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Configurational Assignment of Cyclic Peroxy Metabolites Provides an Insight into Their Biosynthesis: Isolation of Plakortolides, *seco*-Plakortolides, and Plakortones from the Australian Marine Sponge *Plakinastrella clathrata*

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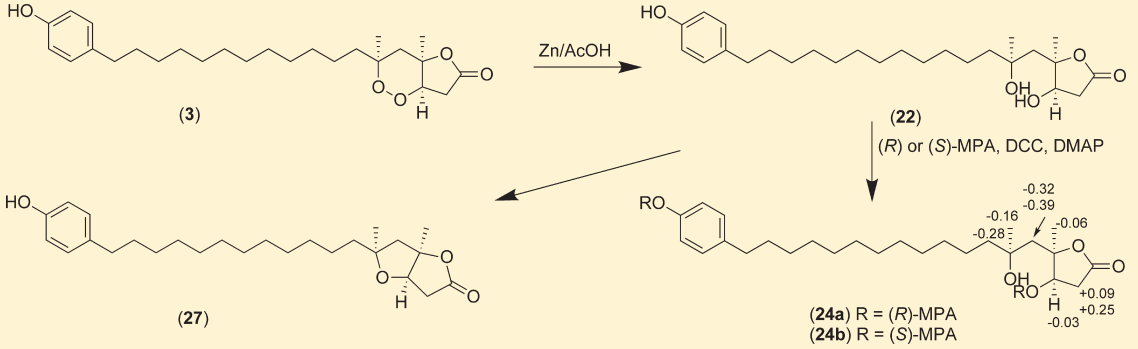
Supporting Information

ABSTRACT:



Sixteen new compounds, comprising nine new plakortolides K−S (**1**−**9**), four *seco*-plakortolides (**10**−**13**), and three plakortones (**14**−**16**), were isolated from the Australian sponge *Plakinastrella clathrata*. Structural elucidation, including relative configurational assignment, was based on extensive spectroscopic analysis, while the absolute configurations of **1**−**4** were deduced from ^{1}H NMR analyses on MPA esters derived from Zn/AcOH reduction products. Diastereomeric sets of plakortolides, e.g., K and L, or M and N, differed in configuration at C-3/C-4 rather than at C-6, a stereochemical result that compromises a biosynthetic pathway involving Diels−Alder cycloaddition of molecular oxygen to a $\Delta^{3,5}$-diunsaturated fatty acid precursor. The biosynthesis may plausibly involve cyclization of a 6-hydroperoxydienoic acid intermediate following stereospecific introduction of the hydroperoxy group into a polyketide-derived precursor. Isolated *seco*-plakortolides converted under mild conditions into plakortones with full retention of configuration, suggesting C-6 hydroxy attack on an α,β-unsaturated lactone intermediate. The NMR data reported for the compound named plakortolide E are inconsistent with the current literature structure and are those of the corresponding *seco*-plakortolide (**19**). The reported conversion of the metabolite into a plakortone ether on storage is consistent with this structural revision.

Cyclic peroxides are a distinctive suite of bioactive metabolites frequently encountered in marine sponges of the genera *Plakortis* and *Plakinastrella*. Individual metabolites can be categorized as belonging to the plakortolide, plakinic acid, plakoric acid, plakortone, or plakortide families.[1] In addition to pronounced cytotoxic, antifungal, and anti-inflammatory activity, recent screening programs have shown that members of these classes of metabolites possess potent antiparasitic activity and so may be promising drug leads against tropical diseases such as malaria, African sleeping sickness, or leishmaniasis.[1,2]

The plakortolide structure is characterized by an aromatic unit connected via a methylene chain to a bicyclic peroxy-lactone ring system. A metabolite with a plakortolide skeleton was briefly described in 1980,[3] but Davidson's 1991 report of the cytotoxic metabolite named plakortolide included the first detailed description of the assignment of relative configuration and used ROESY data and molecular modeling for this purpose.[4] The various plakortolides isolated since these two initial reports exhibit variation in alkyl chain length (generally C_8 or C_{10}, but some C_9 examples are found), methylation and/or unsaturation pattern, and terminal group (either phenyl or *p*-hydroxyphenyl).[4−11] In this paper we report six new plakortolides (**1**−**6**) possessing a C_{12} methylene chain, three C_{10} homologues (**7**−**9**), together with *seco* derivatives **10**−**13**, and the stereochemically related plakortones **14**−**16** from *Plakinastrella clathrata* Kirkpatrick,

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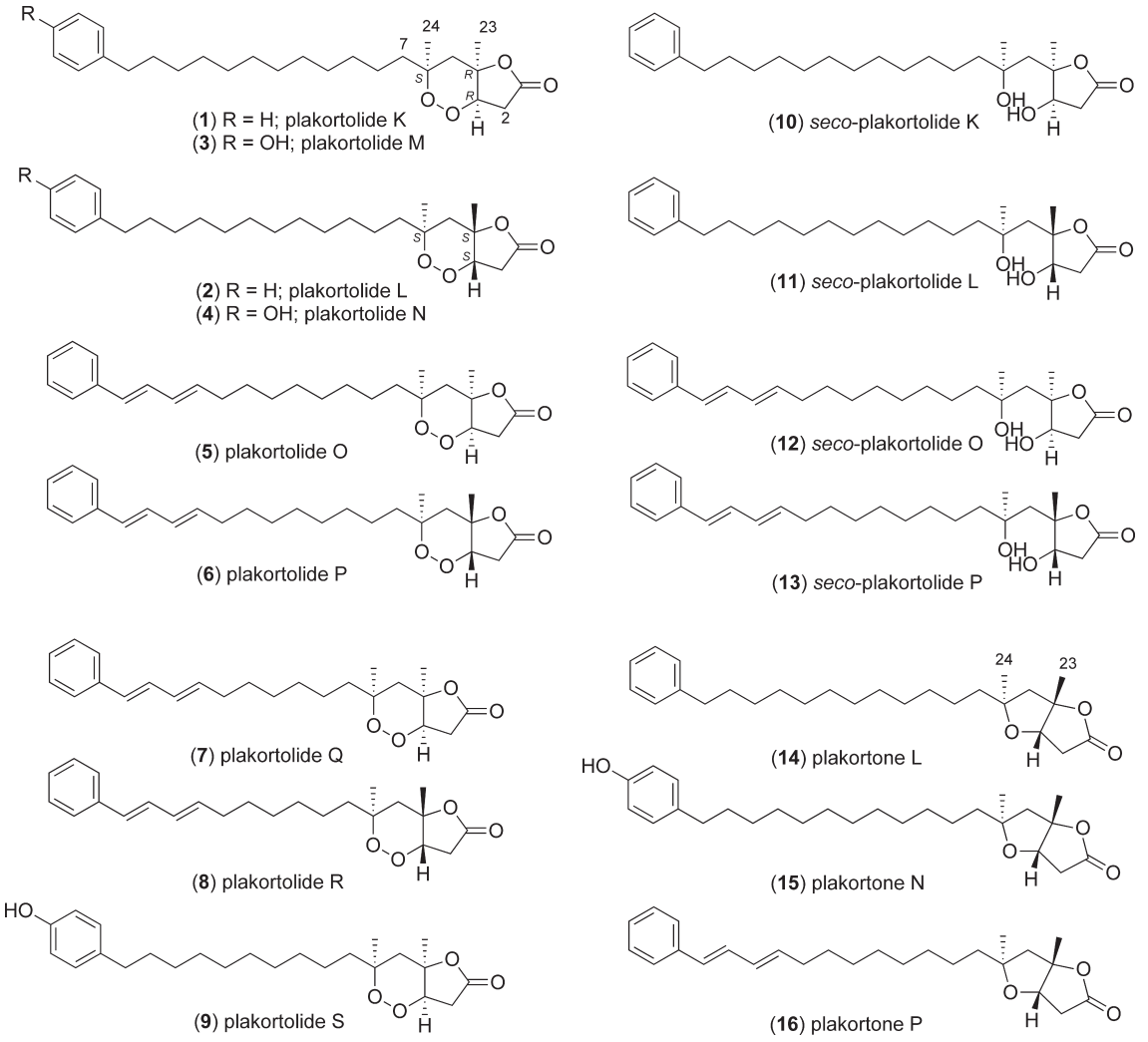
Chart 1



1900. Our study addresses the relative and absolute configurations of the suite of metabolites and reveals that in this particular sponge diastereomeric plakortolides such as **1** and **2** differ in configuration at the linked C-3/C-4 centers, rather than at C-6. These stereochemical results are considered in light of plausible biosynthetic pathways leading to the plakortolides. By comparison of our data with those of plakortolide E (**17**)[6] and its diastereomer (**18**),[7,12] we determine that the NMR data previously ascribed to plakortolide E (**17**) are in fact those of a *seco* analogue, **19**. We also find that *seco*-plakortolides convert easily into ether products possessing a plakortone skeleton.

RESULTS AND DISCUSSION

Structural and Stereochemical Studies. A single large specimen of *P. clathrata* Kirkpatrick, 1900 (Homoscleromorpha) was collected by scuba from the Gneerings Reef offshore from Mooloolaba in South East Queensland. The sponge was extracted with CH_2Cl_2/MeOH (1:1) to give an extract, which was fractionated by NP flash chromatography (CH_2Cl_2/EtOAc) followed by NP-HPLC (hexanes/EtOAc) or by RP-HPLC (CH_3CN/H_2O) to give nine new plakortolides (**1**−**9**), the related *seco* compounds (**10**−**13**), and plakortone ethers (**14**−**16**).

Plakortolides and seco-Plakortolides. Plakortolide K (**1**) was isolated as a colorless oil and had a formula of $C_{26}H_{40}O_4$ inferred from HRESIMS. The ^{1}H NMR and HSQC data (Tables 1 and 2) showed a phenyl ring (δ_H 7.25 (2H), 7.16 (2H), and 7.15 (1H); δ_C 142.8 (s), 127.8 (d), 128.2 (d), and 125.1 (d)), two methyl groups (δ_H 1.36 (s), 1.18 (s); δ_C 25.7, 24.4), one oxygenated methine (δ_H 4.46; δ_C 80.5), two methylenes with a diagnostic AB signal pattern (δ_H 2.88, 2.55; δ_C 33.7 and δ_H 2.25, 1.63; δ_C 40.0), one other identifiable methylene (δ_H 2.58; δ_C 35.6), and a cluster of overlapping methylene signals (δ_H 1.23−1.29; δ_C 29.0−29.2), suggesting the presence of a long alkyl chain in the structure. HMBC data were all fully consistent with the plakortolide skeleton.[4−11] Important correlations were from H-3 (δ_H 4.46) to a lactone carbonyl (C-1; δ_C 174.0), to C-2 (δ_C 33.7), to an oxygenated quaternary carbon (C-4; δ_C 82.3), and to Me-24 (δ_C 25.7); from the Me-23 signal at δ_H 1.36 to C-3 (δ_C 80.5) and C-5 (δ_C 40.0); from H-5 (δ_H 2.25 and 1.63) to Me-23 and Me-24; and from Me-24 (δ_H 1.18) to C-5 and to C-7 (δ_C 36.6). HMBC correlations from H-18 (δ_H 2.58) to an *ipso* aromatic carbon (C-19; δ_C 142.8), to C-20 (δ_C 128.2), and to some individual carbons of the long alkyl chain confirmed that the phenyl ring was attached to the plakortolide skeleton via an alkyl chain, the length of which was C_{12} by HRESIMS.

