further supported by the $^{13}C$ NMR spectral data (Table 1), which contain 21 distinct signals, two of which were assigned to the symmetrically located carbons in a para-disubstituted aromatic ring. The IR spectrum indicated the presence of a $\gamma$-lactone (1768 $cm^{-1}$). The $^{13}C$ NMR and DEPT spectra also contained signals that were attributed

**Table 2.** $^1H$ NMR Data (500 MHz) for **3**−**5**

| proton | **3** $\delta$ (mult, *J* in Hz)[a] | **3** $\delta$ (mult, *J* in Hz) | **4** $\delta$ (mult, *J* in Hz) | **5** $\delta$ (mult, *J* in Hz) |
|---|---|---|---|---|
| 1 | | | | |
| 2 | 2.71 (d, 15.0) | 2.73 (s) | 2.75 (s) | 2.88 (dd, 6.1, 18.6) |
| | 2.68 (d, 15.0) | | | 2.55 (d, 18.6) |
| 3 | | | | 4.46 (d, 6.1) |
| 4α | 2.39 (d, 12.5) | 2.48 (d, 12.5) | 2.40 (d, 12.5) | |
| 4$\beta$ | 1.83 (d, 12.5) | 2.14 (d, 12.5) | 2.25 (d, 12.5) | |
| 5 | | | | 2.25 (d, 15.0) |
| | | | | 1.64 (d, 15.0) |
| 6 | 1.53 (m) | 1.55 (m) | 1.65 (m) | 1.55 (m) |
| | 1.24 (m) | 1.26 (m) | 1.28 (m) | 1.25 (m) |
| 7 | 1.24−1.37 (m) | 1.26 (m) | 1.16−1.53 (m) | 1.23−1.26 (m) |
| 8 | 1.24−1.37 (m) | 1.26 (m) | 1.16−1.53 (m) | 1.23−1.26 (m) |
| 9 | 1.24−1.37 (m) | 1.26 (m) | 1.16−1.53 (m) | 1.23−1.26 (m) |
| 10 | 1.24−1.37 (m) | 1.26 (m) | 1.16−1.53 (m) | 1.23−1.26 (m) |
| 11 | 1.24−1.37 (m) | 1.26 (m) | 1.16−1.53 (m) | 1.23−1.26 (m) |
| 12 | 1.43 (m) | 1.37 (m) | 1.39 (m) | 1.23−1.26 (m) |
| 13 | 2.07 (q, 7.2) | 2.06 (q, 7.2) | 2.05 (q, 7.2) | 1.23−1.26 (m) |
| 14 | 5.62 (ddt, 0.8, 7.2, 14.5) | 5.63 (dt, 7.2, 14.1) | 5.63 (dt, 7.2, 14.1) | 1.55 (m) |
| 15 | 6.09 (ddd, 1.0, 10, 13.9) | 6.02 (m) | 6.02 (m) | 2.50 (t, 7.6) |
| 16 | 6.19 (m) | 6.07 (m) | 6.06 (m) | |
| 17 | 6.19 (m) | 6.07 (m) | 6.06 (m) | 7.01 (d, 8.5) |
| 18 | 6.13 (ddd, 1.0, 10, 14.1) | 6.02 (m) | 6.02 (m) | 6.72 (d, 8.2) |
| 19 | 5.67 (ddt, 0.9, 7.3, 14.5) | 5.69 (dt, 7.3, 14.5) | 5.68 (dt, 7.2, 14.4) | |
| 20 | 1.99 (q, 7.3) | 2.10 (q, 7.3) | 2.08 (q, 7.2)) | 1.36 (s) |
| 21 | 0.91 (t, 7.5) | 0.99 (t, 7.5) | 0.97 (t, 7.5) | 1.18 (s) |
| 22 | 1.40 (s) | 1.47 (s) | 1.45 (s) | |
| 23 | 1.24 (s) | 1.36 (s) | 1.28 (s) | |

