Plakortides M and N, Bioactive Polyketide Endoperoxides from the Caribbean Marine Sponge *Plakortis halichondrioides*

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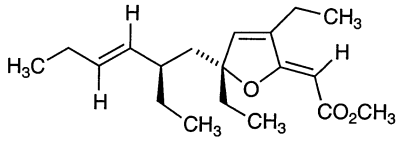
From a small specimen of the marine sponge *Plakortis halichondrioides* collected in Puerto Rico we have isolated the known unsaturated ester methyl (2*Z*,6*R*,8*R*,9*E*)-3,6-epoxy-4,6,8-triethyl-2,4,9-dodecatrienoate (**1**) along with the known cyclic peroxide plakortide F (**2**). In addition, the structures of two new polyketide endoperoxides, namely, plakortide M (**3**) and plakortide N (**4**), were fully characterized by spectroscopic and chemical methods. The absolute stereochemistry of plakortide M methyl ester (**3a**) has been determined by analysis of the (*R*)- and (*S*)-MTPA esters of the acyclic derivative **5** obtained by hydrogenolysis. Plakortide M (**3**) and plakortide N (**4**) exhibited potent cytotoxicity in the NCI human cancer screening program, whereas plakortide M methyl ester, **3a**, displayed strong antimalarial activity against *Plasmodium falciparum*.

Marine sponges of the family Plakiniidae are well known for their ability to produce a plethora of biologically active oxygenated polyketides, cyclic peroxides (1,2-dioxanes and 1,2-dioxolanes), and related metabolites.[1] Specifically, sponges of the genus *Plakortis* are prolific producers of stable cyclic peroxides which are generally assumed to derive from the polyketide pathway. Plakortin, discovered in 1978 by Faulkner in specimens of *Plakortis halichondrioides* collected in Panama and Belize, was the first of this class of compounds to be reported.[2] Since Faulkner's pioneering work, a large series of related and highly bioactive metabolites have been isolated.

As part of an ongoing screening program to identify biologically active secondary metabolites from Caribbean marine animals, we have recently initiated a chemical investigation of the sponge *P. halichondrioides* (Wilson 1902) collected in Puerto Rico. Herein, we report the isolation, structure elucidation, absolute stereochemistry, and biological properties of two new polyketide endoperoxides from this organism, namely, plakortide M (**3**) and plakortide N (**4**). This paper also reports on the isolation of known polyketide esters **1**[3] and **2**,[4,5] which possess similar backbone frameworks and thus appear to be closely related to endoperoxides **3** and **4** from a biogenetic standpoint.



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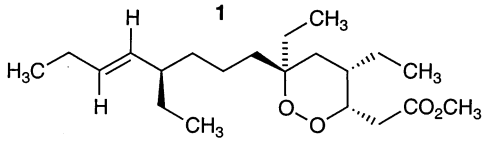

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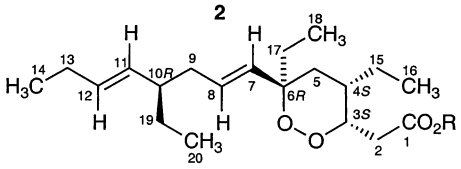
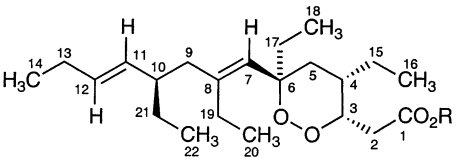


3 R = H
3a R = CH_3



4 R = H
4a R = CH_3

Results and Discussion

A small specimen of *P. halichondrioides* (phylum Porifera, class Demospongiae, order Homoclerophorida, family Plakiniidae) was collected by scuba during an expedition to Mona Island off the west coast of Puerto Rico and immediately frozen. After homogenization, the dry organism (406 g dry wt) was exhaustively extracted with MeOH−$CHCl_3$, 1:1. The crude extract was partitioned between hexane and water, and then a small portion of the organic extract was subjected to chromatography over a column packed with Si gel and eluted with a system of solvents of increasing polarity from hexane to acetone to MeOH. The less polar fractions were further purified by column chromatography over Si gel, and a preliminary spectroscopic analysis revealed that fractions eluted with 100% hexane were mainly composed of the nonpolar polyketide esters **1** and **2**. Repeated chromatography of the more polar fractions eluted with hexane−acetone mixtures led to the isolation of the new polyketide endoperoxides plakortide M (**3**) and plakortide N (**4**) in a pure state, as colorless oils.