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Table 1. ^{1}H NMR Assignments for Plakortolides 1−9[a,b]

position	1	2	3	4	5	6	7	8	9
2a	2.88, dd (18.5, 5.9)	2.89, dd (18.6, 6.1)	2.89, dd (18.6, 6.0)	2.90, dd (18.6, 6.0)	2.88, dd (18.5, 6.0)	2.89, dd (18.6, 6.2)	2.88, dd (18.5, 6.0)	2.89, dd (18.6, 6.2)	2.89, dd (18.8, 6.0)
2b	2.55, br d (18.5)	2.60, br d (18.6)	2.55, br d (18.6)	2.50, br d (18.6)	2.54, br d (18.5)	2.60, br d (18.6)	2.54, br d (18.5)	2.60, br d (18.6)	2.55, br d (18.8)
3	4.46, d (5.9)	4.43, d (6.1)	4.46, d (6.0)	4.43, d (6.0)	4.46, d (6.0)	4.43, d (6.2)	4.46, d (6.0)	4.43, d (6.2)	4.48, d (6.0)
5a	2.25, d (15.0)	2.15, d (15.0)	2.26, d (15.0)	2.15, d (15.0)	2.25, d (15.0)	2.15, d (14.8)	2.26, d (15.0)	2.15, d (14.8)	2.25, d (15.0)
5b	1.63, d (15.0)	1.68, d (15.0)	1.64, d (15.0)	1.69, d (15.0)	1.63, d (15.0)	1.69, d (14.8)	1.64, d (15.0)	1.69, d (14.8)	1.64, d (15.0)
7	1.71, m	1.50−1.46, m	1.71, m	1.50−1.46, m	—[c,d]	1.48, m	1.53, m	1.52−1.48, m	1.53, m
	1.54, m		1.54, m						
8	1.25, m	1.27, m	1.25, m	1.25, m	1.23−1.26, m[c,d]	1.28, m	1.29, m	1.30, m	1.20−1.30, m[c,d]
9	1.23−1.29, m[c,d]	1.24−1.29, m[c,d]	1.22−1.27, m[c,d]	1.23−1.27, m[c,d]	1.23−1.26, m[c,d]	1.25−1.25, m[c,d]	1.23−1.25, m[c,d]	1.26−1.31, m[c,d]	1.20−1.30, m[c,d]
10	1.23−1.29, m[c,d]	1.24−1.29, m[c,d]	1.22−1.27, m[c,d]	1.23−1.27, m[c,d]	1.23−1.26, m[c,d]	1.25−1.25, m[c,d]e	1.23−1.25, m[c,d]	1.26−1.31, m[c,d]	1.20−1.30, m[c,d]
11	1.23−1.29, m[c,d]	1.24−1.29, m[c,d]	1.22−1.27, m[c,d]	1.23−1.27, m[c,d]e	1.23−1.26, m[c,d]	1.25−1.25, m[c,d]	1.23−1.25, m[c,d]	1.26−1.31, m[c,d]	1.20−1.30, m[c,d]
12	1.23−1.29, m[c,d]	1.24−1.29, m[c,d]	1.22−1.27, m[c,d]	1.23−1.27, m[c,d]	1.23−1.26, m[c,d]	1.25−1.25, m[c,d]	2.11, q, 7.0	2.12, q, 7.2	1.20−1.30, m[c,d]
13	1.23−1.29, m[c,d]	1.24−1.29, m[c,d]	1.22−1.27, m[c,d]	1.23−1.27, m[c,d]	1.23−1.26, m[c,d]	1.25−1.25, m[c,d]	5.81, dt (15.0, 7.0)	5.79, dt (15.1, 7.2)	1.20−1.30, m[c,d]
14	1.23−1.29, m[c,d]	1.24−1.29, m[c,d]	1.22−1.27, m[c,d]	1.23−1.27, m[c,d]	2.12, q (7.0)	2.12, q (7.0)	6.18, dd (15.0, 10.5)	6.18, dd (15.1, 10.5)	1.20−1.30, m[c,d]
15	1.23−1.29, m[c,d]	1.24−1.29, m[c,d]	1.22−1.27, m[c,d]	1.23−1.27, m[c,d]	5.81, dt (15.0, 7.0)	5.80, dt (15.0, 7.0)	6.74, dd (15.5, 10.5)	6.73, dd (15.7, 10.5)	1.20−1.30, m[c,d]
16	1.23−1.29, m[c,d]	1.24−1.29, m[c,d]	1.22−1.27, m[c,d]	1.23−1.27, m[c,d]	6.18, dd (15.0, 10.5)	6.18, dd (15.0, 10.5)	6.43, d (15.5)	6.42, d (15.7)	2.51, t (7.5)
17	1.59, m	1.59, m	1.54, m	1.54, m	6.73, dd (15.5, 10.5)	6.73, dd (15.7, 10.5)	7.35, d (7.3)	7.35, d (7.2)	7.01, d (8.5)
18	2.58, t (7.8)	2.57, t (7.6)	2.50, t (7.8)	2.50, t (7.8)	6.42, d (15.5)	6.42, d (15.7)	7.22, t (7.7)	7.29, t (7.7)	6.72, d (8.5)
20	7.16, m	7.16, m	7.02, d (8.5)	7.01, d (8.5)	7.35, d (7.5)	7.35, d (7.3)	7.17, t (7.3)	7.17, t (7.3)	
21	7.25, m	7.25, m	6.72, d (8.5)	6.72, d (8.5)	7.27, t (8.0)	7.28, t (7.7)	1.36, s	1.37, s	1.36, s
22	7.15, m	7.15, m			7.16, t (7.5)	7.17, t (7.3)	1.17, s	1.27, s	1.18, s
23	1.36, s	1.37, s	1.36, s	1.37, s	1.35, s	1.37, s			
24	1.18, s	1.27, s	1.18, s	1.26, s	1.18, s	1.27, s			
22-OH			4.74, br s	4.75, br s					

[a] Chemical shifts (ppm) referenced to $CHCl_3$ (δ_C 7.24). [b] At 500 MHz. [c] Unresolved chemical shifts due to overlapping signals. [d] Signal multiplicity unresolved due to overlapping signals.

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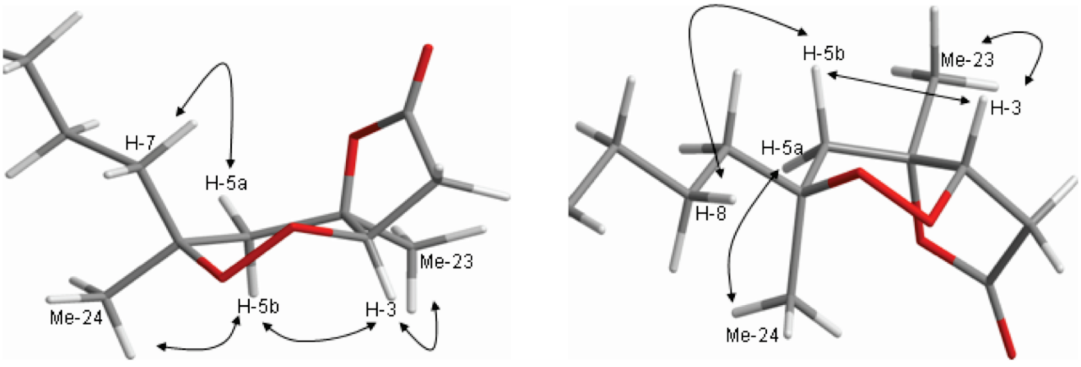
Table 2. ^{13}C NMR Assignments for Plakortolides 1−4 and 6−8[a,b]

position	1	2	3	4	6	7	8
1	174.0	174.1	174.1	174.1	174.1	174.1	174.1
2	33.7	34.2	33.9	34.0	34.2	33.9	34.1
3	80.5	81.0	80.7	80.8	81.0	80.6	80.9
4	82.3	82.5	82.4	82.6	82.6	82.3	82.5
5	40.0	40.4	40.1	40.3	40.5	40.1	40.3
6	80.0	79.9	80.0	80.0	79.9	80.0	79.9
7	36.6	40.8	36.8	40.8	40.7	36.6	40.7
8	23.3	22.8	23.5	22.6	22.8	23.4	22.7
9	29.0−29.2[c]	29.3−29.4[c]	29.1−29.4[c]	29.0−29.2[c]	29.0−29.4[c]	29.0−29.5[c]	28.8−29.6[c]
10	29.0−29.2[c]	29.3−29.4[c]	29.1−29.4[c]	29.0−29.2[c]	29.0−29.4[c]	29.0−29.5[c]	28.8−29.6[c]
11	29.0−29.2[c]	29.3−29.4[c]	29.1−29.4[c]	29.0−29.2[c]	29.0−29.4[c]	29.0−29.5[c]	28.8−29.6[c]
12	29.0−29.2[c]	29.3−29.4[c]	29.1−29.4[c]	29.0−29.2[c]	29.0−29.4[c]	32.6	32.5
13	29.0−29.2[c]	29.3−29.4[c]	29.1−29.4[c]	29.0−29.2[c]	29.0−29.4[c]	135.8	135.5
14	29.0−29.2[c]	29.3−29.4[c]	29.1−29.4[c]	29.0−29.2[c]	32.7	130.3	130.4
15	29.0−29.2[c]	29.3−29.4[c]	29.1−29.4[c]	29.0−29.2[c]	135.8	129.4	129.2
16	29.0−29.2[c]	29.3−29.4[c]	29.1−29.4[c]	29.0−29.2[c]	130.4	129.7	129.8
17	31.3	31.3	31.4	31.5	129.3	137.6	137.5
18	35.6	35.9	34.8	34.6	129.8	125.9	125.9
19	142.8	142.7	135.0	135.0	137.4	128.3	128.3
20	128.2	128.3	129.3	129.2	125.9	126.8	126.9
21	127.8	128.0	114.9	114.7	128.3	25.6	25.7
22	125.1	125.3	153.5	153.3	126.9	24.6	22.2
23	25.7	25.8	25.8	25.6	25.8		
24	24.4	22.2	24.6	22.0	22.2		

[a] Chemical shifts (ppm) taken from 2D spectra referenced to $CDCl_3$ (δ_C 77.0). [b] At 500 MHz. [c] Unresolved chemical shifts taken from HSQC experiments.



1 2

Figure 1. Key NOESY correlations for the bicyclic cores of plakortolides K (**1**) and L (**2**).

Plakortolide L (**2**) eluted earlier than **1** from NP-HPLC, but had the same molecular formula based on HRESIMS measurement. HMBC and HSQC data revealed that compound **2** had a plakortolide skeleton, but a comparison of chemical shifts for the bicyclic core revealed subtle differences in the chemical shifts of C-7 and of the C-24 methyl group (C-7, δ_C 36.6 for **1** vs δ_C 40.8 for **2**; C-24, δ_H 1.18; δ_C 24.4 for **1** vs δ_H 1.27; δ_C 22.2 for **2**) (Tables 1 and 2) that suggested that plakortolides K and L differed in configuration at C-6. Alternatively, they differed in configuration at the C-3/C-4 positions, as had been reported earlier for plakortolide E and its diastereomer by Mosher analysis.[6,7] In NOESY experiments (Figure 1), both compounds **1** and **2** showed a strong dipole coupling between H-3 and H_3-23, confirming the *cis* junction between the lactone and peroxide rings that is required on stereochemical grounds, and also between H-3 and H-5b. In **1**, both H_3-23 and H_3-24 showed a strong NOE correlation to H-5b (δ_H 1.63), while H-5a at δ_H 2.25 showed an NOE to H-7b and to other protons in the side chain. In his stereochemical analysis of plakortolide, Davidson[4] noted that the downfield H-5a signal showed an NOE to the side chain proton H-7b, thereby placing the side chain on the opposite face of both methyls. The C-24 chemical shift data of **1** matched values observed in plakortolide (C-24 δ_H 1.22; δ_C 24.93).[4] In contrast in **2**, H_3-23 and H_3-24 showed strong NOE

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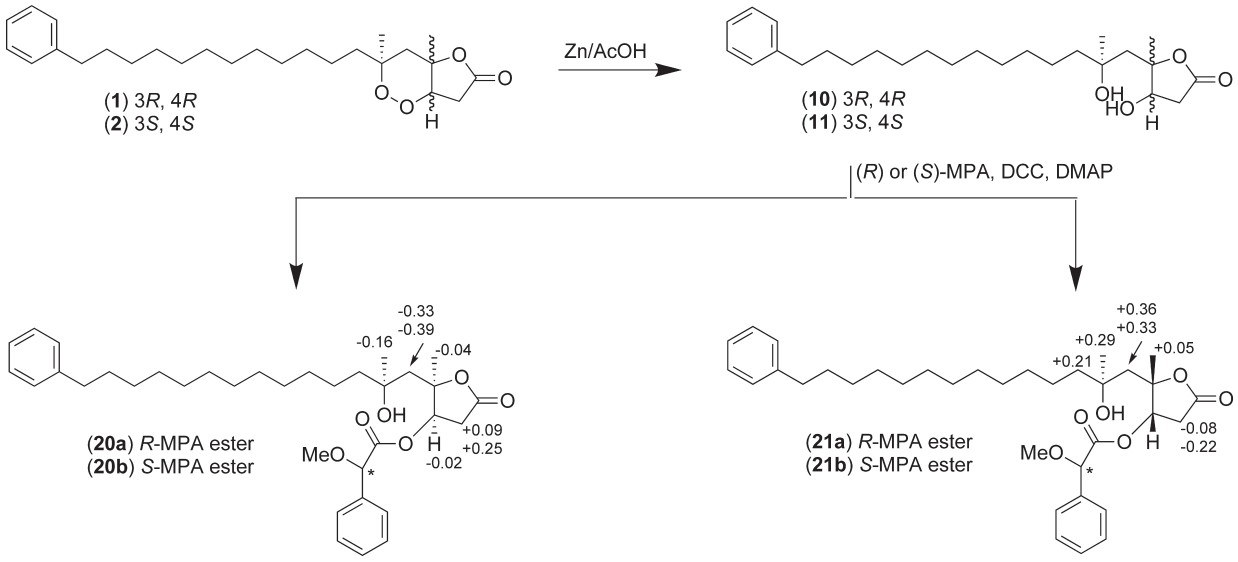

