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Oxidative Processes in the Australian Marine Sponge *Plakinastrella clathrata*: Isolation of Plakortolides with Oxidatively Modified Side Chains

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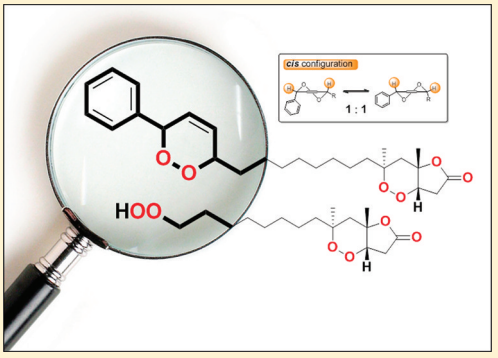
Supporting Information

ABSTRACT: Sixteen new cyclic peroxides (**1**–**16**) with a plakortolide skeleton and the methyl ester derivative of a didehydroplakinic acid (**17**) were isolated from the Australian sponge *Plakinastrella clathrata* Kirkpatrick, 1900. Structural elucidation and configurational assignments were based on spectroscopic analysis and comparison with data for previously isolated plakortolides and revealed both phenyl- and methyl-terminating side chains attached to the plakortolide core. Plakortoperoxides A–D (**5**–**8**) each contained a second 1,2-dioxine ring; a *cis* configuration for the side chain endoperoxide ring was determined by a low-temperature NMR study and by comparison of chemical shift values with those of reported compounds. An enantioselective HPLC study compared natural plakortoperoxide A with a synthetic sample prepared by cyclization of plakortolide P with singlet oxygen and revealed that the natural sample was a mixture of *cis* diastereomers at C-15/C18. Four other cyclic peroxides (**9**–**12**) possessed a C_9-truncated side chain terminating in a formyl or carboxylic acid functionality, suggesting that these metabolites may have been formed by oxidative cleavage of the $\Delta^{9,10}$ bond of diene-functionalized plakortolides. A final group of four metabolites (**13**–**16**) with hydroxy or the rare hydroperoxy functionality unexpectedly revealed a C_8 side chain, while the ester (**17**) represents further structural variation within the growing family of cyclic peroxy sponge metabolites.



Cyclic peroxides are a distinctive suite of bioactive metabolites isolated from marine sponges, notably those from the genera *Plakortis* and *Plakinastrella*.[1] Recently we reported nine new plakortolide metabolites (K–S), each possessing a $Ph(CH_2)_n-$ side chain (n = 10 or 12), together with their reduced (*seco*) derivatives and some stereochemically related plakortone ethers, from the Australian marine sponge *Plakinastrella clathrata* Kirkpatrick, 1900. In our study, we addressed the relative and absolute configurations of the metabolites and revealed that diastereomeric plakortolides in this particular sponge specimen uniformly had a 6*S* configuration and, thereby, differed in configuration at the C-3/C-4 centers. We also provided evidence that plakortone ethers derive from *seco*-plakortolides under mild conditions.[2]

We now describe an additional set of 16 plakortolide metabolites (**1**–**16**) from the same sponge specimen. Three of these plakortolides revealed a $Ph(CH_2)_{12}-$ side chain, while five others had a side chain either C_{15} or C_{17} in length and terminating in a methyl group; additionally, four of these metabolites had an endoperoxide functionality in the side chain. The remaining eight plakortolides contained truncated side chains apparently resulting from side chain oxidative processes. These included two pairs of diastereomeric plakortolides that have a C_9 side chain terminating in either a carboxylic acid or an aldehyde and two pairs of diastereomeric plakortolides that have a C_8 side chain terminating in either a hydroxy or the rare hydroperoxy functionality. An additional ester metabolite (**17**), named by us as a didehydroplakinic acid derivative, lacks the lactone ring associated with the plakortolide core. The biological activities associated with the various metabolites will be reported separately.

RESULTS AND DISCUSSION

Structural and Stereochemical Studies. Fractions from the CH_2Cl_2/MeOH (1:1) extract of *P. clathrata*[2] were subjected to NP-HPLC (hexanes/EtOAc) or to RP-HPLC (CH_3CN/H_2O) to give metabolites **1**–**17**.

Plakortolides. Plakortolide T (**1**) was isolated as a colorless oil and had a formula of $C_{26}H_{38}O_4$ inferred from HR-ESIMS. The metabolite showed ^{1}H NMR and HSQC data (Tables 1 and 2) for a phenyl ring [δ_H 7.32 (2H), 7.26 (2H), 7.16 (1H); δ_C 138.4 (s), 128.5 (d), 126.8 (d), 126.0 (d)], two methyl groups [δ_H 1.37 (s), 1.27 (s); δ_C 26.2, 22.4], one oxygenated methine [δ_H 4.43 (d); δ_C 81.1], two methylenes with a diagnostic AB signal pattern [δ_H 2.89, 2.60; δ_C 34.3 and δ_H

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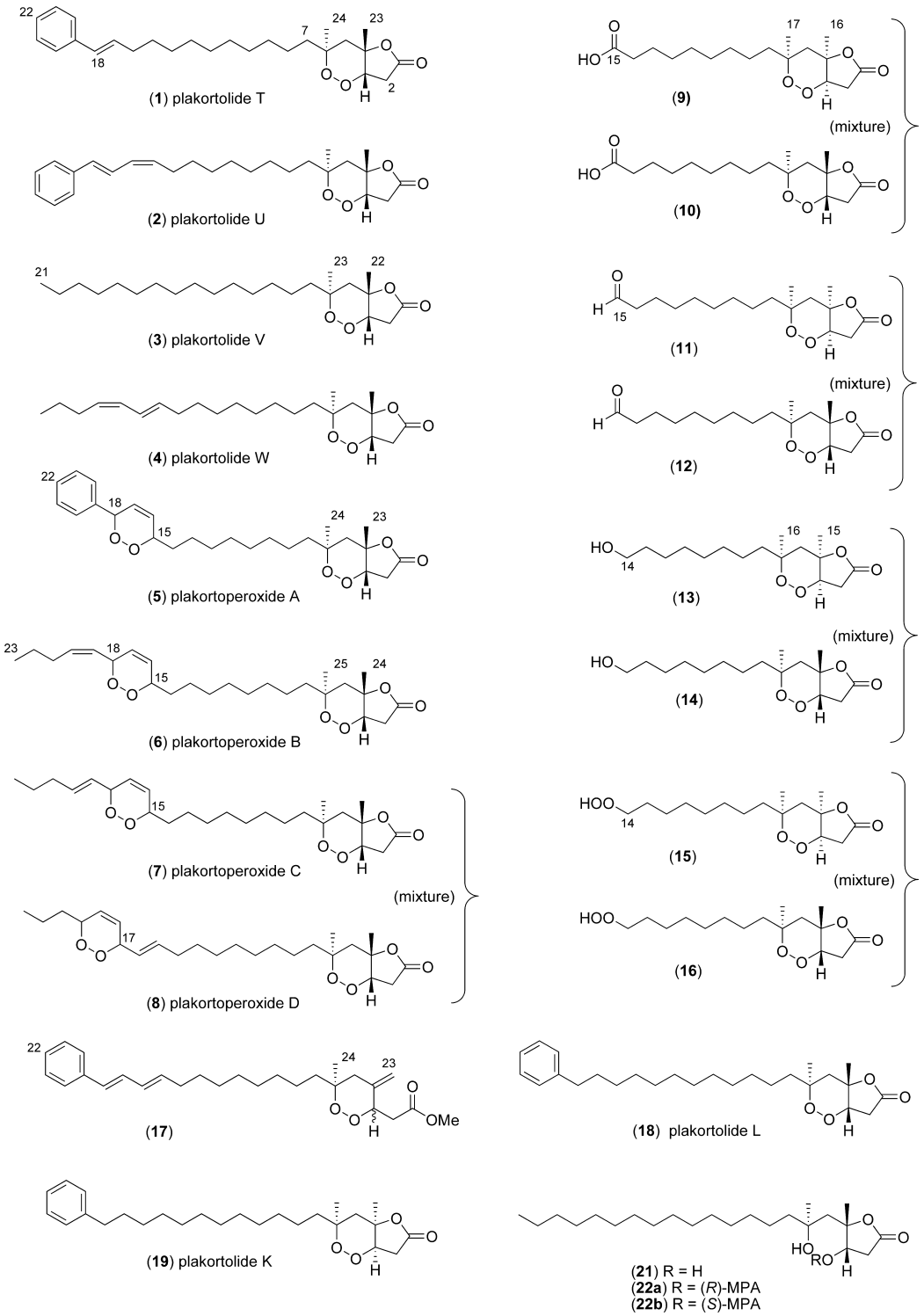
Chart 1