[a] $C_6D_6$.

to a carbonyl ($\delta$ 174.27), two oxygenated quaternary carbons ($\delta$ 82.64 and 80.23), one oxygenated methine carbon ($\delta$ 80.85), and eleven methylene carbons. Correlations present in the $^1$H−$^1$H COSY and proton-detected HMQC spectra of **5** led to the identification of several substructures which were connected by analyzing cross-peaks in the HMBC experiment and by comparison to the data published on related peroxide−lactones.[5−6,11] Important long-range $^1$H−$^{13}$C correlations were as follows: H-3 ($\delta$ 4.46) is coupled to C-1 ($\delta$ 174.27); H-20 ($\delta$ 1.36) is coupled to C-3 ($\delta$ 80.85), C-4 ($\delta$ 82.64), and C-5 ($\delta$ 40.24); H-21 ($\delta$ 1.18) is coupled to C-5 ($\delta$ 40.24), C-6 ($\delta$ 80.23), and C-7 ($\delta$ 36.97). The relative stereochemistry of the peroxide−lactone ring in compound **5** was assigned as shown based on similarities in $^1$H and $^{13}$C NMR chemical shifts with those reported for plakortolide[4] and plakortolide B, C, D,[5] and E.[11] The *p*-hydroxyphenyl terminus of the molecule could be elucidated from the $^1$H NMR signals observed at $\delta$ 7.01 (d, 2H, $J$ = 8.5 Hz), $\delta$ 6.72 (d, 2H, $J$ = 8.2 Hz), and $\delta$ 2.50 (t, 2H, $J$ = 7.6 Hz) and the $^{13}$C NMR signals observed at $\delta$ 153.50 (s), 135.09 (s), 129.45 (d, 2C), 115.02 (d, 2C), and 34.9 (t). The terminal phenol group was joined through an unbranched nine-carbon hydrocarbon chain to the highly oxygenated peroxide−lactone ring.

The free acids **3** and **4** exhibit moderate antifungal activity against *C. albicans* with minimum inhibitory concentrations (MIC) of 25 $\mu$g/mL (SDB) and 3.1 $\mu$g/mL (RPMI) for **3**, and 25 $\mu$g/mL (SDB) and 6.25 $\mu$g/mL (RPMI) for **4**, respectively. Both also showed moderate in vitro inhibition of *A. fumigatus* with $IC_{90}$'s of 25 $\mu$g/mL. Peroxide−lactone **5** gave an MIC of >125 $\mu$g/mL in both SDB and RPMI media against *C. albicans* and an $IC_{90}$ > 125 $\mu$g/mL against *A. fumigatus*.

## Experimental Section

**General Experimental Procedures.** The IR spectra were collected on a Midac M-1200 with Galactic GRAMS/386 software. The $^1$H, COSY, COSYDFTP, 2D *J*-resolved $^1$H NMR, and NOESY, $^{13}$C, DEPT90, DEPT135, HMQC, and HMBC (optimized for 10 Hz) spectra were recorded on a Bruker AMX-500 operating at 500 MHz ($^1$H) and 125 MHz ($^{13}$C). $^1$H chemical shifts are referenced to $CDCl_3$ observed at 7.24 ppm, while $^{13}$C chemical shifts are referenced to $CDCl_3$ observed at 77.0 ppm. The high-resolution FAB mass spectra were obtained on a Finnigan MAT 95 mass spectrometer at University of Florida, Gainesville, FL.