Figure 2. Mosher ester analysis of plakortolides K (**1**) and L (**2**).

correlations to H-5a at δ_H 1.68 and to H-5a at δ_H 2.15, respectively, as does the recently reported plakortolide J. The C-7/C-24 chemical shift data of **2** matched values observed in plakortolide J (C-7 δ_C 41.0; C-24 δ_H 1.28; δ_C 22.4).[11] Thus **1** has the same relative configuration as the original plakortolide, and **2** was either a C-6 epimer of **1** or the C-3/C-4 diastereomer. The C-3 configurations of **1** and **2** were determined by reductive cleavage of the peroxy ring using Zn/AcOH to afford samples of diols **10** and **11**, which were esterified at C-3 to their (*R*)- and (*S*)-*O*-methyl mandelate (MPA) esters **20a**/**20b** and **21a**/**21b**, respectively.[2a,2f,6,7] For the MPA esters **20a**/**20b** from diol **10**, the $\Delta\delta^{RS}$ values (where $\Delta\delta^{RS} = \delta R - \delta S$) were positive for H_2-2 and negative for H-3, H_2-5, H_3-23, and H_3-24, consistent with a 3*R* configuration (Figure 2); in contrast, for the MPA esters **21a**/**21b** from diol **11**, the $\Delta\delta^{RS}$ values were negative for H_2-2 and positive for H_2-5, H_2-7, H_3-23, and H_3-24, consistent with a 3*S* configuration. Together with the relative configurational information, these results established **1** as (3*R*, 4*R*, 6*S*) and **2** as (3*S*, 4*S*, 6*S*). Thus the two stereoisomers differed in their configuration at C-3 and C-4 rather than at C-6, a result that has considerable biosynthetic importance (*vide infra*).

Plakortolides K and L, both with a C_{12} methylene chain, represent homologues of previously published plakortolides that contain a C_{10} methylene chain. The absolute configuration of plakortolide E (**17**), isolated from a Fijian specimen of *Plakortis*, was deduced as (3*R*, 4*R*, 6*R*) by Crews et al. using Mosher ester analysis.[6] A plakortolide metabolite (**18**) from a Philippine sponge *Plakinastrella* sp., with structure and absolute configuration assigned as (3*S*, 4*S*, 6*R*) by Faulkner et al.,[7] was recently subjected to total synthesis by Jung et al. using a biomimetic strategy.[12] These authors named the metabolite as 6-epiplakortolide E even though it possesses the same 6*R* configuration as plakortolide E.[12] Plakortolide K (**1**) has an $[\alpha]_D$ of +8.8, equal and opposite to the literature $[\alpha]_D$ value of −8 reported for **18** by Faulkner et al.,[7] while the NMR data for the core regions matched in all respects. The $[\alpha]_D$ and Mosher results confirm that these two plakortolides have the same relative configuration but are *opposite* in absolute configuration. In contrast, when we compared the data for plakortolide L (**2**) to that of the supposedly homologous structure **17** named plakortolide E,[6] differences in the NMR data for the bicyclic core were immediately apparent, in particular for C-3, C-6, and the C-6 Me group (**2**: C-3, δ_H 4.43, d; δ_C 81.0; C-6 δ_C 79.9; and C-24 δ_H 1.27; δ_C 22.2; for "plakortolide E": C-3: δ_H 4.19, d; δ_C 73.7; C-6 δ_C 72.9; and (using our numbering scheme) C-24 δ_H 1.35; δ_C 29.9). The published NMR data provided by Crews et al.[6] for the Fijian "plakortolide E" metabolite did not match the actual structure **17** that they had reported. Instead, following comparison with NMR data for synthetic diols **10** and **11**, it was apparent that the NMR data were in fact for diol **19**, equivalent to **11** in all respects except for alkyl chain length and absolute configuration.

Serendipitously, a polar fraction from the *P. clathrata* extract showed evidence of ^{1}H NMR signals that were reminiscent of those reported for the Fijian "plakortolide E" sample.[6] Following RP-HPLC purification, the ^{1}H NMR and HSQC data (Tables 3 and 4) for the isolated metabolite showed a phenyl ring, one oxygenated methine proton (δ_H 4.17; δ_C 73.8), two distinctive AB systems (δ_H 2.90, 2.52; δ_C 38.1 and δ_H 2.16, 2.07; δ_C 43.9), two methyl singlets (δ_H 1.42, 1.34; δ_C 26.9, 30.2), and a cluster of overlapping methylene signals for a C_{12} alkyl chain. The HRESIMS data revealed a formula of $C_{26}H_{42}O_4$ that supported a reduced plakortolide structure, and when the HMBC evidence revealed a lactone (δ_C 175.0), the *seco*-plakortolide L (diol) structure **11** was deduced. The ^{1}H NMR data for the sponge-derived *seco*-plakortolide L were identical to those obtained for the sample prepared by Zn/AcOH reduction of **2**. Analogously, the sponge extract also yielded a small sample of *seco*-plakortolide K (**10**), whose ^{1}H NMR data again matched those of the synthetic sample prepared from plakortolide K (**1**). Owing to the small quantities available following purification by RP-HPLC, optical rotation data were not obtained for the sponge-derived samples of diols **10** and **11**. Indeed, during measurements on the samples of *seco*-plakortolides **10** and **11** synthesized from **1** and **2**, we had noted that these two compounds did not give reliable optical rotation readings. The difficulty in obtaining data was not related to any chemical instability of the diols since recovery of samples after $[\alpha]_D$ measurements led only to *seco*-plakortolide products. This is despite the seemingly facile conversion of *seco*-plakortolides to plakortone ethers, as is explained further below.

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Chart 2

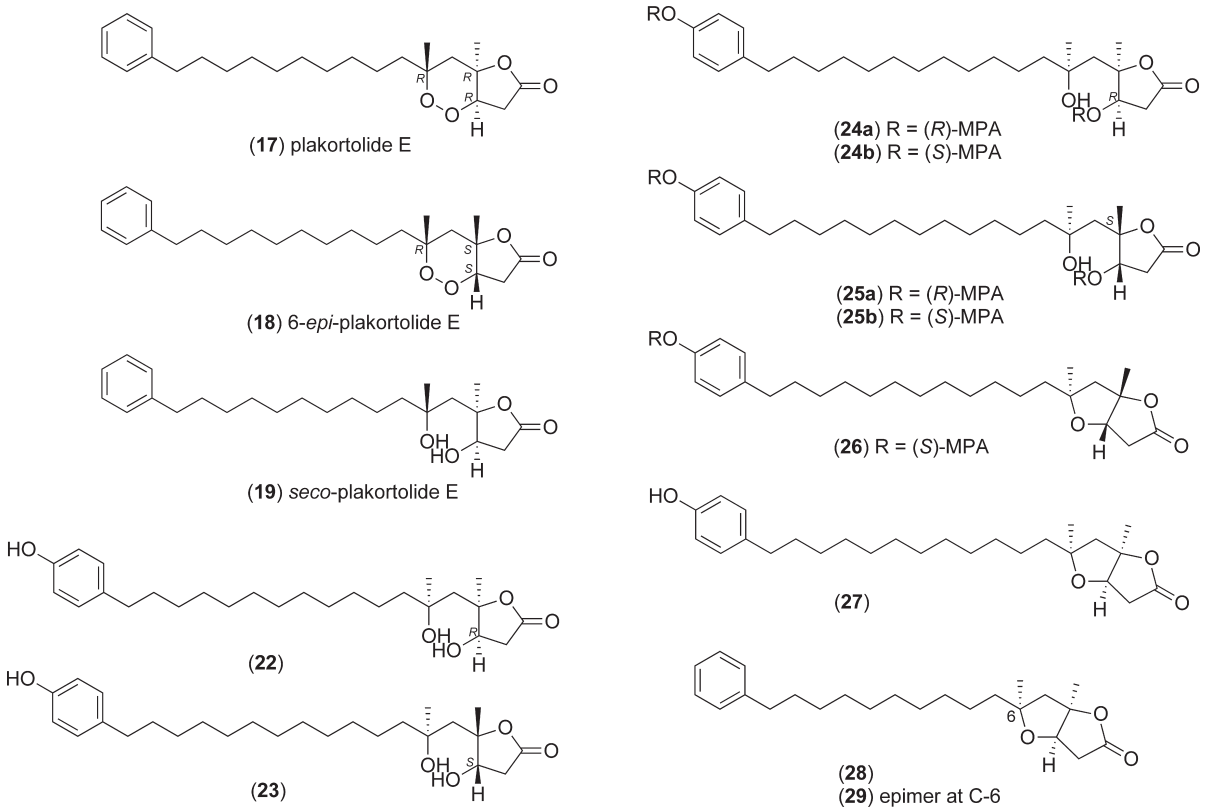

Table 3. ^{1}H NMR Assignments for Sponge-Derived *seco*-Plakortolides 10−13 and Synthetic Diols 22 and 23[a]

position	10[b]	11[b]	12[b]	13[b]	22[c]	23[c]
2a	2.88, dd (18.2, 6.2)	2.90, dd (18.0, 6.5)	2.88, dd (18.0, 6.4)	2.90, dd (18.0, 6.4)	2.89, dd (18.5, 6.0)	2.91, dd (18.5, 6.5)
2b	2.50, dd (18.2, 1.0)	2.52, dd (18.0, 2.0)	2.50, d (18.0)	2.52, d (18.0)	2.51, d (18.5)	2.53, dd (18.5, 1.5)
3	4.21, dd (6.2, 1.0)	4.17, dd (6.5, 2.0)	4.21, d (6.4)	4.17, d (6.4)	4.22, d (6.0)	4.17, dd (6.5, 1.5)
5a	2.36, d (14.9)	2.16, d (15.0)	2.36, d (15.0)	2.15, d (15.0)	2.37, d (14.5)	2.15, d (15.0)
5b	1.86, d (14.9)	2.07, d (15.0)	1.86, d (15.0)	2.08, d (15.0)	1.86, d (14.5)	2.09, d (15.0)
7	1.57−1.51, m	1.62−1.51, m	1.55−1.50, m	1.55−1.50, m	1.54, m	1.52−1.61, m
8	1.28, m	1.28, m	1.28, m	1.28, m	1.28, m	1.28, m
9	1.23−1.29, m[d,e]	1.24−1.29, m[d,e]	1.23−1.30, m[d,e]	1.23−1.30, m[d,e]	1.24−1.28, m[d,e]	1.24−1.28, m[d,e]
10	1.23−1.29, m[d,e]	1.24−1.29, m[d,e]	1.23−1.30, m[d,e]	1.23−1.30, m[d,e]	1.24−1.28, m[d,e]	1.24−1.28, m[d,e]
11	1.23−1.29, m[d,e]	1.24−1.29, m[d,e]	1.23−1.29, m[d,e]	1.23−1.30, m[d,e]	1.24−1.28, m[d,e]	1.24−1.28, m[d,e]
12	1.23−1.29, m[d,e]	1.24−1.29, m[d,e]	1.23−1.30, m[d,e]	1.23−1.30, m[d,e,e]	1.24−1.28, m[d,e]	1.24−1.28, m[d,e]
13	1.23−1.29, m[d,e]	1.24−1.29, m[d,e]	1.23−1.30, m[d,e]	1.23−1.30, m[d,e]	1.24−1.28, m[d,e]	1.24−1.28, m[d,e]
14	1.23−1.29, m[d,e]	1.24−1.29, m[d,e]	2.12, q (7.0)	2.12, q (7.0)	1.24−1.28, m[d,e]	1.24−1.28, m[d,e]
15	1.23−1.29, m[d,e]	1.24−1.29, m[d,e]	5.81, dt (15.0, 7.0)	5.81, dt (15.0, 7.0)	1.24−1.28, m[d,e]	1.24−1.28, m[d,e]
16	1.23−1.29, m[d,e]	1.24−1.29, m[d,e]	6.18, dd (15.0, 10.0)	6.18, dd (15.0, 10.0)	1.24−1.28, m[d,e]	1.24−1.28, m[d,e]
17	1.59, m	1.58, m	6.73, dd (15.8, 10.0)	6.73, dd (15.8, 10.0)	1.55, m	1.55, m
18	2.58, t (7.8)	2.58, t (7.8)	6.42, d (15.8)	6.42, d (15.8)	2.51, t (7.8)	2.51, t (7.8)
20	7.16, m	7.16, m	7.35, d (7.5)	7.35, d (7.5)	7.01, d (8.5)	7.01, d (8.5)
21	7.25, m	7.25, m	7.27, t (8.0)	7.27, t (8.0)	6.72, d (8.5)	6.72, d (8.5)
22	7.15, m	7.15, m	7.17, t (7.5)	7.17, t (7.5)		
23	1.45, s	1.42, s	1.45, s	1.42, s	1.46, s	1.42, s
24	1.34, s	1.33, s	1.34, s	1.34, s	1.35, s	1.34, s
22-OH					4.80, br s	4.80, br s