2.15, 1.69; δ_C 40.6], and a cluster of methylenes, as well as HMBC correlations, that were all consistent with a plakortolide skeleton. In the side chain, there was an *E*-olefinic group [δ_H 6.35, 6.20 (J = 16.0 Hz); δ_C 129.8, 131.3] that was located next to the phenyl ring by HMBC correlations from H-18 at δ_H 6.35 to C-19 (δ_C 138.4) and C-20 (δ_C 126.0). The relative configuration was defined by consideration of the chemical shifts for the bicyclic core that closely matched data for plakortolide L (**18**) rather than for the diastereomeric plakortolide K (**19**) (for **1** C-7, δ_C 41.0; C-24, δ_H 1.27; δ_C 22.4 vs **18**: C-7, δ_C 40.8; C-24, δ_H 1.27; δ_C 22.2 or **19**: C-7 δ_C 36.6; C-24, δ_H 1.18; δ_C 24.4). NOESY data, notably correlations between H-3/H-5b and H-5a/H-24, were fully consistent with this relative configuration.[2] Plakortolide T is thus the 17,18-didehydro analogue of plakortolide L.

The related plakortolide U (**2**) of molecular formula $C_{26}H_{36}O_4$ by HR-ESIMS had an identical core to **1** from $^1H/^{13}C$ NMR data and differed only in the unsaturation pattern

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Table 1. ^{1}H NMR Assignments for Plakortolides 1−8[a,b] and the Ester 17[a,c]

position	1	2	3	4	5	6	7	8	17
2a	2.89, dd (18.5, 6.3)	2.89, dd (18.8, 6.3)	2.89, dd (18.6, 6.1)	2.89, dd (18.6, 6.2)	2.89, dd (18.6, 6.2)	2.89, dd (18.5, 6.0)	2.89, dd (18.6, 6.2)	2.89, dd (18.6, 6.2)	2.70, dd (18.5, 6.3)
2b	2.60, brd (18.5)	2.60, brd (18.8)	2.60, brd (18.6)	2.60, brd (18.6)	2.60, brd (18.6)	2.60, brd (18.5)	2.60, brd (18.6)	2.60, brd (18.6)	2.89, br
3	4.43, d (6.3)	4.43, d (6.3)	4.43, d (6.1)	4.43, d 6.1)	4.43, d (6.2)	4.43, d (6.0)	4.43, d (6.2)	4.43, d (6.2)	4.48, m[c,d]
5a	2.15, d (15.0)	2.15, d (14.5)	2.15, d (14.8)	2.15, d (15.0)	2.15, d (14.8)	2.15, d (15.0)	2.15, d (14.8)	2.15, d (14.8)	2.15, d (15.0)
5b	1.69, d (15.0)	1.69, d (14.5)	1.69, d (14.8)	1.69, d (15.0)	1.69, d (14.8)	1.69, d (15.0)	1.69, d (14.8)	1.69, d (14.8)	1.69, d (15.0)
7	1.50−1.46, m[d,e]	1.49, m	1.49, m	1.46, m[d,e]	1.49, m	1.50, m[d,e]	1.49, m	1.49, m	1.50−1.46, m[d,e]
8	1.27, m[e]	1.40, m	n.d.[f]	n.d.[f]	1.27, m	1.29, m[d,e]	1.29, m[d,e]	1.29, m[d,e]	1.20−1.29, m[d,e]
9	1.23−1.30, m[d,e]	1.26−1.32, m[d,e]	1.22−1.27, m[d,e]	1.22−1.28, m[d,e]	1.23−1.35[d,e]	1.24−1.29, m[d,e]	1.24−1.30, m[d,e]	1.24−1.30, m[d,e]	1.20−1.29, m[d,e]
10	1.23−1.30, m[d,e]	1.26−1.32, m[d,e]	1.22−1.27, m[d,e]	1.22−1.28, m[d,e]	1.23−1.35[d,e]	1.24−1.29, m[d,e]	1.24−1.30, m[d,e]	1.24−1.30, m[d,e]	1.20−1.29, m[d,e]
11	1.23−1.30, m[d,e]	1.26−1.32, m[d,e]	1.22−1.27, m[d,e]	1.22−1.28, m[d,e]	1.23−1.35[d,e]	1.24−1.29, m[d,e]	1.24−1.30, m[d,e]	1.24−1.30, m[d,e]	1.20−1.29, m[d,e]
12	1.23−1.30, m[d,e]	1.26−1.32, m[d,e]	1.22−1.27, m[d,e]	1.22−1.28, m[d,e]	1.26, m	1.24−1.29, m[d,e]	1.24−1.30, m[d,e]	1.24−1.30, m[d,e]	1.20−1.29, m[d,e]
13	1.23−1.30, m[d,e]	1.26−1.32, m[d,e]	1.22−1.27, m[d,e]	1.22−1.28, m[d,e]	1.49, m 1.40, m	1.24−1.29, m[d,e]	1.24−1.30, m[d,e]	1.24−1.30, m[d,e]	1.20−1.29, m[d,e]
14	1.23−1.30, m[d,e]	2.27, q (7.5)	1.22−1.27, m[d,e]	2.06, q (6.8)	1.75, m 1.60, m	1.68, m 1.53, m	1.66, m 1.52, m	2.04, m	2.12, q (7.0)
15	1.45, m[d,e]	5.51, dt (11.5, 7.5)	1.22−1.27, m[d,e]	5.63, dt (15.2, 6.8)	4.54, m	4.42, m	4.46, m	5.76, dt (15.3, 7.0)	5.79, dt (15.3, 7.0)
16	2.18, q (7.3)	6.14, t (11.5)	1.22−1.27, m[d,e]	6.27, ddd (15.2, 10.9, 1.0)	6.09, dt (10.4, 2.1)	5.90, dt (10.3, 2.5)	5.90, m[g]	5.46, m	6.19, brdd (15.3, 10.5)
17	6.20, dt (16.0, 7.3)	7.04, dd (15.5, 11.5)	1.22−1.27, m[d,e]	5.93, t (10.9)	6.05, dt (10.4, 2.1)	5.79, dt (10.3, 2.5)	5.84, m[g]	4.84, m	6.72, dd (15.8, 10.5)
18	6.35, d (16.0)	6.50, d (15.5)	1.22−1.27, m[d,e]	5.28, dt (10.9, 7.7)	5.49, q (2.1)	5.24, brd (8.5)	4.84, m	5.84, m[g]	6.42, d (15.8)
19			1.25, m[d,e]	2.12, q (7.7)		5.39, ddt (10.6, 8.5, 1.0)	5.49, m	5.90, m[g]	
20	7.32, d (8.0)	7.39, d (7.5)	1.25, m[d,e]	1.37, m	7.36, m	5.66, dtd (10.6, 7.5, 1.0)	5.76, dt (15.3, 7.0)	4.46, m	7.32, d (8.0)
21	7.26, t (8.0)	7.29, t (7.5)	0.86, t (6.8)	0.89, t (7.4)	7.34, m	2.10, q (7.5)	2.02, m	1.60, m 1.49, m	7.26, t (8.0)
22	7.16, t (7.0)	7.19, t (7.5)	1.37, s	1.37, s	7.36, m	1.40, m	1.39, m	1.49, m	7.16, t (7.0)
23	1.37, s	1.39, s	1.27, s	1.26, s	1.37, s	0.90, t (7.5)	0.88, t (7.0)	0.91, t (7.0)	4.91, m 4.75, m
24	1.27, s	1.26, s			1.27, s	1.37, s	1.37, s	1.37, s	1.16, s
25						1.26, s	1.26, s	1.26, s	
O-Me									3.68, s

[a]Chemical shifts (ppm) referenced to $CHCl_3$ (δ_H 7.24). [b]At 500 MHz. [c]At 900 MHz. [d]Unresolved chemical shifts due to overlapping signals. [e]Signal multiplicity unresolved due to overlapping signals. [f]Not detected as obscured by other signals. [g]Signals may be interchanged.