The ^{13}C NMR spectrum and HRFABMS of plakortide M (316 mg, 0.8% yield of dry wt) suggested a molecular formula of $C_{20}H_{34}O_4$ for **3** ($[M + Na]^+$ *m/z* 361.2367 obsd, 361.2355 calcd). Inspection of the NMR spectra suggested that the compound was closely related to plakortide F (**2**), first reported by Kashman in 1993 from *P. halichondrioides* from Jamaica (its proposed structure being devoid of relative stereochemistry at C-6 and C-10)[4] and then by Patil, who isolated it in 1996 from a Jamaican specimen of the same organism. The latter group named the com-

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Table 1. ^{1}H NMR (500 MHz), ^{13}C NMR (75 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data for Plakortide M Methyl Ester (**3a**) in $CDCl_3$[a]

position	δ_H, mult, intgr (*J* in Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1		172.3 (s)			H-2, H-2′, H-3, −OCH_3
2	3.03, dd, 1H (9.7, 15.5)	31.4 (t)	H-2′, H-3	H-2′, H-5′	H-3, H-4
2′	2.37, dd, 1H (3.4, 15.5)		H-2, H-3	H-2, H-5′, H-15′	
3	4.44, ddd, 1H (4.0, 4.6, 9.1)	78.9 (d)	H-2, H-2′, H-4	H-2, H-2′, H-4	H-2, H-2′, H-5, H-5′
4	2.17, m, 1H	34.9 (d)	H-3, H-5, H-5′, H-15, H-15′	H-3, H-5, H-7	H-3, H-5, H-5′, H-15′, H_3-16
5	1.76, dd, 1H (4.1, 13.5)	32.6 (t)	H-4, H-5′	H-4, H-5′, H-7	H-3, H-7, H-15, H-15′, H-17, H-17′
5′	1.26, dd, 1H (13.0, 13.5)		H-4, H-5	H-2, H-2′, H-5, H-17	
6		83.6 (s)			H-5, H-5′, H-7, H-8, H-17, H-17′, H_3-18
7	5.69, d, 1H (16.1)[d]	132.9 (d)	H-8	H-4, H-5, H-9	H-8, H-9, H-17, H-17′
8	5.63, ddd, 1H (5.4, 6.5, 16.1)[d]	130.0 (d)	H-7, H-9	H-10[e]	H-7, H-9
9	2.14, m, 2H	38.3 (t)	H-8, H-10	H-7	H-7, H-8
10	1.92, m, 1H	44.5 (d)	H-9, H-11, H-19	H-8[e], H-11[e]	H-9, H-11, H-12, H-19, H_3-20
11	5.14, dd, 1H (8.5, 15.2)	132.9 (d)	H-10, H-12	H-10[e]	H-9, H-10, H-12, H-13
12	5.38, dt, 1H (6.3, 15.2)	132.1 (d)	H-11, H-13		H-10, H-11, H-13, H_3-14
13	1.99, m, 2H	25.6 (t)	H-12, H_3-14		H-11, H-12, H_3-14
14	0.96, t, 3H (7.5)	14.1 (q)	H-13		H-12, H-13
15	1.41, m, 1H	27.5 (t)	H-4, H-15′, H_3-16	H-15′	H_3-16
15′	1.21, m, 1H		H-4, H-15, H_3-16	H-2′, H-15	
16	0.84, t, 3H (7.4)	11.6 (q)	H-15, H-15′		H-15
17	1.43, m, 1H	33.1 (t)	H-17′, H_3-18	H-5′, H-17′	H_3-18
17′	1.32, m, 1H		H-17, H_3-18	H-17	
18	0.82, t, 3H (7.6)	7.1 (q)	H-17, H-17′		H-17, H-17′
19	1.15, m, 2H	25.0 (t)	H-10, H_3-20		H-11, H_3-20
20	0.91, t, 3H (7.4)	11.0 (q)	H-19		H-19
-OMe	3.71, s, 3H	51.9 (q)			

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. [b] ^{13}C NMR multiplicities were obtained from a DEPT experiment. [c] Protons correlated to carbon resonances in ^{13}C column. [d] In $CDCl_3$, H-7 and H-8 appear as signals with almost coincident chemical shifts. Therefore, the δ values shown were obtained in Bz-d_6 solution. [e] NOEs detected in Bz-d_6 solution.

pound plakortide F and established its relative stereochemistry at C-6 but not at C-10. Like plakortide F (**2**), plakortide M possesses a 1,2-dioxane ring bearing an acetic acid functionality at the C-3 position and multiple ethyl substituents at C-4, C-6, and C-10. However, plakortide M, unlike **2**, displays a 4′-ethyl-1′,5′-octadienyl substituent at C-6. The presence of a carboxylic acid functionality is supported by the observation of a ^{13}C NMR resonance at δ 177.6 (s) and strong absorptions at 3600−3000 (broad) and 1718 cm^{-1} in the IR spectrum. The ^{13}C and DEPT NMR spectra provided signals due to 20 carbons, which could be classified as four methyl, seven methylene, seven methine, and two quaternary carbons. The ^{1}H and ^{13}C NMR spectra showed that **3** contained a 3,4,6,6-tetrasubstituted six-membered cyclic peroxide ring system like that present in plakortide F [^{1}H NMR δ 3.04 (dd, 1H, J = 9.6, 15.8 Hz, H-2), 2.41 (dd, 1H, J = 3.4, 15.8 Hz, H-2′), 4.42 (dt, 1H, J = 3.8, 9.0 Hz, H-3), 1.77 (dd, 1H, J = 4.1, 13.5 Hz, H-5), and 1.26 (dd, 1H, J = 12.9, 13.5 Hz, H-5′); ^{13}C NMR δ 78.6 (CH−O, C-3) and 83.6 (C−O, C-6)].