**Animal Material.** The sponge was collected in April 1990 at a depth of 25 ft in the Seychelles on the west coast of Felicite Island (latitude 04°19.28′S, longitude 55°51.95′E), frozen immediately after collection, and kept frozen until used. The sample is identified as *Plakinastrella* sp. (class Demospongiae, order Homosclerophorida, family Plakinidae).[12] The sponge was thickly encrusting, lobate, with a row of oscules, 4 mm in diameter, on a ridge at the top of the sponge. Color in life was dark brown externally and tan internally. The consistency was firm. Spicules are calthrops and diods in at least three size categories. This description most closely matches *Plakinastrella onkodes* from the Caribbean,[13] but it is likely to be a new species. A taxonomic reference specimen is deposited at the Harbor Branch Oceanographic Museum (HBOM catalog number 003:00958, sample number 15-VI-90-3-003).

**Extraction and Purification.** The frozen sponge (103 g wet wt) was diced and extracted with EtOH (5 × 200 mL). The combined EtOH extracts were concentrated to dryness (3.15 g) and partitioned between 1:1 n-BuOH/$H_2O$. The n-BuOH partition was concentrated to dryness (0.73 g) and was further purified via vacuum flash column chromatography on a silica gel (Kieselgel 60H) stationary phase using a step gradient of heptane and heptane/EtOAc as eluent. The fractionation was monitored by antifungal assay against *C. albicans*. The antifungal active fractions which eluted with 80% EtOAc and 100% EtOAc were further separated by HPLC on a silica gel HPLC column (Whatman Partisil 10, 10 × 500 mm) eluted with 1% MeOH in $CH_2Cl_2$ to yield a mixture of compounds **3**, **4**, and **5**. The mixture was further purified using reversed-phase HPLC to obtained pure acids **3** (1.2 mg) and **4** (1.1 mg) and peroxide−lactone **5** (1.4 mg). HPLC conditions: Vydac Protein and Peptide C18 column, 10 × 250 mm, gradient elution, flow rate = 3 mL/min; solvent A methanol−water (1:1 v/v); solvent B 2-propanol; $t$ = 0, A:B (8:2 v/v); $t$ = 30, 100% B, hold at 100% B for 5 min). Compounds were detected by UV absorption at 280 nm.

**Bioassay.** Minimum inhibitory concentrations (MICs) were determined for *C. albicans* by standard microdilution broth method using Sabouraud dextrose broth (SDB)[14] and buffered RPMI (RPMI-1640, 0.165 M MOPS, pH 7.0) as growth media, respectively. MICs for the control drug, 5-fluorocytosine in SDB and RPMI media, are 0.66 and 1.62 $\mu$g/mL, respectively. The MIC is defined as the lowest test concentration of drug giving complete inhibition of growth. $IC_{90}$'s against *A. fumigatus* were determined by standard microdilution broth method using buffered RPMI media (RPMI-1640, 0.165 M MOPS, pH 7.0).[15] Amphotericin B has an $IC_{90}$ of 1 $\mu$g/mL against *A. fumigatus* in this system. The $IC_{90}$ is defined as the concentration of drug giving 90% inhibition of growth.

**Compound 3:** IR ($CHCl_3$) 2925, 2853, 1709, 1460, 1375, 1305, 1210, 994, 796 cm$^{-1}$; $^1$H (Table 2) and $^{13}$C NMR (Table 1); HRFABMS *m/z* 401.2636 (calcd for $C_{23}H_{38}O_4Na$ [M + Na]$^+$, 401.2668).

**Compound 4:** IR ($CHCl_3$) 2928, 2857, 1715, 1463, 1376, 1210, 993 cm$^{-1}$; $^1$H (Table 2) and $^{13}$C NMR (Table 1); HRFABMS *m/z* 379.2816 (calcd for $C_{23}H_{39}O_4$ [M + H]$^+$, 379.2850).

**Compound 5:** IR ($CHCl_3$) 2925, 2854, 1768, 1514, 1443, 1373, 1260, 1218, 1183, 954, 927 cm$^{-1}$; $^1$H (Table 2) and $^{13}$C NMR (Table 1); HRFABMS *m/z* 391.2439 (calcd for $C_{23}H_{35}O_5$ [M + H]$^+$, 391.2486).

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