[a] Chemical shifts (ppm) referenced to $CHCl_3$ (δ_C 7.24). [b] At 750 MHz. [c] At 500 MHz. [d] Unresolved chemical shifts due to overlapping signals. [e] Signal multiplicity unresolved due to overlapping signals.

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Table 4. ^{13}C NMR Assignments for Sponge-Derived *seco*-Plakortolides 10−13 and Synthetic Diols 22 and 23[a]

position	10[b]	11[b]	12[b]	13[b]	22[c]	23[c]
1	174.6	175.0	174.6	174.7	174.9	175.1
2	37.6	38.1	37.6	37.9	37.5	37.9
3	73.3	73.8	73.4	73.8	73.3	73.7
4	90.0	89.7	90.1	89.7	90.3	89.8
5	43.9	43.9	43.9	43.7	43.8	43.6
6	72.5	73.1	72.6	73.1	72.6	73.1
7	46.4	43.7	46.5	43.6	46.4	43.5
8	22.5	24.3	—[d]	—[d]	22.8	24.1
9	29.0−29.9[e]	29.0−29.9[e]	29.0−29.9[e]	29.0−29.9[e]	29.1−29.2[e]	29.3−29.6[e]
10	29.0−29.9[e]	29.0−29.9[e]	29.0−29.9[e]	29.0−29.9[e]	29.1−29.2[e]	29.3−29.6[e]
11	29.0−29.9[e]	29.0−29.9[e]	29.0−29.9[e]	29.0−29.9[e]	29.1−29.2[e]	29.3−29.6[e]
12	29.0−29.9[e]	29.0−29.9[e]	29.0−29.9[e]	29.0−29.9[e]	29.1−29.2[e]	29.3−29.6[e]
13	29.0−29.9[e]	29.0−29.9[e]	29.0−29.9[e]	29.0−29.9[e]	29.1−29.2[e]	29.3−29.6[e]
14	29.0−29.9[e]	29.0−29.9[e]	32.7	32.7	29.1−29.2[e]	29.3−29.6[e]
15	29.0−29.9[e]	29.0−29.e[e]	135.7	135.7	29.1−29.2[e]	29.3−29.6[e]
16	29.0−29.9[e]	29.0−29.9[e]	130.4	130.4	29.1−29.2[e]	29.3−29.6[e]
17	31.3	31.4	129.3	129.3	31.3	31.4
18	35.8	36.1	129.8	129.8	34.7	34.7
19	142.6	142.9	137.4	137.4	134.9	135.0
20	128.2	128.3	125.9	125.9	129.2	129.3
21	128.0	128.2	128.3	128.3	114.8	114.9
22	125.4	125.5	126.9	126.9	153.4	153.4
23	25.9	26.9	26.0	26.7	25.9	26.7
24	28.2	30.2	28.3	30.0	28.2	29.8

[a] Chemical shifts (ppm) taken from 2D spectra referenced to $CDCl_3$ (δ_C 77.0). [b] At 750 MHz. [c] At 500 MHz. [d] Not detected. [e] Unresolved chemical shifts taken from HSQC experiments.

Seven other plakortolide metabolites were isolated from the *Plakinastrella* specimen. The diastereomeric plakortolides M and N, **3** and **4**, were isolated as colorless oils by NP-HPLC, and each had a molecular formula of $C_{26}H_{40}O_5$ inferred from HRESIMS. Both **3** and **4** shared a *p*-disubstituted aromatic ring (e.g., δ_H 7.02 (2H), and 6.72 (2H); δ_C 153.3, 135.0, 129.3, and 114.9 for **3**), consistent with a *p*-hydroxyphenyl end group, but in all other respects the data supported a plakortolide skeleton. The relative configurations of **3** and **4** followed by comparison of the chemical shift values for C-7 and for C-24 with those of **1** and **2**; C-7 was δ_C 36.8 for **3** vs δ_C 40.8 for **4**, while the C-24 values were δ_H 1.18; δ_C 24.6 for **3** vs δ_H 1.26; δ_C 22.0 for **4**. Compound **3** showed an NOE between H-5a and H_2-7 that was diagnostic of the same relative configuration as **1**. As with **1** and **2**, the stereoisomer **3** with *syn* arrangement of methyl groups eluted after its diastereomer **4** during NP-HPLC, but eluted first under RP-HPLC conditions. Confirmation of absolute configuration was obtained with Zn/AcOH reduction to diols **22** and **23** followed by MPA ester formation, providing diesters **24a**/**24b** from **22** and diesters **25a**/**25b** from **23**. From the $\Delta\delta^{RS}$ values of **24a**/**24b**, which were positive for H-2 and negative for H-3, H_2-5, H_2-7, H_3-23, and H_3-24, compound **3** had a 3*R* configuration. Analysis of esters **25a**/**25b** revealed that **4** had negative $\Delta\delta^{RS}$ values for H-2 and positive values for H-3, H_2-5, H_3-23, and H_3-24 and thus had a 3*S* configuration. Therefore **3** was (3*R*, 4*R*, 6*S*) and **4** was (3*S*, 4*S*, 6*S*). The $[\alpha]_D$ values for **3** and **4** were initially measured as −7.2 (*c* 0.37, $CHCl_3$) and −7.9 (*c* 0.19, $CHCl_3$), respectively. The unexpected closeness of the values led us to reisolate a second sample of each diastereomer from another portion of the extract; the $[\alpha]_D$ values for these sample of **3** and **4** were measured as −11 (*c* 0.08, $CHCl_3$) and −13 (*c* 0.05, $CHCl_3$), respectively, illustrating the experimental difficulties associated with $[\alpha]_D$ measurements for this group of compounds.

Four metabolites isolated from the *Plakinastrella* extract with a plakortolide-like core showed evidence of diene functionality by UV data; they were carefully purified by repetitive RP-HPLC. Two diastereomers, **5** and **6**, each with a C_{12} diene side chain from MS data and corresponding to a molecular formula of $C_{26}H_{36}O_4$, were named plakortolides O and P, respectively, while metabolites **7** and **8**, named plakortolides Q and R, both had a C_{10} diene side chain from MS data, corresponding to a molecular formula of $C_{24}H_{32}O_4$. ^{1}H NMR signals at δ_H 5.81 (dt, H-15), 6.18 (dd, H-16), 6.73 (dd, H-17), and 6.42 (dt, H-18) were observed for the diene side chain of **5**, and equivalent signals were observed in the ^{1}H NMR spectra of **6**−**8**. There was insufficient **5** for ^{13}C NMR characterization, but for **6** HMBC signals from the olefinic protons H-17 and H-18 to aromatic carbons at δ_C 137.4 (C-19) and δ_C 125.9 (C-20), from the H-20 signal at δ_H 7.35 to the olefinic carbon at δ_C 129.8 (C-18), from H-18 to δ_C 130.4 (C-16), and from H-17 to δ_C 135.8 (C-15) all secured the conjugated diene next to the aromatic ring. Equivalent HMBC correlations were seen for **7** and **8**. Isomers **5** and **7** showed ^{1}H NMR and ^{1}H/^{13}C NMR data, respectively, for the bicyclic core similar to **1** and **3**, while the data for **6** and **8** matched those of **2** and **4**. In **7**, there were NOE correlations from H_3-24 to H-5b and from H-5a to H_2-7, as had been found earlier for **1**. In contrast, **6** and **8** both showed equivalent NOE correlations to **2**, including from H_3-24 to H-5a. For each of **5**−**8**, the conjugated diene was assigned a (*E*,*E*)-configuration from coupling constant data. The samples of **5**−**8** were individually <0.5 mg, and each

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Table 5. ^{1}H NMR Assignments for Sponge-Derived Plakortones 14−16, and Synthetic Plakortone 27[a,b]

position	14	15	16	27
2a	2.72, dd (18.2, 4.7)	2.73, dd (18.2, 4.8)	2.72, dd (18.0, 4.8)	2.74, dd (15.1, 4.8)
2b	2.65, d (18.2)	2.66, d (18.2)	2.65, br d (18.0)	2.65, d (15.1, 2.0)
3	4.30, d (4.7)	4.30, d (4.8)	4.30, d (4.8)	4.33, d (4.8, 2.0)
5a	2.28, d (14.3)	2.27, d (14.3)	2.26, d (14.3)	2.39, d (14.3)
5b	1.97, d (14.3)	1.97, d (14.3)	1.97, d (14.3)	1.86, d (14.3)
7	1.48, m	1.49, m	1.49	1.53, m
8	1.26, m	1.27, m	1.28, m	1.25, m
9	1.24−1.29, m[c,d]	1.24−1.29, m[c,d]	1.24−1.32, m[c,d]	1.22−1.30, m[c,d]
10	1.24−1.29, m[c,d]	1.24−1.29, m[c,d]	1.24−1.32, m[c,d]	1.22−1.30, m[c,d]
11	1.24−1.29, m[c,d]	1.24−1.29, m[c,d]	1.24−1.32, m[c,d]	1.22−1.30, m[c,d]
12	1.24−1.29, m[c,d]	1.24−1.29, m[c,d]	1.24−1.32, m[c,d]	1.22−1.30, m[c,d]
13	1.24−1.29, m[c,d]	1.24−1.29, m[c,d]	1.24−1.32, m[c,d]	1.22−1.30, m[c,d]
14	1.24−1.29, m[c,d]	1.24−1.29, m[c,d]	2.12, q (6.5)	1.22−1.30, m[c,d]
15	1.24−1.29, m[c,d]	1.24−1.29, m[c,d]	5.80, dt (15.0, 7.0)	1.22−1.30, m[c,d]
16	1.24−1.29, m[c,d]	1.24−1.29, m[c,d]	6.18, dd (15.0, 11.0)	1.22−1.30, m[c,d]
17	1.59, m	1.55, m	6.73, dd (15.5, 11.0)	1.54, m
18	2.58, t (7.8)	2.50, t (7.6)	6.42, d (15.5)	2.49, t (7.9)
20	7.15, m	7.01, d (8.5)	7.35, d (7.5)	7.02, d (8.6)
21	7.25, m	6.72, d (8.5)	7.28, t (7.5)	6.73, d (8.6)
22	7.14, m		7.17, t (7.5)	
23	1.46, s	1.46, s	1.46, s	1.48, s
24	1.24, s	1.25, s	1.25, s	1.21, s
22-OH		4.60, br s		4.75, br s

[a] Chemical shifts (ppm) referenced to $CHCl_3$ (δ_C 7.24). [b] At 500 MHz. [c] Unresolved chemical shifts due to overlapping signals. [d] Signal multiplicity unresolved due to overlapping signals.

contained residual traces of diastereomer that could not be completely removed by RP-HPLC; consequently the $[\alpha]_D$ values reported for them are provisional. As well as **5**−**8**, the sponge extract yielded a mixture of the diene-functionalized *seco*-diols **12** and **13** with the molecular formula $C_{26}H_{38}NaO_4$ inferred by HRESIMS; the NMR data for the two individual components were easily discernible from analysis of 2D spectra and by comparison with the data for **10** and **11**. The ^{1}H NMR signals for H-3 at δ_H 4.21 and at 4.17, each a doublet with J = 6.4 Hz, pinpointed the (3*R*, 4*R*) and (3*S*, 4*S*) diastereomers, respectively.