Analysis of ^{1}H−^{1}H COSY data showed the existence of three proton spin systems. A proton assigned as H-3 (δ 4.42, dt, J = 3.8, 9.0 Hz) was coupled with both a methine signal observed at δ 2.17 (H-4) and the methylene protons observed at δ 3.04 and 2.41 (H_2-2). H-4 was further coupled to two mutually coupled signals observed at δ 1.77 (H-5_{eq}) and 1.26 (H-$5'_{ax}$). The presence of multiple ethyl functionalities in **3** was apparent from the observation of four methyl triplets in the ^{1}H NMR spectrum [δ 0.81 (t, J = 7.6 Hz, Me-18), 0.83 (t, J = 7.3 Hz, Me-16), 0.90 (t, J = 7.4 Hz, Me-20), and 0.95 (t, J = 7.4 Hz, Me-14)], all of which were coupled to methylene groups from the COSY data. The carbons observed at δ 130.0 (d), 132.1 (d), and 132.9 (d, two overlapped signals) in the ^{13}C NMR spectrum suggested the presence of two disubstituted olefins. The corresponding ^{1}H NMR resonances [δ 5.46 (overlapped multiplet, 2H, H-7 and H-8), 5.38 (dt, 1H, J = 6.3, 15.3 Hz, H-12), and 5.14 (dd, 1H, J = 8.4, 15.3 Hz, H-11)] confirmed the presence of the olefin functionalities. The presence of a 4′-ethyl-1′,5′-octadienyl chain could be readily defined from the COSY spectrum. Both double bonds along the 1,5-octadiene alkyl chain were assigned as *trans* on the basis of the ^{1}H−^{1}H scalar coupling constant (J = 15.2 and 16.1 Hz) of plakortide M methyl ester (**3a**). Derivative **3a** was prepared in high yield upon methylation of selected fractions of plakortide M (**3**) with CH_2N_2 followed by purification over a Si gel column. The planar structure of **3** (and that of methyl ester **3a**) was fully established by interpretation of the 2D NMR data (^{1}H−^{1}H COSY, HMQC, and HMBC).

The relative stereochemistry of **3a** was deduced on the basis of the coupling constants, molecular modeling, and the NOESY correlations (Table 1). The protons at C-5 could be resolved into two double doublet signals at δ 1.26 and 1.76 with coupling constants of 13.0, 13.5 Hz, and 4.1, 13.5 Hz, respectively. These coupling constants are typical of methylene protons in a six-membered ring, which are coupled to a single axial proton of H-4 ($J_{H-4ax-H-5ax}$ = 13.0 Hz). Examination of a molecular model supported the orientations of H-3 (equatorial) and H-4 (axial) by the coupling constant of 4.6 Hz ($J_{H-3eq-H-4ax}$). Strong NOE correlations were observed between H-2 and H-5_{ax}, H-3_{eq} and H-4_{ax}, H-4_{ax} and H-5_{eq}, H-4_{ax} and H-7, H-5_{eq} and H-7, and H-5_{ax} and H_2-17, which required a 6α (equatorial)-ethyl configuration (Figure 1). Such a configuration at C-6 in polyketide-derived cyclic peroxides has been reported previously from other sponges of the genus *Plakortis*.[1] The NOESY spectrum also revealed cross-peaks from H-7 (δ 5.69) to the methylene protons at C-9 (δ 2.14), which are consistent with the *E* geometry of the Δ^7 olefin. To determine the absolute stereochemistry, **3a** (the methyl ester of plakortide M) was reduced by catalytic hydrogena-

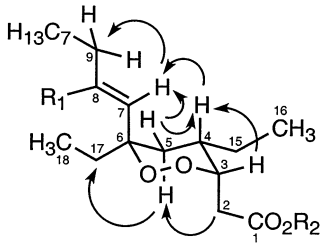
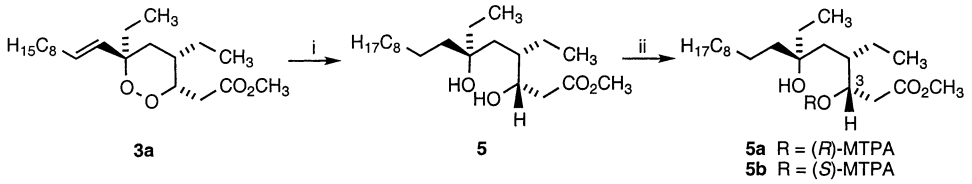


Figure 1. Selected NOE correlations observed for plakortide M methyl ester (**3a**; R_1 = H; R_2 = CH_3) and plakortide N (**4**) (R_1 = CH_2CH_3, R_2 = H).