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Table 2. ^{13}C NMR Assignments for Plakortolides 1−8[a,b] and the Ester 17[a,c]

position	1	2	3	4	5	6	7	8	17
1	174.7	174.2	173.9	174.0	174.1	174.0	174.0	174.0	171.0
2	34.3	34.2	34.2	34.4	34.1	34.3	34.3	34.3	39.4
3	81.1	81.1	81.1	81.0	80.9	81.2	81.2	81.2	79.9
4	83.1	82.6	82.5	82.6	82.6	82.7	82.7	82.7	141.0
5	40.6	40.5	40.5	40.4	40.4	40.6	40.6	40.6	40.4
6	80.3	79.9	79.9	79.9	79.9	79.9	79.9	79.9	82.5
7	41.0	40.9	40.9	40.8	40.8	41.0	41.0	41.0	36.9
8	23.3	22.7	n.d.[d]	n.d.[d]	22.6	23.1[c]	23.1[c]	23.1[c]	29.0−30.0[e]
9	29.2−30.0[e]	28.6−39.9[e]	29.2−30.1[e]	29.0−30.2[e]	29.1−29.6[e]	29.1−30.2[e]	29.1−30.2[e]	29.1−30.2[e]	29.0−30.0[e]
10	29.2−30.0[e]	28.6−39.9[e]	29.2−30.1[e]	29.0−30.2[e]	29.1−29.6[e]	29.1−30.2[e]	29.1−30.2[e]	29.1−30.2[e]	29.0−30.0[e]
11	29.2−30.0[e]	28.6−39.9[e]	29.2−30.1[e]	29.0−30.2[e]	29.1−29.6[e]	29.1−30.2[e]	29.1−30.2[e]	29.1−30.2[e]	29.0−30.0[e]
12	29.2−30.0[e]	28.6−39.9[e]	29.2−30.1[e]	29.0−30.2[e]	29.3	29.1−30.2[e]	29.1−30.2[e]	29.1−30.2[e]	29.0−30.0[e]
13	29.2−30.0[e]	29.2	29.2−30.1[e]	29.0−30.2[e]	25.1	29.1−30.2[e]	29.1−30.2[e]	29.1−30.2[e]	29.0−30.0[e]
14	29.2−30.0[e]	27.9	29.2−30.1[e]	33.0	32.8	33.0	32.6	32.2	32.6
15	29.3	133.3	29.2−30.1[e]	134.6	78.3	78.3	77.8	137.0	135.8
16	33.0	128.7	29.2−30.1[e]	125.6	128.7	127.9	127.9	126.2	130.2
17	131.3	124.5	29.2−30.1[e]	128.7	126.2	127.1	126.6	78.7	129.2
18	129.8	132.0	29.2−30.1[e]	129.8	80.0	74.4	78.7	126.6	129.7
19	138.4	137.6	31.8	29.8	137.4	125.5	126.0	127.9	137.7
20	126.0	126.2	22.5	23.0	128.4	135.7	137.0	77.8	126.0
21	128.5	128.6	13.9	13.7	128.3	29.9	34.2	34.8	128.5
22	126.8	126.9	25.8	26.0	128.6	22.6	21.7	18.5	126.6
23	26.2	25.6	22.2	22.4	25.6	13.7	13.4	13.7	111.3
24	22.4	22.4			22.1	25.9	25.9	25.9	22.2
25						22.4	22.4	22.4	
OMe									51.6

[a]Chemical shifts (ppm) taken from 2D spectra referenced to $CDCl_3$ (δ_C 77.0). [b]At 500 MHz. [c]At 900 MHz. [d]Not detected as obscured by other signals. [e]Unresolved chemical shifts taken from HSQC experiments.

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in the ω-phenyl-terminating C_{12} side chain. The signals for the *E*-olefinic group of **1** were replaced by signals at δ_H 5.51 (dt, H-15), 6.14 (t, H-16), 7.04 (dd, H-17), and 6.50 (d, H-18), assigned as a 15*Z*,17*E*-diene from coupling constant data ($J_{H\text{-}15/H\text{-}16}$ = 11.5 Hz; $J_{H\text{-}17/H\text{-}18}$ = 15.5 Hz); a chemical shift value of δ_C 27.9 for C-14 agreed with the C-15 *Z* configuration. Plakortolide U therefore had the opposite configuration of the C-15/C-16 double bond compared to the previously reported plakortolide P (**20**).[2] The relative configuration was again deduced from the ^{1}H and ^{13}C NMR values for the C-6 methyl group and for C-7. The absolute configuration shown for both **1** and **2** was selected on the basis that a 6*S* configuration had previously been confirmed by Mosher analysis in four different plakortolide metabolites that were representative of both diastereomeric series.[2] We thus considered it biosynthetically reasonable that the absolute configuration would be consistent among the entire group of phenyl-group-terminating plakortolide metabolites isolated from the single specimen of *P. clathrata* used in our study.

The sponge extract further contained a range of plakortolides that did not possess a phenyl end group. The first of these was plakortolide V (**3**), with a molecular formula of $C_{23}H_{42}O_4$ from HR-ESIMS, supporting a saturated C_{15} side chain. The ^{1}H NMR data revealed signals for a terminal methyl group (δ_H 0.86, t) and a long-chain alkyl side chain, but in all other respects the ^{1}H/^{13}C NMR and HMBC data confirmed a plakortolide skeleton. The relative configuration of **3** followed directly from comparison of the chemical shift values for C-7 and for the C-6 methyl group (here numbered as C-23) with those of plakortolide L. NOESY data showed correlations between H-3/H-5b and between H-5a/C-6Me as expected given the *trans* arrangement of methyl groups. The absolute configuration of **3** was determined by reductive cleavage of the peroxy ring using H_2/Pd/C to give diol **21**, which was esterified at C-3 as the (*R*)- and (*S*)-*O*-methyl mandelate (MPA) esters **22a**/**22b**;[3,4] the $\Delta\delta^{RS}$ values were negative for H_2-2 and positive for H_2-5, H_2-7, H_3-22, and H_3-23, consistent with a 3*S* configuration. Together with the relative configurational information, this Mosher analysis confirmed **3** as 3*S*,4*S*,6*S*-configured, thus belonging to the same configurational series as the phenyl end group-functionalized plakortolides.

Plakortolide W (**4**), with the molecular formula $C_{23}H_{38}O_4$ by HR-ESIMS, also had a C_{15} side chain (δ_H 0.89 (t, H-21)), but differed from **3** in that side chain unsaturation was present. Signals at δ_H 5.63 (dt, H-15), 6.27 (ddd, H-16), 5.93 (t, H-17), and 5.28 (dq, H-18) were assigned to a 15*E*,17*Z*-diene from coupling constant data ($J_{H\text{-}15/H\text{-}16}$ = 15.2 Hz; $J_{H\text{-}17/H\text{-}18}$ = 10.9 Hz). The ^{13}C values for C-14 and C-19 further confirmed the double-bond configurations (C-14: δ_C 33.0; C-19: δ_C 29.8). In the HMBC spectrum, there were correlations from the terminal methyl (H-21) to C-20 (δ_C 23.0) and C-19 (δ_C 29.8), from H-18 to C-15 (δ_C 134.6), C-16 (δ_C 125.6), and C-20, and from H-17 to C-19, while 1D-TOCSY data showed correlations through H-20 and H-19 to H-18 upon irradiation of H-21. The relative configuration of the bicyclic ring was again established from the ^{1}H and ^{13}C NMR values for C-7 and the C-6 methyl group. Plakortolide W thus has the opposite configuration for each side chain double bond compared to plakortolide U.

Plakortoperoxides. Four metabolites isolated from the *P. clathrata* extract each had an additional two oxygen atoms in their molecular formula, suggestive of a second 1,2-dioxine ring. Two of these metabolites, namely, **5** and **6**, were successfully purified by repetitive HPLC; however the remaining two peroxy compounds, **7** and **8**, could not be separated, either by HPLC or by silver nitrate-based chromatography, and so were characterized as a mixture.