The final plakortolide isolated was named plakortolide S (**9**) and from the molecular formula of $C_{24}H_{36}O_5$ inferred from HRESIMS had a C_{10} methylene side chain terminating in a *p*-hydroxyphenyl group. ^{1}H NMR signals for the *p*-hydroxyphenyl moiety were in agreement with those of **3** and **4**. On the basis of comparison of the ^{1}H NMR data, including 1D-NOE, for the bicyclic core with those of **1**, **3**, and **7**, plakortolide S possessed the same relative configuration. The absolute configuration shown was inferred on the reasonable expectation of a 6*S* configuration. There was insufficient sample to acquire the ^{13}C NMR data of **9**. Plakortolide S is a homologue of plakortolide M and also of the metabolite with a C_9 alkyl chain, named plakortolide F.[9,11]

Plakortones. Plakortones A−F are a series of biologically active ethyl-branched furanolactones isolated from Caribbean sponges.[14,15] In addition to plakortolides and diol metabolites, the Australian *P. clathrata* extract showed evidence of the presence of plakortone-like metabolites, but with methyl rather than ethyl substituents. Plakortone-like products were also obtained as byproducts of the Mosher esterification reactions. Plakortone L (**14**), so-named because it belongs to the same structural and stereochemical series as **2** and **11**, was first noted as a minor component of the sponge extract accompanying the various plakortolide metabolites and from which it was separated by RP-HPLC. The molecular formula of **14**, measured as $C_{26}H_{40}O_3$ by HRESIMS, revealed one oxygen less than for plakortolides **1** and **2**. The ^{1}H NMR and HSQC data (Tables 5 and 6) again showed a phenyl ring (δ_H 7.25 (2H), 7.15 (2H), and 7.14 (1H); δ_C 142.8 (s), 128.3 (d), 128.2 (d), and 125.5 (d)), two methyl groups (δ_H 1.46 (s), 1.24 (s); δ_C 23.1, 26.7), one oxygenated methine (δ_H 4.30; δ_C 81.8), two methylenes with a diagnostic AB signal pattern (δ_H 2.72, 2.65; δ_C 37.2 and δ_H 2.28, 1.97; δ_C 49.1), plus signals suggesting the methylene chain. Important HMBC correlations were from H-3 at δ_H 4.30 to C-2 at δ_C 37.2, to a lactone carbonyl (δ_C 175.2, C-1), and to C-4 at δ_C 94.5; from H_3-23 (δ_H 1.46) to the methine carbon (δ_C 81.8, C-3), to C-4, and to a methylene carbon (δ_C 49.1, C-5); and from H_3-24 (δ_H 1.24) to C-5, C-6 (δ_C 84.8), and C-7 (δ_C 41.8), all of which were consistent with a bicyclic plakortone core. The NMR data for **14** matched literature data for plakortones A−F,[14] apart from chemical shift variations consistent with the replacement of the ethyl substituents by methyl groups. In a recent synthetic study on plakortones C−F, Kitching et al. described the use of NOE data to distinguish between diastereomeric plakortones; they observed that in the diastereomer with a *cis* arrangement of the C-4 and C-6 ethyl groups one of the two H-5 protons shows an NOE to both ethyl groups, whereas if the ethyl groups are *trans*-configured, then each H-5 proton shows an NOE to a different ethyl group.[15] For **14**, irradiation of H-5a at δ_H 2.28 led to enhancement of H_3-24, while irradiation of H-5b at δ_H 1.97 led to enhancement of H_3-23, supporting the relative

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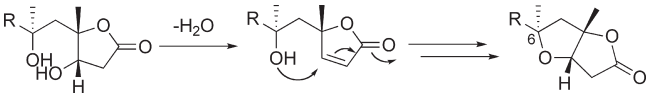
Table 6. ^{13}C NMR Assignments for Sponge-Derived Plakortones 14−16 and Synthetic Plakortone 27[a,b]

position	14	15	16	27
1	175.2	175.1	175.1	175.5
2	37.2	36.8	36.9	36.4
3	81.8	81.6	81.7	80.8
4	94.5	94.5	94.6	94.3
5	49.1	48.7	48.8	48.9
6	84.8	84.8	84.7	84.6
7	41.8	41.6	41.6	41.5
8	24.4	23.9	24.1	24.2
9	29.4−30.0[c]	29.4−30.0[c]	29.0−29.8[c]	28.5−30.0[c]
10	29.4−30.0[c]	29.4−30.0[c]	29.0−29.8[c]	28.5−30.0[c]
11	29.4−30.0[c]	29.4−30.0[c]	29.0−29.8[c]	28.5−30.0[c]
12	29.4−30.0[c]	29.4−30.0[c]	29.0−29.8[c]	28.5−30.0[c]
13	29.4−30.0[c]	29.4−30.0[c]	29.0−29.8[c]	28.5−30.0[c]
14	29.4−30.0[c]	29.4−30.0[c]	32.5	28.5−30.0[c]
15	29.4−30.0[c]	29.4−30.0[c]	135.8	28.5−30.0[c]
16	29.4−30.0[c]	29.4−30.0[c]	130.4	28.5−30.0[c]
17	31.5	31.2	129.2	31.2
18	36.0	34.6	129.8	34.8
19	142.8	134.9	137.4	135.0
20	128.3	129.1	126.0	129.3
21	128.2	114.7	128.3	115.1
22	125.5	153.2	126.9	153.7
23	23.1	22.7	22.8	23.4
24	26.7	26.4	26.5	25.8

[a] Chemical shifts (ppm) taken from 2D spectra referenced to $CDCl_3$ (δ_C 77.0). [b] At 500 MHz. [c] Unresolved chemical shifts taken from HSQC experiments.

Scheme 1. Conversion of *seco*-Plakortolides into Plakortones Involving Michael-Type Attack on a Butenolide Intermediate



configuration shown. The presence of traces of plakortone L (**14**) was also noticed (in particular by the distinctive signal for H-3 in the ^{1}H NMR) during the parallel esterification reactions of diol **11** with (*R*)- and (*S*)-MPA, but no attempt was made to isolate the ether product. The facile conversion of *seco*-plakortolides into ether products under mildly basic conditions suggests that an attractive mechanistic proposal for ether formation involves dehydration followed by C-6 hydroxy attack on the α,β-unsaturated lactone intermediate (Scheme 1).

Also isolated from the sponge extract was plakortone N (**15**), analogous to plakortolide N (**4**) and likely derived from the ring-opened diol **23** even though this was not found in the sponge extract. For **15**, the *trans* arrangement of the C-23 and C-24 methyls was confirmed by 1D-NOE studies involving the diastereomeric H-5 protons (as outlined above for **14**). During the Mosher chemistry on the synthetic sample of diol **23**, plakortone N (**15**) was isolated as a byproduct of reaction with both (*R*)- or (*S*)-MPA, as was the plakortone derivative **26** esterified at the *p*-hydroxyphenyl group during the (*S*)-MPA reaction. In a similar fashion, diol **22**, during reaction with (*R*)- or with (*S*)-MPA, also yielded **27** by HRESIMS data and whose ^{1}H NMR data closely resembled, but were not identical to, those of **15**. Irradiation of H-5b at δ_H 1.86 gave NOEs onto both H_3-23 (at δ_H 1.48) and H_3-24 (at δ_H 1.21), whereas there was no observable NOE onto either methyl group on irradiation of H-5a at δ_H 2.39. These NOE data were in accordance with a *cis* arrangement for the two methyl substituents in **27**,[15] as expected from the relative configuration of **22** (and its parent **3**). The sponge extract also yielded a diene-functionalized plakortone ether (**16**) with a C_{12} side chain from HRESIMS and whose ^{1}H and ^{13}C NMR data were entirely as expected by comparison with **14** and **15** (for the bicyclic core) and with **6**−**8** and **12**/**13** (for the diene-functionalized side chain). The relative configuration shown was confirmed by a 1D-NOE study as outlined earlier for **14**.

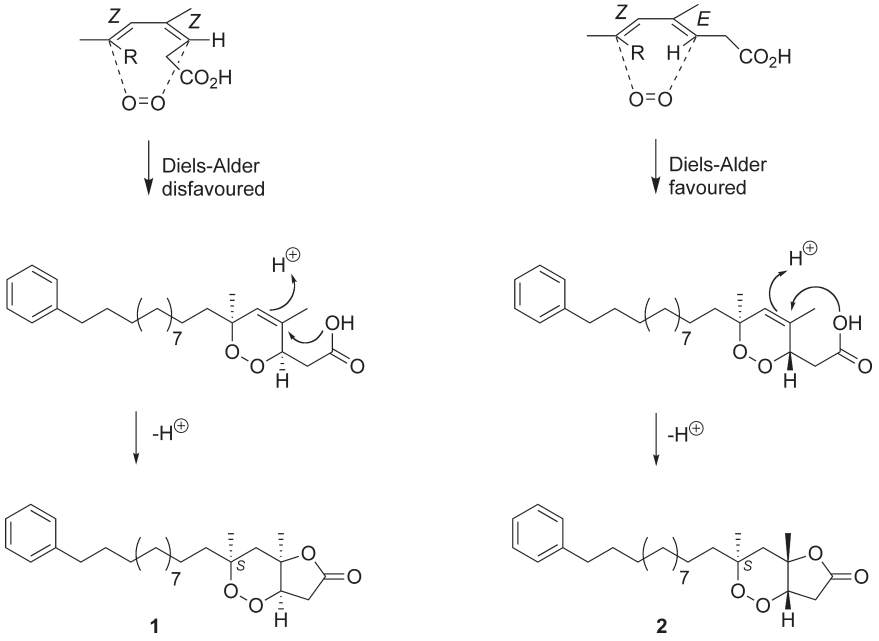
Because the synthetic samples of plakortones **14** and **15** were obtained from plakortolides **2** and **4**, they consequently have a (3*S*, 4*S*, 6*S*) configuration, whereas plakortone **27**, derived by synthetic modification of plakortolide **3**, has a (3*R*, 4*R*, 6*S*) configuration. In their recent work on plakortone synthesis, Kitching et al. proposed that the (furano)lactone configuration determines the sign of optical rotation; ethyl-substituted plakortones of known (3*S*, 4*S*, 6*S*) configuration all show a negative $[\alpha]_D$ value.[15] For **15**, the $[\alpha]_D$ values of the sponge-derived sample and the synthetic sample were measured as −49 (*c* 0.007, $CHCl_3$) and −23 (*c* 0.01, $CHCl_3$), respectively, with the difference in values likely stemming from the small samples available.

The isolation of plakortones from *P. clathrata* led us to consider further the chemistry of the metabolite named plakortolide E. In their paper, Crews et al.[6] reported EIMS data for plakortolide E, which revealed only three oxygen atoms. This was explained by the authors on the basis that organic peroxides can show a M^+-O fragmentation, as has been well documented in the peroxide literature.[13] They also characterized a rearrangement product containing three oxygens and assigned the plakortone structure **28**, which they suggested derived directly from plakortolide E after extended storage. However the ether rearrangement product could alternatively originate from diol **19**, the now-established structure of the "plakortolide E" sample in their sponge, except that (via the mechanism shown in Scheme 1) this should deliver the epimeric **29** rather than **28**. This stereochemical discrepancy remains to be explained. Although the ROESY data published by Crews et al. is somewhat ambiguous, comparison of the published ^{1}H NMR data for their compound with those of **14**−**16**, and **27** (Table 5) matched better for **28** rather than **29** (H-3: δ_H 4.35[6] vs δ_H 4.30 for **14** or δ_H 4.33 for **27**; H-5: δ_H 2.40 and 1.87[6] vs δ_H 2.28 and 1.97 for **14** or δ_H 2.39 and 1.86 for **27**); the ^{13}C NMR data (Table 6) did not readily distinguish the diastereomers.