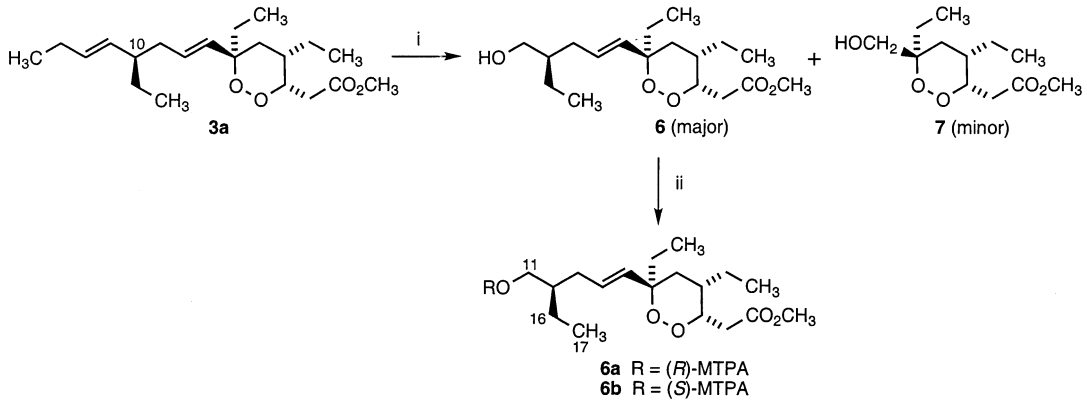
tion with 10% Pd/C to give the diol **5**, which was in turn esterified at C-3 by (*R*)- or (*S*)-MTPA acid in dry CH_2Cl_2 as shown in Figure 2.[6] Upon analysis of the Mosher's derivatives **5a** and **5b**, the absolute configuration of **5** at C-3 was *S*. Whereby coupled with the relative geometry described above, this procedure established the complete absolute stereochemistry about the peroxide ring in **3a** as 3*S*,4*S*,6*R*. To determine the absolute geometry at remote chiral center C-10 in **3a**, a procedure based on the ^{1}H NMR chemical shift difference of the hydroxymethylene protons in diastereomeric (+)- and (−)-MTPA esters was employed.[7,8] This method has been proposed to assign the absolute stereochemistry at C-2 of primary β-methyl alcohols. Accordingly, compound **3a** was transformed into alcohol derivatives **6** (major) and **7** (minor) by mild ozonolysis[3,9] followed by reduction with $NaBH_4$ as shown in Figure 3. Treatment of **6** with (+)- and (−)-MTPA acid gave the 11-(+)-MTPA (**6a**) and 11-(−)-MTPA (**6b**) esters, respectively. In the ^{1}H NMR spectra the 11-methylene protons of the (+)-MTPA derivative (**6a**) appeared as two well-separated double doublets at δ 4.32 and 4.16, whereas those of the (−)-MTPA ester (**6b**) were very close at δ 4.26 and 4.21. These data were in excellent agreement with those previously reported in models and suggested the *R* configuration at C-10 of **3a**. As the same diol **5** ($[\alpha]_D$= −44.2°) was obtained from plakortide F under the same reaction conditions, the absolute configuration of **2** was also assigned to be 3*S*,4*S*,6*R*,10*R*. Interestingly, a cyclic peroxide acid with the same constitution as plakortide M but epimeric at C-3 and devoid of relative stereochemistry at C-10 has been recently reported by Wright from a Jamaican specimen of *P. halichondrioides*.[10]

Plakortide N (**4**), $[\alpha]_D^{24}$ −275.0° (*c* 0.52, $CHCl_3$), had the molecular formula $C_{22}H_{38}O_4$ established by HRFABMS of the $[M + Na]^+$ ion (*m/z* 389.2678 found; 389.2668 calcd). The IR absorption at 1711 cm^{-1} together with the large band between 3400 and 3000 cm^{-1} supported the presence of the carboxylic acid functionality [δ 176.4 (s) in the ^{13}C NMR spectrum], as well as a weak but significant band at 1193 cm^{-1} indicative of a peroxidic linkage. The ^{13}C NMR spectrum of **4** showed 22 carbon resonances including signals for two carbons bearing oxygen at δ 84.4 (s) and 78.5 (d) and one trisubstituted [δ 142.5 (s) and 126.9 (d)] and one disubstituted [δ 133.2 (d) and 131.7 (d)] double bond. The downfield-shifted resonance ascribable to H-3 (δ 4.43, ddd, J = 3.6, 4.8, 9.0 Hz) correlated with the H_2-2 protons (δ 3.06 and 2.42) and with the methine signal at δ 2.12 (H-4). The latter hydrogen also showed cross-peaks with the mutually coupled signals at δ 1.71 (H-5) and 1.27 (H-5′) as well as with the methylene protons at δ 1.18 and 1.16 (H_2-15), both in turn coupled to the methyl group at δ 0.91 (Me-16). The branched alkyl chain was unambiguously ascertained by 2D NMR experiments that allowed elucidation of the C-9 through C-14 fragment (Table 2). Upon analysis of the COSY spectrum, H-10 further correlated to the methylene protons at δ 1.42 and 1.16 (H_2-21), both in turn coupled to the methyl triplet at δ 0.84 (Me-22). The remaining proton signals were attributed to two isolated ethyl groups resonating at δ 1.56 (H-17), 1.23 (H-17′), 0.85 (Me-18) and δ 2.20 (H_2-19), and 0.97 (Me-20). The HMBC data (Table 2) allowed the partial structures to be connected and completed the planar structure of **4**. The NOESY spectrum of **4** showed cross-peaks between one H_2-2 proton (δ 3.06) and H-5_{ax} (δ 1.27), H-3_{eq} and H-4_{ax},



i. H_2 (10%) Pd/C in MeOH, r.t., 10 h (88%); ii. (*R*)- or (*S*)-MTPA Acid in CH_2Cl_2, DCC, DMAP, r.t., 15 h.