The first diperoxide, named plakortoperoxide A (**5**), had a molecular formula of $C_{26}H_{36}O_6$ by HR-ESIMS and exhibited all the anticipated NMR features associated with a plakortolide core linked by an alkyl chain to a phenyl group. The *trans* arrangement of the C-4 and C-6 methyl groups in **5** followed from ^{13}C NMR comparison with other plakortolides. However, four additional signals, at δ_H 4.54 (m, H-15), 6.09 (dt, H-16), 6.05 (dt, H-17), and 5.49 (q, H-18), were apparent in the ^{1}H NMR spectrum, and comparison of these chemical shifts with those of model cyclic peroxides[5,6] suggested a second 1,2-dioxine moiety. The associated ^{13}C NMR signals [δ_C 78.3 (C-15), 128.7 (C-16), 126.2 (C-17), and 80.0 (C-18)] were located using HSQC data, while HMBC correlations from H-20 to C-18 and from H-18 to δ_C 128.4 (C-20) positioned the peroxy ring adjacent to the phenyl ring.

The relative configuration of the side chain endoperoxide moiety was suggested as *cis* since the chemical shift value for H-15 (δ_H 4.54) was close to that observed for the equivalent position in *cis*-3,6-dipropyl-1,2-dioxa-4-cyclohexene (δ_H 4.66)[5a] or *cis*-3,6-dibutyl-1,2-dioxa-4-cyclohexene (δ_H 4.44)[5b] and to data for synthetic model peroxides prepared by us. The *cis* or *trans* configuration of endoperoxides can be distinguished by low-temperature ^{1}H NMR study since in the *cis* stereoisomer two half-chair conformations are possible, whereas in the *trans* isomer a single half-chair conformer is favored (Figure 1).[5] A

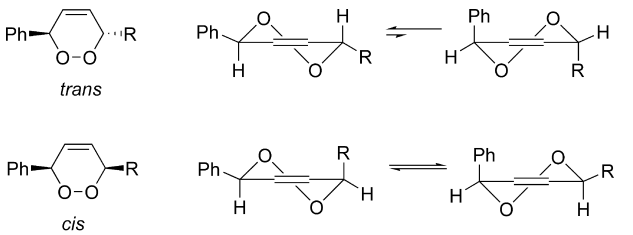

Figure 1. Half-chair conformations of *cis*- and *trans*-1,4-disubstituted endoperoxides.

portion of the spectrum (25 °C) of **5** in acetone-d_6 is shown in Figure 2a; in contrast, at −87 °C (Figure 2b), two sets of NMR signals in a ratio of ∼1:1 were apparent for H-15 and H-18 of **5**, consistent with the interconversion of two half-chairs for the side chain endoperoxide ring. These pairs of NMR signals coalesced at −60 °C. The spectrum at −87 °C also revealed two sets of signals for H-2a/b, H-3, H-5a/b, and the 4- and 6-methyl groups compared to the spectrum recorded at 25 °C. The conformational implications for the plakortolide core are currently under evaluation. Cyclization of plakortolide P (**20**), isolated in our earlier study,[2] with singlet oxygen in DCM at 5 °C in the presence of rose bengal bis(triethylammonium) salt as photosensitizer (Scheme 1) led to a synthetic sample of plakortoperoxide A (**5**) in 62% yield and with spectroscopic data identical to natural material. The synthetic material could be separated into the two diastereomers, **5a** and **5b** (1:1 ratio), by enantioselective HPLC using a DAICEL Chiralpak AD column with UV detection at 254 nm; this experiment served as a standard for the stereochemical analysis of natural **5**, which then revealed that the naturally isolated material was likewise a mixture (∼1:1) of the two diastereomers **5a** and **5b**. The two

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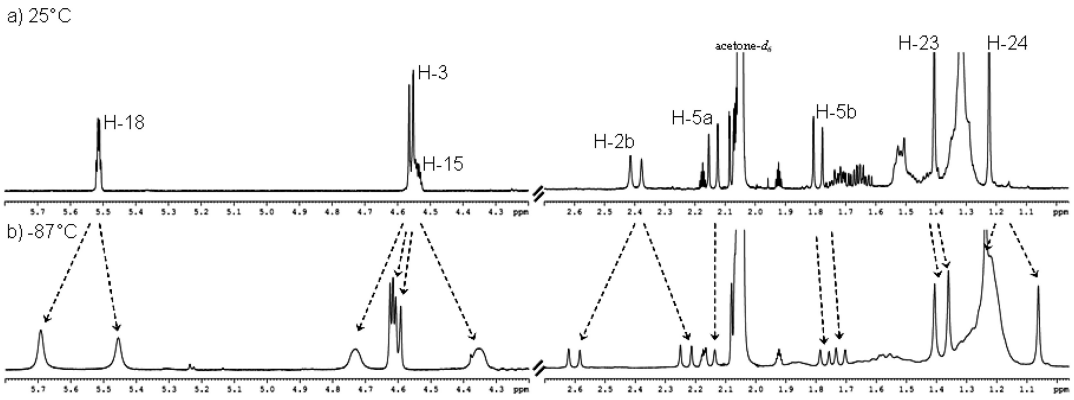
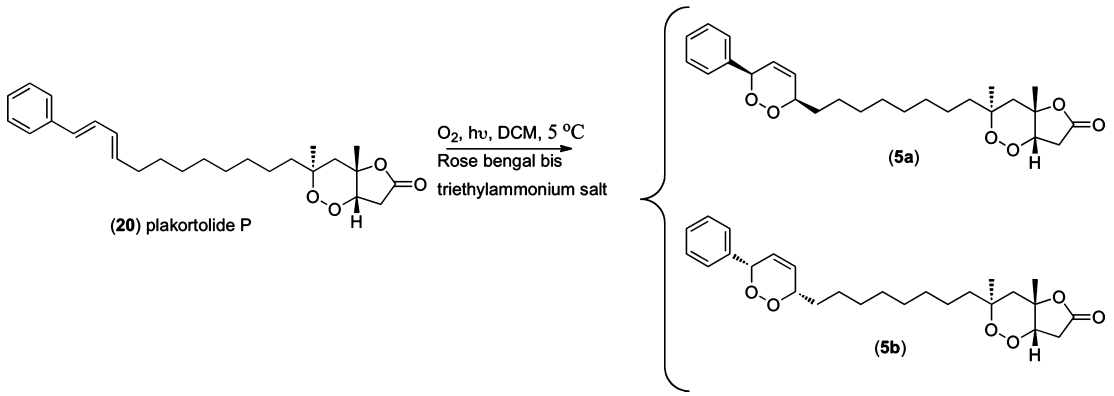


Figure 2. ^{1}H NMR spectra of plakortoperoxide A (**5**) in acetone-d_6 at (a) 25 °C; (b) −87 °C.

Scheme 1. Preparation of Synthetic Plakortoperoxide A (5) as a Mixture (1:1) of Diastereomers 5a and 5b for Comparison with Natural Material



individual diastereomers were named plakortoperoxides A1 and A2, respectively.

The second endoperoxide, named plakortoperoxide B (**6**), had a molecular formula of $C_{25}H_{40}O_6$ by HR-ESIMS and, therefore, had a different carbon chain length from any of the other plakortolides isolated from *P. clathrata*. Inspection of the ^{1}H/^{13}C NMR data revealed the absence of a phenyl ring; instead signals at δ_H 0.90 for H-23 supported a methyl terminus, and on the basis of the MS data, there was a C_{17} side chain. 1D-TOCSY correlations from H-23 through H-22 and from H-21 to H-20 (δ_H 5.66) and H-19 (δ_H 5.39) established a C-19/C-20 olefinic group, assigned as *Z* from the 10.6 Hz coupling constant. Signals at δ_H 4.42 (m, H-15), 5.90 (dt, H-16), 5.79 (t, H-17), and 5.24 (brd, H-18) were for an endoperoxide moiety that could be positioned by HMBC correlations from H-20 to C-18 (δ_C 74.5), together with correlations from H-18 to C-20 (δ_C 135.7) and to C-16 (δ_C 127.9) as adjacent to the *Z*-olefin. The *cis* configuration of the side chain endoperoxide was inferred from chemical shift comparison with H-15 of **5** and of model endoperoxides,[5] while the relative configuration of the plakortolide ring was again inferred from a ^{13}C NMR chemical shift comparison with co-occurring plakortolides. Although the stereochemical homogeneity was not investigated by enantioselective HPLC, plakortoperoxide B is also likely to be a mixture of C-15/C-18-*cis*-diastereomers that could individually be named as plakortoperoxides B1 and B2.