Various plakortolides have been reported to have cytotoxic activity. The original plakortolide was reported as active,[4] as were plakortolides B, D, and E[5,6] (but note that the published data for plakortolide E may potentially refer to the *seco* product instead). Recent screening has revealed that plakortolide F possesses potent cytotoxic activity, but shows modest antimalarial and antitubercular activity.[11] Assessment of the biological activities associated with the suite of metabolites isolated in the current study has been complicated by the small amounts of metabolite available for screening.

Biosynthesis. Biosynthetic speculation on the formation of plakortolides, and related cyclic peroxides,[16] has centered on a

Scheme 2. Biosynthetic Pathway to the Plakortolides Involving Diels−Alder Cycloaddition of Molecular Oxygen



Diels−Alder cycloaddition of the singlet state of molecular oxygen to a $\Delta^{3,5}$-dienoic acid precursor, followed by lactonization of the cyclic peroxyacid products.[12] Indeed, a number of synthetic studies, including one on the structure named 6-epi-plakortolide E,[12] describe a singlet oxygen-mediated approach to cyclic peroxide products.[17] Although the elegance of the Diels−Alder proposal is undeniable, our stereochemical results are not in accordance with the well-established stereochemical outcomes of Diels−Alder processes. Determination of a 6*S* configuration in four different plakortolide metabolites suggests that this specific configuration may be conserved throughout the suite of metabolites produced by this particular sponge species. Scheme 2 shows a stereochemical proposal for the formation of **1** and **2** that involves a dienoic acid, or equivalent enzyme-bound precursor, and further depicts an attack of molecular oxygen at C-6 from the bottom face so as to generate a 6*S* configuration. As shown, cycloaddition to a (3*E*,5*Z*)-dienoic acid provides plakortolide L (**2**) with a *trans* arrangement of C-23 and C-24 Me groups. However, to generate the alternative plakortolide configuration, that is with a *cis* arrangement of Me groups as in **1**, and *at the same time* retain the 6*S* configuration, requires that (i) either the configuration of the dienoic acid precursor is changed to a (3*Z*,5*Z*)-configuration or (ii) molecular oxygen delivery is from the top face on a (3*E*,5*E*)-precursor.

Either mechanistic scenario seems unlikely. First, the *s-cis* conformation of a (3*Z*,5*Z*)-dienoic acid precursor is sterically congested and, therefore, disfavored as a substrate for a cycloaddition reaction, and second, the facial selectivity of the enzymatic Diels−Alder process seems likely to be retained within the same pathway in the same sponge species.

For the plakortones, despite their synthesis from diol precursors as outlined above, biosynthetic speculation has centered on their formation from open-chain hydroxyenoic acids by Michael-type addition followed by lactonization.[1] This leads us to consider a biosynthetic pathway (Scheme 3) for the diastereomeric plakortolides that first involves cyclization of the 6*S*-hydroperoxydienoic acid equivalent **30** and, thus, implies a stereospecific oxidation at the C-6 center; in contrast, the subsequent nucleophilic attack of the hydroperoxy group onto C-3 of the α,β-unsaturated carboxylic acid derivative is not stereospecific. Attack of the carboxy group onto the carbocation derived from protonation of the 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. There is some literature relevant to the suggested biosynthesis. Oxidative transformation of fatty acids is the first step in the formation of bioactive oxylipins involved in cellular signaling processes.[18] The process is initiated by lipoxygenases or cyclooxygenases that control the introduction of a hydroperoxy group via stereoselective hydrogen abstraction from a (1*Z*,4*Z*)-diene unit within the fatty acid chain, followed by introduction of oxygen to the opposite face; the fatty acid product has a (1*Z*,3*E*)-diene configuration. In some cases, the introduced peroxy group cyclizes onto an appropriately located double bond to generate an endoperoxide, e.g., in prostaglandin biosynthesis.[18] Related enzymatic transformations in unicellular algae provide polyunsaturated aldehydes implicated in the host chemical defense.[19] It may well be that a similar oxidative pathway operates on methyl-substituted fatty acid substrates such as those leading to the plakortolides. In Scheme 3, a (2*Z*,5*E*)-dienoic acid is suggested to convert to a (2*Z*,4*Z*)-intermediate; the double-bond transposition and isomerization is comparable to that in oxylipin biosynthesis. Other stereoisomers of the precursor dienoic acid could also reasonably participate in such a pathway, but the order of some of the steps may differ from those in Scheme 3, e.g., lactonization may precede endoperoxide formation.

Most pertinent however is the model of Ovenden and Capon that rationalizes the biosynthesis of norterpene cyclic peroxides, such as the sigmosceptrellins, and some related ketones and dienes. The key steps are first stereospecific hydroperoxidation at C-6 of an $\Delta^{2,5}$-dienoic acid followed by Michael-type attack on an α,β-unsaturated carboxylic acid.[20] Noteworthy, given the obvious mechanistic parallels between the Ovenden/Capon proposal and that shown in Scheme 3, are the differing biosynthetic

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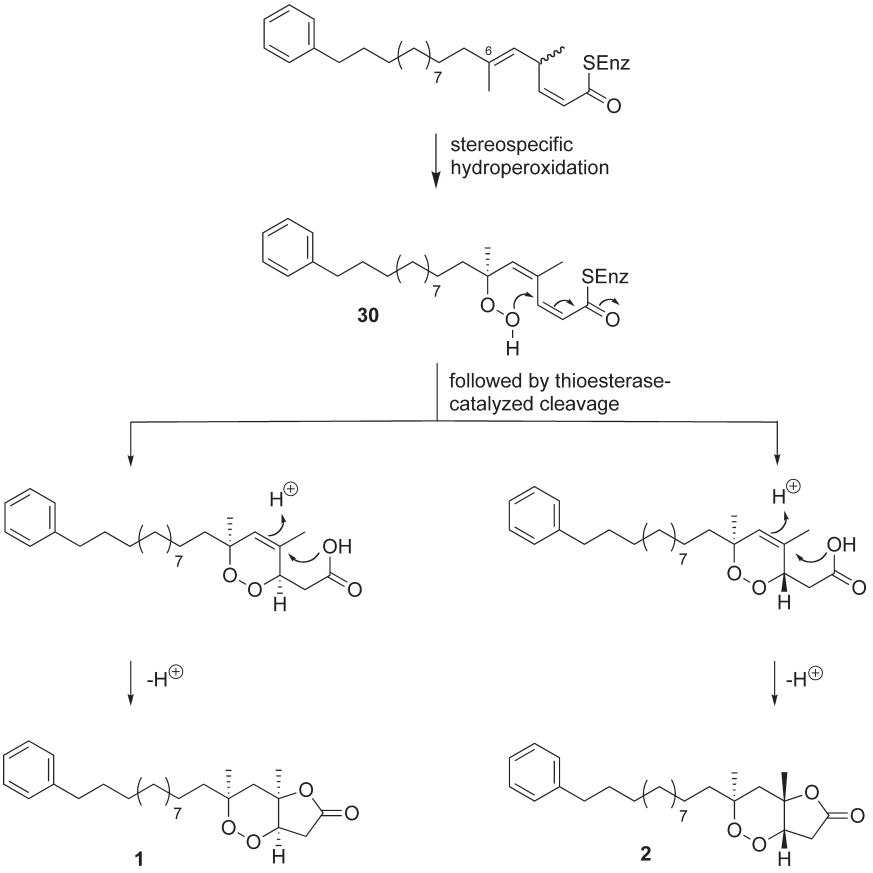
Scheme 3. Biosynthetic Pathway to the Plakortolides Involving Stereoselective Hydroperoxidation at C-6 Followed by Michael-Type Attack on an α,β-Unsaturated Carboxylic Acid Derivative



origins of the dienoic acid substrates. Likely, the sigmosceptrellins are of isoprenoid origin, whereas the plakortolides are derived via a polyketide pathway. We note that the polyketide biosynthetic pathway for plakortolides **1**−**9**, as well as other C_{10}-chain-linked plakortolides, involves phenylacetate, while plakortolide products with a C_9 alkyl chain[9] require a benzoate chain starter unit. The role that stereospecific oxidative transformations may play in the biosynthesis of bioactive marine peroxides of terpene, and now polyketide, origin clearly merits experimental study.

CONCLUSIONS

Sixteen new compounds including the nine plakortolides K−S (**1**−**9**), four *seco*-plakortolides (**10**−**13**), and three plakortones (**14**−**16**) were isolated from the Australian sponge *P. clathrata* Kirkpatrick, 1900 and characterized by extensive 2D NMR studies. Diastereomeric sets of plakortolides, e.g., K and L, or M and N, differed in configuration at C-3/C-4 rather than at C-6, which leads us to propose that the biosynthetic pathway may plausibly involve cyclization of 6-hydroperoxydienoic acid intermediates following stereospecific introduction of a C-6 hydroperoxy group into a polyketide-derived precursor. Various *seco*-plakortolides converted under mild conditions into plakortones with retention of configuration, indicating that their formation could involve dehydration followed by C-6 hydroxy attack on an α,β-unsaturated lactone product. We find that the NMR data reported for the compound named plakortolide E are inconsistent with the literature structure presented for it and are likely those of the corresponding *seco*-plakortolide (**19**). The reported conversion of "plakortolide E" into a plakortone ether on storage is fully consistent with this structural revision. The small magnitude of $[\alpha]_D$ values associated with many of the peroxy metabolites suggests that it would be speculative to assign either absolute or relative configuration on the basis of $[\alpha]_D$ values alone. Mosher ester analysis on reduced (diol) products is required to secure absolute configuration, while careful analysis of the ^1H and ^{13}C NMR values for C-7 and C-24, together with relevant NOE data, guide determination of relative configuration. On the basis of the current study, a number of literature plakortolide structures merit stereochemical reinvestigation.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained using a JASCO P-2000 polarimeter. UV data were acquired on a Perkin-Elmer Lambda 35 spectrometer. 1D and 2D NMR spectra were acquired using a Bruker DRX-500 or Bruker DMX-750 instrument. NMR spectra were obtained in $CDCl_3$ at room temperature and were internally referenced to $CHCl_3$ at δ_H 7.24 or $CDCl_3$ at δ_C 77.0. ESIMS were determined using a Bruker Esquire HCT 3D ion trap spectrometer (ESI mode). HRESIMS were recorded on a Bruker MicrOTof-Q spectrometer (Dionex UltiMate 3000 micro LC system, ESI mode). NP-HPLC was carried out using a Waters 515 pump with a Waters (10μ) μPorasil 7.8 × 300 mm semipreparative column and a Gilson 132 series RI detector with HPLC grade hexanes/EtOAc as solvent. RP-HPLC was

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carried out using an Agilent 1100 HPLC equipped with a 1100 series variable-wavelength UV detector and with a Phenomenex Gemini (5μ) C_{18} 10 × 250 mm semipreparative column or a Phenomenex Gemini (5μ) C_{18} 4.6 × 250 mm analytical column. HPLC grade CH_3CN/Milli Q H_2O was used as solvent.

Biological Material. The sponge *Plakinastrella clathrata* Kirkpatrick, 1900 (subclass: Homoscleromorpha; order: Homosclerophorida; family: Plakinidae) was collected from Coral Gardens dive site at the Inner Gneerings reef, a group of shoals near Mooloolaba (Australia), using scuba at a depth of 10−15 m on January 20, 2007. Samples were taken back to the laboratory on ice, where they were stored at −20 °C until extraction. The sponge sample was of a rubbery consistency and had a thin black outer surface. The internal color was initially chalk-colored but changed to a dull gray color on storage. A voucher specimen (QM G331502) is lodged at the Queensland Museum.