Figure 2. Conversion of plakortide M methyl ester (**3a**) to diol **5** and its MTPA derivatives.



i. O_3 in MeOH, $NaBH_4$, -78°C, 20 min (64%); ii. (*R*)- or (*S*)-MTPA Acid in CH_2Cl_2, DCC, DMAP, r.t., 15 h.

Figure 3. Conversion of plakortide M methyl ester (**3a**) to alcohol **6** and its MTPA derivatives.

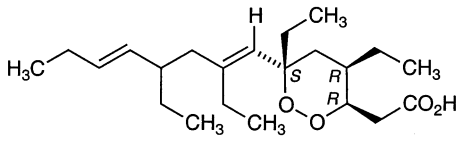
Table 2. ^{1}H NMR (500 MHz), ^{13}C NMR (75 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data for Plakortide N (**4**) in $CDCl_3$[a]

position	δ_H, mult, intgr (*J* in Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1		176.4 (s)			H-2, H-2′
2	3.06, dd, 1H (9.6, 15.9)	31.1 (t)	H-2′, H-3	H-2′, H-3, H-5′	
2′	2.42, dd, 1H (3.4, 15.9)		H-2, H-3	H-2, H-3, H-15, H-15′	
3	4.43, ddd, 1H (3.6, 4.8, 9.0)	78.5 (d)	H-2, H-2′, H-4	H-2, H-2′, H-4	H-2, H-5, H-5′
4	2.12, m, 1H	35.2 (d)	H-3, H-5, H-5′, H-15, H-15′	H-3, H-5, H-7	H-3, H-5, H-5′, H-15, H-15′, H_3-16
5	1.71, dd, 1H (4.0, 13.3)	35.7 (t)	H-4, H-5′	H-4, H-5′, H-7	H-3, H-7, H-15, H-15′
5′	1.27, dd, 1H (13.0, 13.3)		H-4, H-5	H-2, H-17	
6		84.4 (s)			H-7, H-17, H-17′, H_3-18
7	5.14, br s, 1H	126.9 (d)		H-4, H-5, H-9	H-5′, H-17′, H-19
8		142.5 (s)			H-7, H-19, H_3-20
9	2.13, 1.99, m, 2H	42.5 (t)	H-10	H-7	H-7, H-10, H-11, H-19
10	1.97, m, 1H	42.5 (d)	H-9, H-11, H-21	H-11	H-9, H-11, H-12, H-21, H_3-22
11	5.11, dd, 1H (8.3, 15.3)	133.2 (d)	H-10, H-12	H-10	H-9, H-12, H-13
12	5.37, dt, 1H (6.2, 15.3)	131.7 (d)	H-11, H-13		H-11, H-13, H_3-14
13	1.97, m, 2H	25.6 (t)	H-12, H_3-14		H-11, H-12, H_3-14
14	0.95, t, 3H (7.5)	14.0 (q)	H-13		H-12, H-13
15	1.18, m, 1H	25.0 (t)	H-4, H-15′, H_3-16	H-2′, H-15′	H-5′, H_3-16
15′	1.16, m, 1H		H-4, H-15, H_3-16	H-2′, H-15	
16	0.91, t, 3H (7.4)	11.0 (q)	H-15, H-15′		H-15
17	1.56, m, 1H	32.9 (t)	H-17′, H_3-18	H-5′, H-17′, H-19	H_3-18
17′	1.23, m, 1H		H-17, H_3-18	H-17, H-19	
18	0.85, t, 3H (7.6)	7.7 (q)	H-17, H-17′		
19	2.20, m, 2H	22.7 (t)	H_3-20	H-17, H-17′	H-7, H_3-20
20	0.97, t, 3H (7.5)	12.1 (q)	H-19		H-19
21	1.42, 1.16, m, 2H	27.8 (t)	H-10, H_3-22		H_3-22
22	0.84, t, 3H (7.5)	11.6 (q)	H-21		H-10, H-21

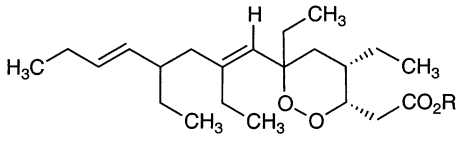
[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. [b] ^{13}C NMR multiplicities were obtained from a DEPT experiment. [c] Protons correlated to carbon resonances in ^{13}C column.