The remaining pair of plakortoperoxides was characterized as a 1:1 mixture. A molecular formula of $C_{25}H_{40}O_6$ was apparent by HR-ESIMS, supporting a C_{17} side chain. In plakortoperoxide C (**7**), the side chain endoperoxide was apparent from ^{1}H NMR signals at δ_H 4.46 (m, H-15), 5.90 (m, H-16), 5.84 (m, H-17), and 4.84 (m, H-18) and was adjacent to an *E*-olefinic moiety [δ_H 5.49 (m, H-19), 5.76 (dt, H-20); $J_{H\text{-}19/H\text{-}20}$ = 15.3 Hz]. In the HMBC, there were cross-peaks from the terminal methyl (δ_H 0.88, H-23) to C-22 (δ_C 21.7) and C-21 (δ_C 34.2), from H-22 to C-20 (δ_C 137.0), and from H-21 to C-19 (δ_C 126.0). Additional HMBC cross-peaks from H-20 to C-18 at δ_C 78.7 confirmed the location of the 1,2-dioxine moiety between C-15 and C-18. The assignment was consistent with the observed TOCSY spin system from H-23 to H-19. The downfield value for C-18 in **7** of δ_C 78.7 compared to δ_C 74.4 in **6** supported the configurational assignments, although unusually the opposite trend was noted in the proton values (H-18 in **7** is δ_H 4.84 compared to δ_H 5.24 in **6**). In plakortoperoxide D (**8**), TOCSY correlations observed from H-23 (δ_H 0.91) to H-22, H-21, and an oxymethine at δ_H 4.46 (H-20) revealed that the endoperoxide ring and alkene moiety were reversed compared to **7**. The 15.3 Hz coupling between H-15 and H-16, together with a chemical shift value of δ_C 32.2 for C-14, supported a 15*E* double bond. HMBC correlations from both H-15 and H-16 to methylene carbons of the side chain were in accordance with the structure of **8**. A *cis* configuration for the side chain endoperoxide was apparent from the chemical shift values of H-15 (δ_H 4.46) in **7** and of H-20 (δ_H 4.46) in **8**, while in each metabolite the plakortolide core had a *trans* arrangement of the C-4 and C-6 methyl groups by ^{13}C NMR. Each plakortoperoxide was assumed to be a diastereomeric mixture.

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Table 3. ^{1}H NMR Assignments for Side Chain-Oxidized Plakortolides 9−16[a,b]

position	9	10	11	12	13	14	15	16
2a	2.88, dd (18.5, 6.0)	2.89, dd (18.5, 6.1)	2.89, dd (18.5, 5.9)	2.89, dd (18.6, 6.2)	2.89, dd (18.5, 6.0)	2.89, dd (18.5, 6.0)	2.89, dd (18.5, 5.7)	2.89, dd (18.5, 6.2)
2b	2.53, brd (18.5)	2.59, brd (18.5)	2.54, brd (18.3)	2.60, brd (18.6)	2.53, brd (18.5)	2.60, brd (18.5)	2.54, brd (18.5)	2.60, brd (18.5)
3	4.45, d (6.0)	4.43, d (6.1)	4.46, d (5.9)	4.44, d (6.2)	4.45, d (6.0)	4.42, d (6.0)	4.45, d (5.7)	4.43, d (6.2)
5a	2.24, d (14.9)	2.14, d (14.8)	2.25, d (14.9)	2.15, d (14.9)	2.24, d (15.0)	2.16, d (15.0)	2.25, d (15.0)	2.15, d (14.5)
5b	1.63, d (14.9)	1.68, d (14.8)	1.63, d (14.9)	1.68, d (14.9)	1.63, d (15.0)	1.68, d (15.0)	1.64, d (15.0)	1.68, d (14.5)
7	1.70, m	1.51, m	1.70, m	1.47, m	n.d.[c]	n.d.[c]	n.d.[c]	1.49, m
	1.51, m	1.46, m	1.52, m					
8	1.25, m[d,e]	1.25, m[d,e]	1.25, m[d,e]	1.25, m[d,e]	1.25, m[d,e]	1.25, m[d,e]	1.28, m[d,e]	1.28, m[d,e]
9	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.23−1.30, m[d,e]	1.23−1.30, m[d,e]
10	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.23−1.30, m[d,e]	1.23−1.30, m[d,e]
11	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.23−1.30, m[d,e]	1.23−1.30, m[d,e]
12	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.22−1.32, m[d,e]	1.23−1.30, m[d,e]	1.23−1.30, m[d,e]
13	1.62, m[d,e]	1.62, m[d,e]	1.61, m[d,e]	1.61, m[d,e]	n.d.[c]	n.d.[c]	1.61, m[d,e]	1.61, m[d,e]
14	2.32, t (7.5)	2.32, t (7.5)	2.40, td (7.5, 1.8)	2.40, td (7.5, 1.8)	3.61, brt (6.5)	3.61, brt (6.5)	4.00, t (6.7)	4.00, t (6.7)
15			9.74, t (1.8)	9.74, t (1.8)	1.36, s	1.37, s	1.35, s	1.37, s
16	1.35, s	1.36, s	1.36, s	1.37, s	1.18, s	1.27, s	1.17, s	1.26, s
17	1.16, s	1.25, s	1.17, s	1.26, s				
−OOH							7.32[f]	7.33[f]

[a]Chemical shifts (ppm) referenced to $CHCl_3$ (δ_H 7.24). [b]Frequency at 500 MHz. [c]Not detected, as obscured by other signals. [d]Unresolved chemical shifts due to overlapping signals. [e]Signal multiplicity unresolved due to overlapping signals. [f]Interchangeable.

Table 4. ^{13}C NMR Assignments for Side Chain-Oxidized Plakortolides 9−16[a,b]

position	9	10	11	12	13	14	15	16
1	174.0	174.1	173.9	174.1	174.0	174.1	174.1	174.1
2	34.1	34.3	34.2	34.2	34.1	34.3	33.9	34.1
3	80.8	81.2	80.7	81.1	80.6	81.2	80.6	80.9
4	82.5	82.8	82.3	82.7	82.5	82.8	82.3	82.5
5	40.3	40.6	40.3	40.7	40.3	40.6	40.1	40.5
6	80.1	80.2	79.9	79.9	80.1	80.2	80.0	79.9
7	36.8	41.0	36.8	40.9	36.8	41.0	36.6	40.8
8	23.0	23.0	23.2	23.2	23.0	23.0	22.8	22.8
9	29.0−29.9[c]	29.0−29.9[c]	28.8−29.9[c]	28.8−29.9[c]	29.0−29.9[c]	29.0−29.9[c]	29.1−29.9[c]	29.1−29.9[c]
10	29.0−29.9[c]	29.0−29.9[c]	28.8−29.9[c]	28.8−29.9[c]	29.0−29.9[c]	29.0−29.9[c]	29.1−29.9[c]	29.1−29.9[c]
11	29.0−29.9[c]	29.0−29.9[c]	28.8−29.9[c]	28.8−29.9[c]	29.0−29.9[c]	29.0−29.9[c]	29.1−29.9[c]	29.1−29.9[c]
12	29.0−29.9[c]	29.0−29.9[c]	28.8−29.9[c]	28.8−29.9[c]	29.0−29.9[c]	29.0−29.9[c]	25.7	25.7
13	24.7	24.7	22.0	22.0	32.5	32.5	27.3	27.3
14	33.7	33.7	43.7	43.7	62.9	62.9	77.0	77.0
15	177.4	177.4	202.9	202.9	25.8	25.8	25.7	25.7
16	25.9	25.9	25.9	25.9	24.7	22.3	24.5	22.2
17	24.9	22.5	24.8	22.2				

[a]Chemical shifts (ppm) referenced to $CDCl_3$ (δ_C 77.0). [b]Frequency at 500 MHz. [c]Unresolved chemical shifts taken from HSQC experiments.