Extraction and Purification. The specimen of *P. clathrata* (wet weight 112 g) was diced and extracted exhaustively with CH_2Cl_2/MeOH (1:1) (3 × 200 mL). The extract was filtered through cotton wool and evaporated under reduced pressure to give an aqueous residue, which was partitioned sequentially with EtOAc (3 × 60 mL) and *n*-BuOH (60 mL). The EtOAc fraction was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a brown-colored extract (1.3 g), which was analyzed by TLC and ^{1}H NMR. A portion of the EtOAc extract was then 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 NP9, which eluted from 100% CH_2Cl_2 to CH_2Cl_2/EtOAc (5:1), was purified using semipreparative NP-HPLC with isocratic hexanes/EtOAc (3:1), yielding plakortolide L (**2**) (0.8 mg), plakortolide K (**1**) (2.4 mg), and plakortolide O (**5**) (0.45 mg) in order of elution. The next flash column fraction, NP10, which eluted from ∼CH_2Cl_2/EtOAc (3:1), was processed by semipreparative RP-HPLC, first 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, and then by repeat injection onto an analytical column using a gradient solvent system of 90% CH_3CN/10% H_2O to 100% CH_3CN to afford plakortolide Q (**7**) (0.5 mg), plakortolide R (**8**) (0.7 mg), plakortone R (**16**) (0.4 mg), and plakortone L (**14**) (0.4 mg) in order of elution. Flash column fraction NP11, which eluted from ∼CH_2Cl_2/EtOAc (3:1), was chromatographed by semipreparative NP-HPLC using hexanes/EtOAc (3:1), to give first plakortolide P (**6**) (0.9 mg), plakortone N (**15**) (0.6 mg), plakortolide N (**4**) (2.9 mg), plakortolide M (**3**) (5.6 mg), and plakortolide S (**9**) (0.6 mg). Flash column fraction NP14, which eluted from ∼CH_2Cl_2/EtOAc (1:1), was further purified by analytical RP-HPLC using a solvent gradient of 90% CH_3CN/10% H_2O to 100% CH_3CN over 10 min, then using 100% CH_3CN for 30 min, to afford first a mixture of *seco*-plakortolides O (**12**) and P (**13**) (0.3 mg), then two further fractions (0.5 mg and 0.3 mg) that contained *seco*-plakortolide K (**10**) and *seco*-plakortolide L (**11**).

Plakortolide K (1): colorless oil; $[\alpha]_D$ +8.8 (*c* 0.03, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 1 and 2; HRESIMS *m/z* 439.2805 $[M + Na]^+$ (calcd for $C_{26}H_{40}NaO_4$, 439.2819).

Plakortolide L (2): colorless oil; $[\alpha]_D$ −4.6 (*c* 0.16, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 1 and 2; HRESIMS *m/z* 439.2809 $[M + Na]^+$ (calcd for $C_{26}H_{40}NaO_4$, 439.2819).

Plakortolide M (3): colorless, amorphous solid; $[\alpha]_D$ −7.2 (*c* 0.37, $CHCl_3$); a second isolate had $[\alpha]_D$ −11 (*c* 0.08, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 1 and 2; ^{1}H and ^{13}C NMR (C_6D_6, 500 MHz) δ_H 6.95 (2H, d, *J* = 8.3 Hz, H-20), 6.62 (2H, d, *J* = 8.3 Hz, H-21), 3.71 (1H, d, *J* = 5.8, H-3), 2.47 (2H, t, *J* = 7.7, H-18), 2.21 (1H, br d, *J* = 18.4, H-2a), 2.07 (1H, dd, *J* = 18.4, 6.2 Hz, H-2b), 1.89 (1H, m, H-7a), 1.82 (1H, d, *J* = 14.9, H-5a), 1.56 (1H, m, H-7b), 1.55 (2H, m, H-17), 1.29−1.24 (16H, m, H-9 to H-16), 1.09 (1H, d, *J* = 14.9, H-5b), 0.99 (3H, s, H-24), 0.64 (3H, s, H-23); δ_C 173.1 (C-1), 154.4 (C-22), 135.0 (C-19), 129.4 (C-20), 115.1 (C-21), 81.3 (C-4), 80.6 (C-3), 79.5 (C-6), 40.1 (C-5), 36.9 (C-7), 35.0 (C-18), 33.6 (C-2), 31.7 (C-17), 29.5−29.0 (C-9 to C-16), 25.1 (C-23), 24.5 (C-24); HRESIMS *m/z* 455.2775 $[M + Na]^+$ (calcd for $C_{26}H_{40}NaO_5$, 455.2768).

Plakortolide N (4): colorless, amorphous solid; $[\alpha]_D$ −7.9 (*c* 0.19, $CHCl_3$); a second isolate had $[\alpha]_D$ −13 (*c* 0.05, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 1 and 2; ^{1}H and ^{13}C NMR (C_6D_6, 500 MHz) δ_H 6.93 (2H, d, *J* = 8.4 Hz, H-20), 6.56 (2H, d, *J* = 8.6 Hz, H-21), 3.68 (1H, d, *J* = 5.7, H-3), 2.47 (2H, t, *J* = 7.8, H-18), 2.31 (1H, br d, *J* = 18.4, H-2a), 2.10 (1H, dd, *J* = 18.4, 6.2 Hz, H-2b), 1.71 (1H, d, *J* = 14.9, H-5a), 1.55 (2H, m, H-17), 1.46 (1H, m, H-7a), 1.29 (1H, m, H-7b), 1.29−1.24 (16H, m, H-9 to H-16), 1.20 (1H, d, *J* = 14.9, H-5b), 1.24 (3H, s, H-24), 0.68 (3H, s, H-23); δ_C 173.1 (C-1), 154.3 (C-22), 135.0 (C-19), 129.3 (C-20), 115.1 (C-21), 81.2 (C-4), 80.9 (C-3), 80.0 (C-6), 41.0 (C-7), 40.5 (C-5), 35.1 (C-18), 33.8 (C-2), 31.8 (C-17), 29.5−29.0 (C-9 to C-16), 25.1 (C-23), 22.2 (C-24); HRESIMS *m/z* 455.2776 $[M + Na]^+$ (calcd for $C_{26}H_{40}NaO_5$, 455.2768).

Plakortolide O (5): colorless oil; $[\alpha]_D$ −26 (*c* 0.004, $CHCl_3$); UV (CH_3CN) λ_{max} (log ε) 285 (2.66) nm; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; HRESIMS *m/z* 435.2519 $[M + Na]^+$ (calcd for $C_{26}H_{36}NaO_4$, 435.2506).

Plakortolide P (6): colorless oil; $[\alpha]_D$ −24 (*c* 0.06, $CHCl_3$); UV (CH_3CN) λ_{max} (log ε) 287 (2.68) nm; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 1 and 2; HRESIMS *m/z* 435.2518 $[M + Na]^+$ (calcd for $C_{26}H_{36}NaO_4$, 435.2506).

Plakortolide Q (7): colorless oil; $[\alpha]_D$ +5.4 (*c* 0.01, $CHCl_3$); UV (CH_3CN) λ_{max} (log ε) 286 (3.60) nm; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 1 and 2; HRESIMS *m/z* 407.2184 $[M + Na]^+$ (calcd for $C_{24}H_{32}NaO_4$, 407.2193).

Plakortolide R (8): colorless oil; $[\alpha]_D$ +7.6 (*c* 0.05, $CHCl_3$); UV (CH_3CN) λ_{max} (log ε) 284 (3.32) nm; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 1 and 2; HRESIMS *m/z* 407.2179 $[M + Na]^+$ (calcd for $C_{24}H_{32}NaO_4$, 407.2193).

Plakortolide S (9): colorless oil; $[\alpha]_D$ −7.0 (*c* 0.04, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 1 and 2; HRESIMS *m/z* 427.2457 $[M + Na]^+$ (calcd for $C_{24}H_{36}NaO_5$, 427.2455).

***seco*-Plakortolide K (10):** colorless oil; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 3 and 4; HRESIMS *m/z* 441.2977 $[M + Na]^+$ (calcd for $C_{26}H_{42}NaO_4$, 441.2975).

***seco*-Plakortolide L (11):** colorless oil; $[\alpha]_D$ −11 (*c* 0.02, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 3 and 4; HRESIMS *m/z* 441.2980 $[M + Na]^+$ (calcd for $C_{26}H_{42}NaO_4$, 441.2975).

***seco*-Plakortolide O (12):** colorless oil; UV (CH_3CN) λ_{max} (log ε) 286 (3.22) nm; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 3 and 4; HRESIMS *m/z* 437.2673 $[M + Na]^+$ (calcd for $C_{26}H_{38}NaO_4$, 437.2662).

***seco*-Plakortolide P (13):** colorless oil; UV (CH_3CN) λ_{max} (log ε) 286 (3.22) nm; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 3 and 4; HRESIMS *m/z* 437.2673 $[M + Na]^+$ (calcd for $C_{26}H_{38}NaO_4$, 437.2662).

Plakortone L (14): colorless oil; $[\alpha]_D$ −20.1 (*c* 0.019, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 5 and 6; HRESIMS *m/z* 423.2857 $[M + Na]^+$ (calcd for $C_{26}H_{40}NaO_3$, 423.2870).

Plakortone N (15): colorless oil; $[\alpha]_D$ −49 (*c* 0.007, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 5 and 6; HRESIMS *m/z* 439.2805 $[M + Na]^+$ (calcd for $C_{26}H_{40}NaO_4$, 439.2819).

Plakortone P (16): colorless oil; $[\alpha]_D$ −43 (*c* 0.007, $CHCl_3$); UV (CH_3CN) λ_{max} (log ε) 286 (3.60) nm; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 5 and 6; HRESIMS *m/z* 419.2556 $[M + Na]^+$ (calcd for $C_{26}H_{36}NaO_4$, 419.2557).

Reductive Cleavage of Plakortolide K (1) and Plakortolide L (2). Plakortolide K (**1**) (0.4 mg, 1.0 μmol) was dissolved in anhydrous Et_2O (1 mL), then treated with AcOH (50 μL) and an

excess of Zn metal (30 mg). The reaction was stirred for 16 h at room temperature, then filtered, and the solution dried under reduced pressure to obtain a synthetic sample of *seco*-plakortolide K (**10**) (0.4 mg) in close to quantitative yield. Plakortolide L (**2**) (1.8 mg, 4.3 μmol) was subjected to the same experimental procedure to provide a synthetic sample of *seco*-plakortolide L (**11**) (1.5 mg).

***seco*-Plakortolide K (10):** colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) was identical to the natural sample of *seco*-plakortolide K; LRESIMS *m/z* 441.3 $[M + Na]^+$.

***seco*-Plakortolide L (11):** colorless oil; $[\alpha]_D$ −10.9 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) was identical to the natural sample of *seco*-plakortolide L; LRESIMS *m/z* 441.3 $[M + Na]^+$.

Preparation of MPA Esters of *seco*-Plakortolide K (20a/20b) and *seco*-Plakortolide L (21a/21b). The sample of *seco*-plakortolide K (**10**) (0.4 mg) obtained by Zn/AcOH reduction of plakortolide K was divided in half, and each sample (approximately 0.2 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). The reaction was stirred overnight at room temperature before filtering through a small plug of silica eluting with $CHCl_3$ (5 mL). The solvent was then removed *in vacuo*, and the product MPA esters were then individually purified by RP-HPLC using an analytical column and eluting with CH_3CN/H_2O (9:1) to obtain respectively the (*R*)-MPA ester (**20a**) (0.1 mg) and (*S*)-MPA ester (**20b**) (0.1 mg). A sample of *seco*-plakortolide L (**11**) (1.5 mg), obtained by Zn/AcOH reduction of plakortolide L, was likewise divided in half, and each sample (approximately 0.8 mg) reacted with either (*R*)- or (*S*)-MPA using the same procedure yielding (*R*)-MPA ester (**21a**) (0.4 mg) and (*S*)-MPA ester (**21b**) (0.5 mg). Trace amounts of plakortone L (**14**) (approximately 0.1 mg) were identified during the RP-HPLC of the esterification products derived from **11**.

(*R*)-MPA ester (20a): colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33−7.42 (5H, m, MPA phenyl protons), 7.25 (2H, m, H-21), 7.16 (2H, m, H-20), 7.15 (1H, m, H-22), 5.19 (1H, dd, *J* = 6.5, 1.5 Hz, H-3), 4.74 (1H, s, C*H* of MPA), 3.36 (3H, s, OMe), 3.03 (1H, dd, *J* = 18.5, 6.5 Hz, H-2a), 2.58 (2H, t, 7.5 Hz, H-18), 2.47 (1H, dd, *J* = 18.5, 1.5 Hz, H-2b), 1.49 (3H, s, H-23), 1.49 (1H, d, *J* = 15.0 Hz, H-5a), 1.39 (1H, d, *J* = 15.0 Hz, H-5b), 1.24−1.32 (22H, m, H-7 to H-17), 1.10 (3H, s, H-24); HRESIMS *m/z* 589.3498 $[M + Na]^+$ (calcd for $C_{35}H_{50}NaO_6$, 589.3500).