and between H-4_{ax} and H-5_{eq} (Table 2). Consistent with these observations, $J_{H-4ax-H-5ax}$ exhibited a large coupling constant (13.0 Hz) expected for interactions between axial protons, whereas $J_{H-4ax-H-5eq}$ was significantly smaller (4.0 Hz). This agreed with a 3,4-*cis* disubstituted 1,2-dioxane ring adopting a chair conformation as shown in Figure 1. In addition, NOEs between H-7 and H-4 and between H-5_{ax} (δ 1.27) and one H_2-17 proton (δ 1.56) allowed establishment of the axial orientation of the larger alkyl chain at C-6. Finally, the *E* geometry of Δ^{11} was assigned on the basis of a large coupling constant (J = 15.3 Hz) between H-11 (δ 5.11) and H-12 (δ 5.37), whereas the Δ^7 trisubstituted double bond was determined as *E* because of intense NOEs between H-7 (δ 5.14) and both H_2-9 (δ 2.13 and 1.99), as well as between H_2-17 (δ 1.56 and 1.23) and H_2-19 (δ 2.20). Furthermore, since the ^{1}H NMR spectrum of the peroxy ring in **4** is almost identical to that of plakortide M (**3**), the relative stereochemistry at C-3, C-4, and C-6 of **4** must respectively be the same as that conferred on **3**. Thus, it may be concluded that **4** has either an absolute stereochemistry of 3*S*,4*S*,6*R* or the absolute stereochemistry of its enantiomer, 3*R*,4*R*,6*S*. The optical rotation obtained for plakortide N, $[\alpha]_D$ −275.0° (*c* 0.52, $CHCl_3$), is, however, the same in sign and similar in magnitude to the value recorded for plakortide M, $[\alpha]_D$ −249.0° (*c* 1.4, $CHCl_3$). Coupling these findings with the likelihood that the optical rotations of these compounds tend to be governed by their peroxy ring moiety,[11] it is quite possible that the peroxy ring of **4** has the same absolute stereochemistry as **3**. A side-by-side comparison of the structure and optical rotation of plakortide F (**2**), $[\alpha]_D$ −159.5° (*c* 0.9, $CHCl_3$),[12] with those of **3** and **4** further supports this assignment. Since these compounds share the same biogenetic pathway, it is highly likely that the C-10 ethyl group in **4** is also the same. Accordingly, it is fair to suggest that 3*S*,4*S*,6*R*,10*R* is the most likely absolute configuration of plakortide N.

Except for the optical rotation, the spectral properties of plakortide N (**4**) were similar to those of compounds **8** and **9**, isolated from an Okinawan sponge *Plakortis* sp., $[\alpha]_D$ +49.8° (*c* 0.5, $CHCl_3$),[6] and the Palauan sponge *Plakortis* aff. *angulospiculatus*, $[\alpha]_D$ +164° (*c* 2.4, $CHCl_3$),[13] respectively. From these data, in particular the different polarimetric properties, we surmise that **4**, **8**, and **9** should be diastereomeric. On the other hand, the spectral properties and optical rotation of **4a** (the methyl ester of plakortide N obtained by methylation with CH_2N_2), $[\alpha]_D$ −151.3° (*c* 1.4, $CHCl_3$), are in close agreement with those of **10**, $[\alpha]_D$ −224° (*c* 1.0, $CHCl_3$), isolated from a specimen of *P. halichondrioides* from Belize.[3] These data suggest that compound **10** (reported with incomplete relative stereochemistry as shown) could in fact be the same as plakortide N methyl ester.



8



9 R = H
10 R = CH_3

Plakortides M (**3**) and N (**4**) were evaluated in the National Cancer Institute (NCI) three-cell line, one-dose, primary anticancer assay and subsequently in the NCI's in vitro antitumor screen consisting of 60 human tumor cell lines. These compounds exhibited potent cytotoxic activity against a number of cancer cell lines but were not selective (Table 3). In vitro antituberculosis screening of plakortide M methyl ester (**3a**) and plakortide N methyl ester (**4a**) against *Mycobacterium tuberculosis* H_{37}Rv at a

Table 3. Selected in Vitro Antitumor Screen Data of Compounds **3** and **4**

panel/cell line (IC_{50} μM)	**3**	**4**
leukemia		
CCRF-CEM	0.2	0.1
RPMI-8226	1.3	<0.01
SR	0.4	0.1
non-small cell lung cancer		
A549/ATCC	1.8	0.1
HOP-62	1.6	0.9
NCI-H460	1.8	0.2
colon cancer		
COLO 205	1.5	1.3
HCT-116	2.0	0.4
HT29	1.4	0.5
CNS cancer		
SF-295	1.8	0.3
SF-539	1.8	1.5
U251	1.7	0.5
melanoma		
LOX IMVI	0.5	0.03
M14	6.8	1.6
SK-MEL-2	11.1	1.7
ovarian cancer		
IGROV1	0.6	0.06
OVCAR-3	2.0	1.1
SK-OV-3	1.3	1.9
renal cancer		
786-0	1.6	1.3
ACHN	1.7	0.2
UO-31	1.4	0.01
prostate cancer		
PC-3	1.6	0.3
DU-145	1.9	0.4
breast cancer		
MCF7	2.9	1.8
NCI/ADR-RES	1.8	0.4
T-47D	2.2	1.3