Side Chain-Truncated Plakortolides. The final group of plakortolide metabolites isolated from the *Plakinastrella* extract all showed evidence of side chain oxidation. Following repetitive RP-HPLC, four fractions were obtained, each of which was a mixture of diastereomers. The first fraction, a 1:2 mixture of diastereomers **9** and **10** by NMR, had a formula of $C_{17}H_{28}O_6$ inferred from HR-ESIMS data, supporting a C_9 side chain, and the two individual structures were named carboxyplakortolides-1 and -2. The second fraction, containing diastereomers **11** and **12** in a 1:2 ratio, named formylplakortolides-1 and -2, respectively, likewise had a C_9 side chain from MS data that corresponded to a molecular formula of $C_{17}H_{28}O_5$. These four metabolites showed ^{1}H/^{13}C NMR data (Tables 3 and 4) as well as HMBC correlations from H-3 (at δ_H 4.45, 4.43, 4.46, and 4.44 in **9**−**12**, respectively) to the lactone C-1, to C-4, and to the C-4 Me, which were fully consistent with a plakortolide skeleton. The ^{1}H NMR spectrum of metabolites **9** and **10** revealed a downfield signal (δ_H 2.32) that showed an HMBC signal to a carboxyl group (δ_C 177.4). Isomers **11** and **12** showed side chain NMR signals (**11**: δ_H 9.74; δ_C 202.9; **12**: δ_H 9.74; δ_C 202.9) that were diagnostic of an aldehyde and that showed HMBC correlations from an adjacent downfield triplet proton signal (δ_H 2.40 for both **11** and **12**). Metabolites **9** and **11** showed similar ^{1}H/^{13}C NMR data, respectively, for the bicyclic core as plakortolide K (**9** and **11**; C-7, δ_C 36.8 for each vs δ_C 36.6 for plakortolide K; C-6 Me; δ_H 1.16, δ_C 24.9 and δ_H 1.17, δ_C 24.8, respectively, vs δ_H 1.18; δ_C 24.2 for plakortolide K). Similarly the data for **10** and **12** matched those of plakortolide L (**10** and **12**; C-7, δ_C 41.0 and 40.9, respectively, vs δ_C 40.8 for plakortolide L; C-6 Me; δ_H 1.25; δ_C 22.5 and δ_H 1.26; δ_C 22.2, respectively, vs δ_H 1.27; δ_C 22.2 for plakortolide L).[2] Inspection of NOESY data

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revealed correlations between H-3/H-5b and H-5b/C-6Me for **9** and between H-3/H-5b and H-5a/C-6Me for **10**, as expected for *cis* and *trans* methyl-configured plakortolides, respectively.[2]

The two remaining fractions yielded sets of diastereomers (**13**−**16**) each possessing a C_8 side chain by HR-ESIMS data. Diastereomers **13** and **14** (1:2 ratio), named hydroxyplakortolides-1 and -2, had the molecular formula $C_{16}H_{28}O_5$. NMR signals at δ_H 3.61 linked to a carbon at δ_C 62.9 suggested a hydroxy functionality. The pair of diastereomers **15** and **16** (3:5 ratio), with a molecular formula of $C_{16}H_{28}O_6$, had an additional oxygen atom compared to **13** and **14**, implying a hydroperoxy functionality. ^{1}H NMR signals at δ_H 4.00 (t, J = 6.0 Hz) linked to a carbon at δ_C 77.0 were observed in the side chain of both **15** and **16** and matched literature data for the terminal methylene of primary alkyl hydroperoxides.[7] There were also two singlets at δ_H 7.32 and 7.33 that could be assigned to the hydroperoxy protons.[8] Reduction of the hydroperoxy group to the corresponding alcohol was not attempted owing to the small amount of material available and the presence of the metal or Ph_3P-sensitive plakortolide core.[9] The two hydroperoxides were named hydroperoxyplakortolides-1 and -2, respectively. These are the first examples of primary alkyl hydroperoxides isolated from marine sponges, although we note that a primary allylic hydroperoxide has been reported from the Okinawan cnidarian *Anthopleura pacifica* Uchida.[7a] A comparison of ^{13}C NMR data confirmed the relative configurations of **13** and **15** matched those of plakortolide K, while **14** and **16** had the same relative configuration as plakortolide L.

Isolation of a Methyl Ester of a Didehydroplakinic Acid. A nonpolar compound, **17**, eluted before the various plakortolide metabolites in Si flash chromatography and gave a sodiated molecular ion peak (m/z 449.2653) by HR-ESIMS that corresponded to a molecular formula of $C_{27}H_{38}O_4$. ^{1}H and HSQC spectra showed the expected signals for a phenyl ring [δ_H 7.35 (2H), 7.27 (2H), and 7.17 (1H); δ_C 128.5, 126.6, and 126.0], an oxy-methine signal (δ_H 4.88; δ_C 79.9), and two characteristic AB methylene groups (δ_H 2.70, dd and 2.89, br; δ_C 39.4 and δ_H 2.15, d and 1.69, d; δ_C 40.4) in addition to the cluster of methylene signals typical for an alkyl side chain. In contrast to the various plakortolides, there was only one methyl group (δ_H 1.16; δ_C 22.2), but instead there were signals for an exomethylene group (δ_H 4.91 and 4.75; δ_C 111.3) and a methoxy group (δ_H 3.68, δ_C 51.6). The methoxy group and the signal at δ_H 2.70 (H-2) showed HMBC correlations to an ester carbonyl (δ_C 171.0, C-1), revealing that the plakortolide lactone ring had been opened. HMBC correlations from the exomethylene (δ_H 4.91 and 4.75) to the C-4 quaternary carbon (δ_C 141.0), to C-3 (δ_C 79.9), and to the isolated C-5 methylene carbon (δ_C 40.4) placed the exocyclic double bond at C-4, while correlations from the methyl signal at δ_H 1.16 to C-5 (δ_C 40.4), C-7 (δ_C 36.9), and C-6 (δ_C 82.5) all confirmed a C-6 methyl group. In the side chain, there were also characteristic signals (δ_H 6.72, 6.42, 6.19, and 5.79; δ_C 135.8, 130.2, 129.7, and 129.2) for a conjugated (*E*,*E*)-diene system that matched data for the reported plakortolides O-R;[2] the diene was positioned next to the terminal phenyl ring from HMBC data. The absolute configuration at C-6 was tentatively assigned as *S* on the basis of the conserved stereochemistry of the biosynthetic precursor for the "phenyl" group of plakortolides. The configuration at C-3 was not determined. Ester **17** shows structural resemblance to a cytotoxic peroxy-functionalized acid isolated from the New Guinea sponge *Callyspongia* sp.[10]

Biosynthesis. In our earlier publication,[2] we presented a biosynthetic route to the diastereomeric plakortolides that involves nucleophilic attack of a 6*S*-hydroperoxy group onto C-3 of an α,β-unsaturated carboxylic acid derivative. Attack of the carboxylic group onto the carbocation derived from protonation of the C-4/C-5 alkene then generates the bicyclic system, with the stereochemical outcome at C-4 necessarily linked to that at C-3 owing to the steric constraints of the fused lactone. This biosynthetic proposal contrasts with the generally accepted mechanism for endoperoxide formation involving Diels−Alder addition of singlet oxygen to a diene. Experimental studies have shown that Diels−Alder reactions with singlet oxygen are not always concerted[5a] and that both *E*,*E* and *E*,*Z* dienes generate *cis* adducts.[5,6] Our detailed study of *P. clathrata* has provided plakortolides with side chains containing *E*,*E*-dienes (plakortolides O−R[2]) as well as an *E*,*Z*-diene in plakortolides U (**2**) and W (**4**); thus plakortolide U (**2**) is a plausible precursor of plakortoperoxide A (**5**), while each of plakortoperoxides B−D presumably arises from a plakortolide with a $\Delta^{15,17,19}$ triene functionality. Although we did not isolate any directly, evidence for plakortolides with side chain trienes was noticed in some HPLC fractions on comparison of ^{1}H NMR data with those of the recently reported epiplakinic acid F methyl ester.[11] It is difficult to assess whether the plakortoperoxides are genuine natural products or artifacts of side chain oxidation during isolation. The literature reports one other example of a diperoxy polyketide, notably isolated along with some related dienes.[12] Some of the new plakortolides isolated in our study possessed a methyl end group rather than the phenyl end group, and so their biosynthesis likely involves acetate rather than phenylacetate as a chain starter unit.