(*S*)-MPA ester (20b): colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33−7.42 (5H, m, MPA phenyl protons), 7.25 (2H, m, H-21), 7.16 (2H, m, H-20), 7.15 (1H, m, H-22), 5.21 (1H, dd, *J* = 6.5, 1.5 Hz, H-3), 4.78 (1H, s, C*H* of MPA), 3.39 (3H, s, OMe), 2.94 (1H, dd, *J* = 18.5, 6.5 Hz, H-2a), 2.58 (2H, t, 7.5 Hz, H-18), 2.22 (1H, dd, *J* = 18.5, 1.5 Hz, H-2b), 1.82 (1H, d, *J* = 15.0 Hz, H-5a), 1.78 (1H, d, *J* = 15.0 Hz, H-5b), 1.53 (3H, s, H-23), 1.26 (3H, s, H-24), 1.24−1.32 (22H, m, H-7 to H-17); HRESIMS *m/z* 589.3597 $[M + Na]^+$ (calcd for $C_{35}H_{50}NaO_6$, 589.3500).

(*R*)-MPA ester (21a): colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33−7.42 (5H, m, MPA phenyl protons), 7.25 (2H, m, H-21), 7.16 (2H, m, H-20), 7.15 (1H, m, H-22), 5.19 (1H, dd, *J* = 6.5, 2.0 Hz, H-3), 4.79 (1H, s, C*H* of MPA), 3.40 (3H, s, OMe), 2.94 (1H, dd, *J* = 18.5, 6.5 Hz, H-2a), 2.58 (2H, t, 8.0 Hz, H-18), 2.25 (1H, dd, *J* = 18.5, 2.0 Hz, H-2b), 1.92 (1H, d, *J* = 15.0 Hz, H-5a), 1.76 (1H, d, *J* = 15.0 Hz, H-5b), 1.59 (2H, m, H-17), 1.53 (3H, s, H-23), 1.47 (2H, m, H-7), 1.24−1.32 (20H, m, H-8 to H-16), 1.13 (3H, s, H-24); HRESIMS *m/z* 589.3518 $[M + Na]^+$ (calcd for $C_{35}H_{50}NaO_6$, 589.3500).

(*S*)-MPA ester (21b): colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33−7.42 (5H, m, MPA phenyl protons), 7.25 (2H, m, H-21), 7.16 (2H, m, H-20), 7.15 (1H, m, H-22), 5.19 (1H, dd, *J* = 6.2, 1.5 Hz, H-3), 4.74 (1H, s, C*H* of MPA), 3.36 (3H, s, OMe), 3.02 (1H, dd, *J* = 18.6, 6.2 Hz, H-2a), 2.58 (2H, t, 7.8 Hz, H-18), 2.47 (1H, dd, *J* = 18.6, 1.5 Hz, H-2b), 1.59 (1H, d, *J* = 15. 0 Hz, H-5a), 1.59 (2H, m, H-17), 1.48 (3H, s, H-23), 1.40 (1H, d, *J* = 15.0 Hz, H-5b), 1.24−1.32 (20H, m, H-7 to H-16), 0.84 (3H, s, H-24); HRESIMS *m/z* 589.3520 $[M + Na]^+$ (calcd for $C_{35}H_{50}NaO_6$, 589.3500).

Plakortone L (14): colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) was identical to the sponge-derived sample of plakortone ether L; LRESIMS *m/z* 423.3 $[M + Na]^+$.

Reductive Cleavage of Plakortolide M (3) and Plakortolide N (4). Samples of plakortolide M (**3**) (2.3 mg) or plakortolide N (**4**) (2 mg) were subjected to Zn/AcOH reduction using the same experimental procedure as above to provide samples of *seco*-plakortolide M (**22**) (1.3 mg) and *seco*-plakortolide N (**23**) (1.1 mg).

***seco*-Plakortolide M (22):** colorless oil; $[\alpha]_D$ +21 (*c* 0.09, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 3 and 4; HRESIMS *m/z* 457.2925 $[M + Na]^+$ (calcd for $C_{26}H_{42}NaO_4$, 457.2924).

***seco*-Plakortolide N (23):** colorless oil; $[\alpha]_D$ +13 (*c* 0.08, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 3 and 4; HRESIMS *m/z* 457.2939 $[M + Na]^+$ (calcd for $C_{26}H_{42}NaO_4$, 457.2924).

Preparation of MPA Diesters (24a/b and 24a/b) of *seco*-Plakortolide M (22) and *seco*-Plakortolide N (23) from 3 and 4. Mosher ester preparation used the same experimental procedures as for **20a**/**20b** and **21a**/**21b** except that three equivalents of reagents were used to ensure esterification of the terminal phenolic group. The Mosher ester products were purified by RP-HPLC using an analytical column and eluting with CH_3CN/H_2O (9:1) to obtain respectively the (*R*)-MPA ester (**24a**) (0.2 mg) and (*S*)-MPA ester (**24b**) (0.2 mg) from *seco*-plakortolide M (**22**) and the (*R*)-MPA ester (**25a**) (0.2 mg) and (*S*)-MPA ester (**25b**) (0.1 mg) from *seco*-plakortolide N (**23**). During the HPLC purifications, samples of plakortone N (**15**), the 22-*O*-(*S*)-MPA ester of plakortone N (**26**), and plakortone M (**27**), each <0.2 mg in weight, were isolated from the reactions with **23** and **22**, respectively.

(*R*)-MPA diester (24a): colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 (2H, m, MPA phenyl protons), 7.33−7.41 (8H, m, MPA phenyl protons), 7.10 (2H, d, *J* = 8.5 Hz, H-20), 6.86 (2H, d, *J* = 8.5 Hz, H-21), 5.18 (1H, dd, *J* = 6.2, 1.2 Hz, H-3), 4.97 (1H, s, C*H* of MPA), 4.74 (1H, s, C*H* of MPA), 3.49 (3H, s, OMe), 3.36 (3H, s, OMe), 3.03 (1H, dd, *J* = 18.6, 6.2 Hz, H-2a), 2.54 (2H, t, 7.6 Hz, H-18), 2.47 (1H, dd, *J* = 18.6, 1.2 Hz, H-2b), 1.55 (2H, m, H-17), 1.49 (1H, d, *J* = 15.1 Hz, H-5a), 1.39 (1H, d, *J* = 15.1 Hz, H-5b), 1.48 (3H, s, H-23), 1.22−1.28 (14H, m, H-9 to H-16), 1.09 (3H, s, H-24), 1.08 (2H, m, H-7), 1.07 (2H, m, H-8); HRESIMS *m/z* 753.3975 $[M + Na]^+$ (calcd for $C_{44}H_{58}NaO_9$, 753.3973).

(*S*)-MPA diester (24b): colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 (2H, m, MPA phenyl protons), 7.33−7.41 (8H, m, MPA phenyl protons), 7.10 (2H, d, *J* = 8.5 Hz, H-20), 6.86 (2H, d, *J* = 8.5 Hz, H-21), 5.21 (1H, dd, *J* = 6.3, 1.5 Hz, H-3), 4.97 (1H, s, C*H* of MPA), 4.78 (1H, s, C*H* of MPA), 3.48 (3H, s, OMe), 3.39 (3H, s, OMe), 2.94 (1H, dd, *J* = 18.6, 6.3 Hz, H-2a), 2.54 (2H, t, 7.9 Hz, H-18), 2.22 (1H, dd, *J* = 18.6, 1.5 Hz, H-2b), 1.81 (1H, d, *J* = 15. 0 Hz, H-5a), 1.78 (1H, d, *J* = 15.1 Hz, H-5b), 1.54 (2H, m, H-17), 1.54 (3H, s, H-23), 1.25 (3H, s, H-24), 1.36 (2H, m, H-7), 1.22−1.26 (18H, m, H-8 to H-16); HRESIMS *m/z* 753.3952 $[M + Na]^+$ (calcd for $C_{44}H_{58}NaO_9$, 753.3973).

(*R*)-MPA diester (25a): colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 (2H, m, MPA phenyl protons), 7.33−7.40 (8H, m, MPA phenyl protons), 7.10 (2H, d, *J* = 8.5 Hz, H-20), 6.86 (2H, d, *J* = 8.5 Hz, H-21), 5.19 (1H, dd, *J* = 6.4, 1.8 Hz, H-3), 4.97 (1H, s, C*H* of MPA), 4.78 (1H, s, C*H* of MPA), 3.48 (3H, s, OMe), 3.39 (3H, s, OMe), 2.94 (1H, dd, *J* = 18.6, 6.4 Hz, H-2a), 2.53 (2H, t, 7.8 Hz, H-18), 2.25 (1H, dd, *J* = 18.5, 1.8 Hz, H-2b), 1.91 (1H, d, *J* = 15.1 Hz, H-5a), 1.76 (1H, d, *J* = 15.1 Hz, H-5b), 1.48−1.55 (4H, m, H-7 and H-8), 1.55 (3H, s, H-23), 1.22−1.28 (14H, m, H-9 to H-16), 1.13 (3H, s, H-24); HRESIMS *m/z* 753.3947 $[M + Na]^+$ (calcd for $C_{44}H_{58}NaO_9$, 753.3973).

(*S*)-MPA diester (25b): colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 (2H, m, MPA phenyl protons), 7.33−7.41 (8H, m, MPA phenyl protons), 7.10 (2H, d, J = 8.5 Hz, H-20), 6.86 (2H, d, J = 8.5 Hz, H-21), 5.19 (1H, dd, J = 6.2, 1.5 Hz, H-3), 4.97 (1H, s, C*H* of MPA), 4.74 (1H, s, C*H* of MPA), 3.48 (3H, s, OMe), 3.36 (3H, s, OMe), 3.03 (1H, dd, J = 18.6, 6.2 Hz, H-2a), 2.54 (2H, t, 7.7 Hz, H-18), 2.48 (1H, dd, J = 18.6, 1.5 Hz, H-2b), 1.58 (1H, d, J = 15.1 Hz, H-5a), 1.54 (2H, m, H-17), 1.39 (1H, d, J = 15.1 Hz, H-5b), 1.47 (3H, s, H-23), 1.22−1.26 (20H, m, H-7 to H-16), 0.83 (3H, s, H-24); HRESIMS m/z 753.3977 $[M + Na]^+$ (calcd for $C_{44}H_{58}NaO_9$, 753.3973).

22-*O*-(*S*)-MPA ester of plakortone N (26): colorless oil; $[\alpha]_D$ +6.4 (c 0.007, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 (1H, m, MPA phenyl protons), 7.33−7.41 (4H, m, MPA phenyl protons), 7.09 (2H, d, J = 8.6 Hz, H-20), 6.86 (2H, d, J = 8.6 Hz, H-21), 4.97 (1H, s, C*H* of MPA), 4.29 (d, J = 4.7 Hz, H-3), 3.48 (3H, s, OMe), 2.72 (1H, dd, J = 18.2, 4.8 Hz, H-2a), 2.65 (1H, d, J = 18.1 Hz, H-2b), 2.53 (2H, t, 7.5 Hz, H-18), 2.28 (1H, d, J = 14.3 Hz, H-5a), 1.96 (1H, d, J = 14.3 Hz, H-5b), 1.54 (2H, m, H-17), 1.46 (3H, s, H-23), 1.22−1.26 (20H, m, H-7 to H-16), 1.24 (3H, s, H-24); HRESIMS m/z 587.3350 $[M + Na]^+$ (calcd for $C_{35}H_{48}NaO_4$, 587.3343).

Plakortone N (15): colorless oil; $[\alpha]_D$ −23 (c 0.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) was identical to the sponge-derived sample of plakortone ether N; LRESIMS m/z 439.29 $[M + Na]^+$.

Plakortone M (27): colorless oil; $[\alpha]_D$ +47 (c 0.007, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz), see Tables 5 and 6; HRESIMS m/z 439.2814 $[M + Na]^+$ (calcd for $C_{26}H_{40}NaO_4$, 439.2819).

■ ASSOCIATED CONTENT

Supporting Information. Figures S1−S45. ^{1}H and selected 2D NMR data for compounds **1**−**16**, **20a**/**b**, **21a**/**b**, **22**, **23**, **24a**/**b**, **25a**/**b**, and **27**. This material is available free of charge via the Internet at http://pubs.acs.org.

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