concentration of 6.25 μg/mL showed no significant inhibitory activity. Compound **3a** also proved to be inactive as a potential inhibitor of the cell cycle regulators cdc2/cyclin B kinase and cdc25 phosphatase.[14] On the other hand, cyclic peroxide esters **3a** and **4a** were tested for the inhibition of *Plasmodium falciparum*, the parasite responsible for the most severe forms of malaria.[15] Among these, compound **3a** demonstrated the most toxic effect (IC_{50} 8 μg/mL). Interestingly, it seems that the presence of an ethyl group at C-8 in **4a** leads to a significant loss in antimalarial activity (i.e., for **4a** the $IC_{50} \geq 50$ μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter and the infrared spectra with a Nicolet Magna 750 FT-IR spectrophotometer. ^{1}H NMR spectral data were generated with a 500 MHz Bruker DPX-500 FT-NMR spectrometer, and the ^{13}C NMR spectral data and ^{1}H−^{1}H COSY, NOESY, DEPT, HMQC, and HMBC experiments were measured with a 300 MHz Bruker DPX-300 FT-NMR spectrometer. FABMS were carried out in a VG AutoSpec (Fisons). GC/MS analyses were recorded at 70 eV using a Hewlett-Packard 5972A MS ChemStation equipped with a 30 m × 0.25 mm special performance capillary column (HP-5MS) of polymethylsiloxane cross-linked with 5% phenyl methylpolysiloxane. Analyses were performed using the following conditions: initial temperature, 130 °C; rate of increase, 3 °C/min; final temperature, 260 °C. Column chromatography was performed on Si gel (35−75 mesh). TLC analyses were carried out using glass Si gel plates, and spots were visualized by exposure to I_2 vapors or heating Si gel plates sprayed with 5% H_2SO_4 in EtOH. Diazomethane was prepared in-house according to literature procedures.[16] Diazald, $NaBH_4$, (*R*)- and (*S*)-MTPA acid, DMAP, and DCC were purchased from Aldrich Chemical Co. All solvents used were spectral grade or were distilled from glass prior to use. Lowest energy conformers were searched using the MMFF force field implemented in the McSpartan *Pro* program (Wavefunction, Inc.). The trivial names assigned to compounds **3** and **4** are based on previous work by Patil and Hamann.[5,17] The percentage yield of each compound is based on the weight of the dry sponge MeOH−$CHCl_3$ extract.

Collection, Extraction, and Isolation. The sponge specimen was collected by scuba from shallow reef waters off Mona Island, Puerto Rico, in April 1992 and frozen shortly after collection. A taxonomic reference specimen is deposited at the Chemistry Department of the University of Puerto Rico, sample number MI-III-Ph-1992. The freeze-dried animal (406 g) was cut into small pieces and blended with 1:1 MeOH−$CHCl_3$ (3 × 1 L). The combined organic extracts were filtered and then concentrated, and the residue obtained (69 g) was partitioned between hexane (3 × 300 mL) and water (200 mL). Rotoevaporation of the combined hexane extract followed by overnight storage under high vacuum produced 29.6 g of a dark brown, oily residue. A portion of the hexane extract (16.4 g) was flash-chromatographed over Si gel (116 g) with mixtures of hexane−acetone of increasing polarity (0−100%) and then with mixtures of acetone−MeOH (0−100%). Fractions were pooled on the basis of their TLC and NMR profile to yield 22 primary fractions, denoted as I−XXII. Fraction IV (2.7 g) was purified on a Si gel (130 g) column using 100% hexane as eluant, affording known methyl esters **1** (953 mg; 2.5% yield)[3] and **2** (76 mg; 0.2% yield).[4,5] Fraction V (2.0 g) was rechromatographed over Si gel (60 g) and eluted with a 10% mixture of hexane−$CHCl_3$ to yield nine subfractions. Subfraction 5 (208 mg) was subjected twice to column chromatography over Si gel (8 g) using 15% EtOAc in hexane laced with a few drops of acetic acid, affording plakortide N (**4**) (38.8 mg; 0.1% yield). Successive column chromatography of fraction VI (2.4 g) on Si gel (83 g) with 30% hexane in $CHCl_3$ and then Si gel (5 g) with a mixture of 15% EtOAc in hexane containing a few drops of acetic acid yielded plakortide M (**3**) (316 mg, 0.8% yield).

Methyl (2*Z*,6*R*,8*R*,9*E*)-3,6-Epoxy-4,6,8-triethyl-2,4,9-dodecatrienoate (1): The $[\alpha]_D$, ν_{max}, λ_{max}, ^{1}H and ^{13}C NMR, and HREIMS were identical in all respects to those previously reported in the literature.[3]

Plakortide F (2). The $[\alpha]_D$, IR, and ^{1}H ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra of cyclic peroxide **2** were essentially identical in all respects to those previously reported in the literature.[4,5]