Carboxylic acids **9** and **10** and aldehydes **11** and **12**, all with a C_9 side chain, apparently derive by oxidative cleavage of the $\Delta^{15,16}$ double bond of a parent plakortolide. For comparison, 10-carboxy-11,12,13,14-tetranorplakortide Q has recently been reported along with plakortide Q and an 11,12-didehydro-13-oxo analogue from a *Plakortis* sp.,[13] while manadoperoxide C might derive from oxidative cleavage of the $\Delta^{12,13}$ double bond of the co-occurring manadoperoxides A and B.[3] However C−C single bond cleavage is known and commonly involves a cytochrome P450-catalyzed oxidation proceeding via a diol intermediate.[14]

More intriguing from a biosynthetic perspective are the alcohols **13** and **14**, and particularly the hydroperoxides **15** and **16** with a C_8 side chain. In Nature, hydroperoxides may be formed by an "ene" reaction involving molecular oxygen or by radical abstraction of a hydrogen atom followed by addition of molecular oxygen.[9] These pathways generate a double bond adjacent to the position of hydroperoxidation in both terpene[15] and fatty acid/oxylipins.[15,16] In plant species, and perhaps also in this *Plakinastrella* sponge, hydroperoxide reductases often detoxify reactive peroxy products by converting them to inactive alcohols.[17] In plants and diatoms, hydroperoxide lyases release unsaturated aldehydes with a shortened chain length that are implicated in the ecological success of the host system.[16] However the absence of any alkene functionality in the side chains of hydroperoxy compounds **15** and **16** defies all of these established biosynthetic pathways. When considered together with the stereoselective hydroperoxidation at C-6 of plakortolide precursors that was explored in our earlier paper,[2] the presence of such a diverse suite of oxidatively modified

plakortolide structures hints at an extraordinary peroxidative-based enzymatic chemistry in this marine sponge species that warrants further investigation.

CONCLUSIONS

Four new plakortolides (**1**–**4**) with either a side chain diene functionality or a saturated side chain have been isolated from the Australian sponge *P. clathrata* Kirkpatrick, 1900, and characterized by extensive 2D NMR studies. An additional four metabolites, the plakortoperoxides A–D (**5**–**8**), all shared a second peroxy ring in their side chain, and the relative configuration of the side chain endoperoxide was deduced as *cis* from mechanistic considerations, by low-temperature NMR study, and by comparison with literature models. Each plakortoperoxide was shown to be a mixture of two diastereomers by enantioselective HPLC. A number of these peroxy metabolites possessed a methyl end group rather than the common phenyl end group, and so their biosynthesis likely involves acetate rather than phenylacetate as a chain starter unit. A final group of eight metabolites with a plakortolide core contained a truncated side chain, either C_9 in length and terminating in either a carboxylic acid (**9**, **10**) or a formyl (**11**, **12**) functionality or alternatively C_8 in length and terminating in either a hydroxy (**13**, **14**) or the rare hydroperoxy (**15**, **16**) group. The sponge extract also contained an ester (**17**), which represents an additional structural variation in the complex family of peroxy-containing metabolites.

EXPERIMENTAL SECTION

General Experimental Procedures. These have been reported previously.[2] NMR spectra obtained in acetone-d_6 at room temperature were internally referenced to acetone (^{1}H, 2.05 ppm; ^{13}C, 29.8 ppm). Analytical enantioselective HPLC was performed on an Agilent 1200 Series liquid chromatograph system equipped with both a UV detector (set at 254 nm) and an ALP detector (advanced laser polarimeter, PDR-Chiral Inc.) using a Chiralpak AD column (4.6 × 250 mm, DAICEL Chemical Co.) and with isocratic ^{i}PrOH/hexanes (3:17) at a flow rate of 0.5 mL per min.

Biological Material. This has been described previously.[2]

Extraction and Purification. A portion of the EtOAc extract from *P. clathrata*[2] was subjected to NP flash chromatography with gradient elution (hexanes → CH_2Cl_2 → EtOAc → MeOH) to give 20 combined fractions (based on TLC profiles) and coded NP1–NP20. Fraction NP7, which eluted from 100% CH_2Cl_2, was further purified using analytical RP-HPLC with 100% CH_3CN to give the ester (**17**) (0.4 mg). Fraction NP9, which eluted from 100% CH_2Cl_2 to CH_2Cl_2/EtOAc (5:1), was subjected to additional NP flash chromatography with gradient elution (hexanes → EtOAc) to give five fractions, coded as NP9a to NP9e. Fraction NP9a was purified by NP-HPLC using EtOAc/hexanes (3:1) to give plakortolide W (**3**) (0.6 mg). Fraction NP9c was also purified by NP-HPLC using EtOAc/hexanes (3:1) to afford a mixture (1:2 by NMR) of formylplakortolide-1 (**11**) and -2 (**12**) (1.0 mg) and a mixture (3:5 by NMR) of hydroperoxyplakortolide-1 (**15**) and -2 (**16**) (0.5 mg). Fraction NP9e was purified by RP-HPLC using gradient elution (50% CH_3CN/50% H_2O to 100% CH_3CN over 30 min), yielding first a mixture (1:2) of carboxyplakortolide-1 (**9**) and -2 (**10**) (1.6 mg), followed by a mixture (1:2) of hydroxyplakortolide-1 (**13**) and -2 (**14**) (0.5 mg). Fraction NP10, which eluted from CH_2Cl_2/EtOAc (3:1), was further chromatographed using RP-HPLC with a solvent gradient of 95% CH_3CN/5% H_2O to 100% CH_3CN over 10 min followed by 100% CH_3CN for 30 min to give 16 HPLC fractions (HP10a–HP10p) based on the UV profiles. Fraction HP10c was chromatographed on analytical RP-HPLC using a solvent gradient of 85% CH_3CN/15% H_2O to 95% CH_3CN/5% H_2O over 10 min, followed by 95% CH_3CN/5% H_2O for 10 min to afford a mixture (1:1) of plakortoperoxides C (**7**) and D (**8**) (0.5 mg) followed by plakortoperoxide B (**6**) (0.1 mg). Fraction HP10g was chromatographed on analytical RP-HPLC using a solvent gradient of 85% CH_3CN/15% H_2O to 96% CH_3CN/4% H_2O over 10 min, followed by 96% CH_3CN/4% H_2O for 10 min, yielding plakortolide U (**2**) (0.3 mg). Fraction HP10j was chromatographed on NP-HPLC using hexanes/EtOAc (85:15) to give plakortolide W (**4**) (0.8 mg) and plakortolide T (**1**) (2.8 mg). Finally HP10p was rechromatographed on NP-HPLC using hexanes/EtOAc (3:1) to afford plakortoperoxide A (**5**) (0.8 mg).

*Plakortolide T (**1**):* colorless oil; $[\alpha]_D$ −15 (*c* 0.05, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 1 and Table 2; HR-ESIMS *m*/*z* 437.2663 $[M + Na]^+$ (calcd for $C_{26}H_{38}NaO_4$, 437.2662).

*Plakortolide U (**2**):* colorless oil; $[\alpha]_D$ +26 (*c* 0.02, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 1 and Table 2; HR-ESIMS *m*/*z* 435.2508 $[M + Na]^+$ (calcd for $C_{26}H_{36}NaO_4$, 435.2506).

*Plakortolide V (**3**):* colorless oil; $[\alpha]_D$ +15 (*c* 0.05, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 1 and Table 2; HR-ESIMS *m*/*z* 405.2991 $[M + Na]^+$ (calcd for $C_{23}H_{42}NaO_4$, 405.2975).

*Plakortolide W (**4**):* colorless oil; $[\alpha]_D$ −7 (*c* 0.05, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 1 and Table 2; HR-ESIMS *m*/*z* 401.2662 $[M + Na]^+$ (calcd for $C_{23}H_{38}NaO_4$, 401.2662).

*Plakortoperoxide A (**5**):* colorless oil; $[\alpha]_D$ −13 (*c* 0.06, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 1 and Table 2; ^{1}H and ^{13}C NMR (acetone-d_6, 500 MHz) δ_H 7.41 (2H, m, H-20), 7.39 (1H, m, H-22), 7.36 (2H, m, H-21), 6.19 (1H, ddd, *J* = 10.3, 2.5, 2.5 Hz, H-16), 6.11 (1H, ddd, *J* = 10.3, 2.3, 2.3 Hz, H-17), 5.51 (1H, dd, *J* = 2.5, 2.3 Hz, H-18), 4.55 (1H, d, *J* = 6.2 Hz, H-3), 4.44 (1H, m, H-15), 3.14 (1H, dd, *J* = 18.5, 6.2 Hz, H-2a), 2.40 (1H, d, *J* = 18.5 Hz, H-2b), 2.14 (1H, d, *J* = 14.9 Hz, H-5a), 1.80 (1H, d, *J* = 14.9 Hz, H-5b), 1.72 (1H, m, H-14a), 1.56 (1H, m, H-14b), 1.52 (2H, m, H-7), 1.41 (3H, s, H-23), 1.38–1.28 (10H, m, H-9 to H-13), 1.35–1.32 (2H, m, H-8), 1.23 (3H, s, H-24); δ_C 174.4 (C-1), 138.9 (C-19), 129.4 (C-16), 129.1 (C-20), 128.9 (C-21), 128.9 (C-22), 127.0 (C-17), 83.0 (C-4), 81.8 (C-3), 80.5 (C-18), 80.1 (C-6), 78.7 (C-15), 41.4 (C-7), 40.8 (C-5), 34.5 (C-2), 33.4 (C-14), 31.0–29.0 (C-9 to C-13), 25.4 (C-23), 23.4 (C-8), 22.5 (C-24); HR-ESIMS *m*/*z* 467.2404 $[M + Na]^+$ (calcd for $C_{26}H_{36}NaO_6$, 467.2404).

*Plakortoperoxide B (**6**):* colorless oil; $[\alpha]_D$ −51 (*c* 0.01, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 1 and Table 2; HR-ESIMS *m*/*z* 459.2719 $[M + Na]^+$ (calcd for $C_{25}H_{40}NaO_6$, 459.2717).

*Mixture of plakortoperoxide C (**7**) and plakortoperoxide D (**8**):* colorless oil; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 1 and Table 2; HR-ESIMS *m*/*z* 459.2719 $[M + Na]^+$ (calcd for $C_{25}H_{40}NaO_6$, 459.2717).

*Mixture of carboxyplakortolide-1 (**9**) and carboxyplakortolide-2 (**10**):* colorless oil; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 4; HR-ESIMS *m*/*z* 351.1773 $[M + Na]^+$ (calcd for $C_{17}H_{28}NaO_6$, 351.1778).

*Mixture of formylplakortolide-1 (**11**) and formylplakortolide-2 (**12**):* colorless oil; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 4; HR-ESIMS *m*/*z* 335.1818 $[M + Na]^+$ (calcd for $C_{17}H_{28}NaO_5$, 335.1829).

*Mixture of hydroxyplakortolide-1 (**13**) and hydroxyplakortolide-2 (**14**):* colorless oil; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 4; HR-ESIMS *m*/*z* 323.1829 $[M + Na]^+$ (calcd for $C_{16}H_{28}NaO_5$, 323.1829).

*Mixture of hydroperoxyplakortolide-1 (**15**) and hydroperoxyplakortolide-2 (**16**):* colorless oil; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 3 and Table 4; HR-ESIMS *m*/*z* 339.1784 $[M + Na]^+$ (calcd for $C_{16}H_{28}NaO_6$, 339.1778).

*Ester **17**:* colorless oil; $[\alpha]_D$ −4 (*c* 0.02, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Table 1 and Table 2; HR-ESIMS *m*/*z* 449.2653 $[M + Na]^+$ (calcd for $C_{27}H_{38}NaO_4$, 449.2662).

Reductive Cleavage of Plakortolide V (3). A mixture of plakortolide V (**3**) (0.8 mg, 2.1 μmol) and 10% Pd on charcoal in EtOAc (1 mL) was stirred under H_2 at room temperature for 16 h. The reaction mixture was filtered through a short plug of silica (0.8 g), eluting with 2 mL of $CHCl_3$, then dried under reduced pressure to obtain a synthetic sample of *seco*-plakortolide V (**21**) (0.6 mg, 75%).

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***seco*-Plakortolide V (21):** colorless oil; $[\alpha]_D$ +15 (*c* 0.05, $CHCl_3$); ^1H and ^{13}C NMR ($CDCl_3$, 500 MHz) δ_H 4.17 (1H, dd, *J* = 6.8, 1.8 Hz, H-3), 2.90 (1H, dd, *J* = 18.2, 6.8 Hz, H-2a), 2.53 (1H, dd, *J* = 18.2, 1.8 Hz, H-2b), 2.17 (1H, d, *J* = 15.0 Hz, H-5a), 2.08 (1H, d, *J* = 15.0 Hz, H-5b), 1.62 (1H, m, H-7a), 1.52 (1H, m, H-7b), 1.42 (3H, s, H-22), 1.34 (3H, s, H-23), 1.22−1.31 (26H, m, H-8 to H-20), (0.86 (3H, t, *J* = 6.8 Hz, H-21); δ_C 174.8 (C-1), 89.7 (C-4), 73.7 (C-3), 73.1 (C-6), 43.6 (C-5), 43.5 (C-7), 37.8 (C-2), 31.7 (C-19), 29.0−29.9 (C-9 to C-18), 29.9 (C-23), 26.6 (C-22), 24.2 (C-8), 22.5 (C-20), 13.9 (C-21); HR-ESIMS *m*/*z* 407.3134 (calcd for $C_{23}H_{44}NaO_4$, 407.3132).

Preparation of MPA Esters of *seco*-Plakortolide V (22a/22b). The sample of *seco*-plakortolide V (**21**) (0.6 mg) obtained from the catalytic (Pd/C) reduction of plakortolide V was divided in half, and each sample (approximately 0.3 mg) was treated with either (*R*)- or (*S*)-MPA (1 mg, 2 equiv), followed by DCC (1 mg, 2 equiv) and DMAP (0.6 mg, 2 equiv) in dry CH_2Cl_2 (0.5 mL). Each reaction was stirred overnight at room temperature before filtering through a small plug of silica (0.8 g) eluting with $CHCl_3$ (5 mL). The solvent was removed *in vacuo*, and the MPA esters were individually purified by RP-HPLC using an analytical column and eluting with CH_3CN/H_2O (9:1) to give the (*R*)-MPA ester (**22a**) (0.1 mg, 30%) and (*S*)-MPA ester (**22b**) (0.1 mg, 30%), respectively.

*(R)-MPA ester (**22a**):* colorless oil; ^1H NMR ($CDCl_3$, 500 MHz) δ_H 7.33−7.42 (5H, m, MPA phenyl protons), 5.19 (1H, dd, *J* = 6.4, 1.8 Hz, H-3), 4.79 (1H, s, C*H* of MPA), 3.39 (3H, s, OMe), 2.94 (1H, dd, *J* = 18.6, 6.4 Hz, H-2a), 2.25 (1H, dd, *J* = 18.6, 1.8 Hz, H-2b), 1.91 (1H, d, *J* = 15.0 Hz, H-5a), 1.76 (1H, d, *J* = 15.0 Hz, H-5b), 1.55 (3H, s, H-22), 1.20−1.32 (28H, m, H-7 to H-22), 1.13 (3H, s, H-23), 0.86 (3H, t, *J* = 7.1 Hz, H-21); HR-ESIMS *m*/*z* 555.3666 (calcd for $C_{32}H_{52}NaO_6$, 555.3656).

*(S)-MPA ester (**22b**):* colorless oil; ^1H NMR ($CDCl_3$, 500 MHz) δ_H 7.33−7.42 (5H, m, MPA phenyl protons), 5.19 (1H, dd, *J* = 6.3, 1.3 Hz, H-3), 4.74 (1H, s, C*H* of MPA), 3.36 (3H, s, OMe), 3.03 (1H, dd, *J* = 18.5, 6.3 Hz, H-2a), 2.48 (1H, dd, *J* = 18.5, 1.3 Hz, H-2b), 1.58 (1H, d, *J* = 15.0 Hz, H-5a), 1.48 (3H, s, H-22), 1.40 (1H, d, *J* = 15.0 Hz, H-5b), 1.20−1.30 (28H, m, H-7 to H-22), 0.86 (3H, t, *J* = 7.0 Hz, H-21), 0.83 (3H, s, H-23); HR-ESIMS *m*/*z* 555.3664 (calcd for $C_{32}H_{52}NaO_6$, 555.3656).

Biomimetic Synthesis of Plakortoperoxide A (5) and Comparison with Natural Material. A solution of plakortolide P^2 (**20**) (9.4 mg) in anhydrous DCM (2 mL) at 5 °C was irradiated with light from a 500 W tungsten-halogen lamp in the presence of rose bengal bis(triethylammonium) salt (0.1 equiv), and oxygen bubbled through the solution for 3 h. The solution was concentrated to dryness, and the residue purified by NP flash chromatography using $CHCl_3$ to give a synthetic sample of plakortoperoxide A (**5**) (6.3 mg, 62%), $[\alpha]_D$ −23 (*c* 0.09, $CHCl_3$), identical to natural material by ^1H NMR and MS data.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures **S1**−**S28**. ^1H and selected 2D NMR data for compounds **1**−**17** and ^1H NMR spectra for compounds **21** and **22a**/**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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