Plakortide M (3) [(3*S*,4*S*,6*R*,7*E*,10*R*,11*E*)-3,6-epidioxy-4,6,10-triethyltetradeca-7,11-dienoic acid]: colorless oil; $[\alpha]_D^{24}$ −249.0° (*c* 1.4, $CHCl_3$); IR (film) 3600−3000 (br), 2967, 2933, 2876, 1718, 1462, 1379, 1291, 1189, 1007, 969 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.46 (overlapped m, 2H, H-7 and H-8), 5.38 (dt, 1H, J = 6.3, 15.3 Hz, H-12), 5.14 (dd, 1H, J = 8.4, 15.3 Hz, H-11), 4.42 (dt, 1H, J = 3.8, 9.0 Hz, H-3), 3.04 (dd, 1H, J = 9.6, 15.8 Hz, H-2), 2.41 (dd, 1H, J = 3.4, 15.8 Hz, H-2′), 2.19−1.85 (complex m, 6H, H-4, H-9, H-9′, H-10, H-13, H-13′), 1.77 (dd, 1H, J = 4.1, 13.5 Hz, H-5), 1.26 (dd, 1H, J = 12.9, 13.5 Hz, H-5′), 1.56−1.08 (complex m, 6H, H-15, H-15′, H-17, H-17′, H-19, H-19′), 0.95 (t, 3H, J = 7.4 Hz, Me-14), 0.90 (t, 3H, J = 7.4 Hz, Me-20), 0.83 (t, 3H, J = 7.3 Hz, Me-16), 0.81 (t, 3H, J = 7.6 Hz, Me-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.6 (s, C-1), 31.4 (t, C-2), 78.6 (d, C-3), 34.8 (d, C-4), 32.6 (t, C-5), 83.6 (s, C-6), 132.9 (d, C-7), 130.0 (d, C-8), 38.2 (t, C-9), 44.5 (d, C-10), 132.9 (d, C-11), 132.1 (d, C-12), 25.6 (t, C-13), 14.1 (q, C-14), 27.5 (t, C-15), 11.6 (q, C-16), 33.1 (t, C-17), 7.1 (q, C-18), 24.9 (t, C-19), 10.9 (q, C-20); HRFAB-MS (3-NBA) m/z $[M + Na]^+$ calcd for $C_{20}H_{34}O_4Na$ 361.2355, found 361.2367.

Methylation of Plakortide M (3). To a solution of plakortide M (**3**) (31.6 mg, 0.093 mmol) in $CHCl_3$ (10 mL) was added a solution of diazomethane in ether (20 mL), and the resulting mixture was stirred at 25 °C for 1 h. Excess reagent and solvents were removed by rotoevaporation, and following overnight storage under high vacuum, the oily residue obtained was purified by Si gel (1.6 g) column chromatography with 5% acetone in hexane to yield plakortide M methyl ester

(**3a**) as a colorless oil (31 mg, 94%): $[\alpha]_D^{24}$ −343.0° (*c* 0.72, $CHCl_3$); IR (film) 2963, 2928, 2874, 2852, 1741, 1594, 1461, 1436, 1379, 1281, 1194, 970 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HRFAB-MS (3-NBA) *m/z* $[M + Li]^+$ calcd for $C_{21}H_{36}O_4Li$ 359.2773, found 359.2786.

Reduction of 3a. A solution of **3a** (8.8 mg, 0.025 mmol) in MeOH (4 mL) was stirred under H_2 and 10% Pd on charcoal at room temperature for 10 h. The filtered solution was dried under vacuum to afford pure compound **5** (7.9 mg, 88%): colorless oil; $[\alpha]_D^{20}$ −44.2° (*c* 1.2, $CHCl_3$); IR (film) 3316 (br), 2959, 2875, 2862, 1742, 1463, 1439, 1376, 1294, 1175, 1061, 1022, 978 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.18 (dt, 1H, *J* = 2.7, 10.9 Hz, H-3), 3.71 (s, 3H, $-CO_2CH_3$), 2.58 (dd, 1H, *J* = 10.8, 16.2 Hz, H-2), 2.36 (dd, 1H, *J* = 2.1, 16.2 Hz, H-2′), 1.89 (m, 1H, H-4), 1.54−1.45 (br envelope), 1.38 (dd, 1H, *J* = 2.5, 15.2 Hz, H-5), 1.30−1.12 (br envelope), 0.94 (t, 3H, *J* = 7.4 Hz, Me-16), 0.88 (t, 6H, *J* = 7.2 Hz, Me-14 and Me-18), 0.83 (t, 3H, *J* = 7.1 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.1 (s), 73.7 (s), 70.5 (d), 51.8 (q), 39.8 (d), 38.8 (d), 38.5 (t), 37.8 (t), 35.8 (t), 33.8 (t), 33.1 (t), 32.8 (t), 28.9 (t), 26.6 (t), 26.0 (t), 23.1 (t), 21.2 (t), 14.1 (q), 12.5 (q), 10.9 (q), 7.8 (q); EIMS *m/z* 311 $[M - H_2O - C_2H_5]^+$ (11), 200 (11), 199 (92), 167 (11), 144 (18), 142 (24), 103 (24), 83 (23), 69 (43), 57 (100).

Absolute Stereochemistry of 3a at C-3. Preparation of (*R*)- and (*S*)-MTPA Esters 5ab. To a solution of diol **5** (9.4 mg, 0.026 mmol), dicyclohexylcarbodiimide (27.0 mg, 0.13 mmol), and (dimethylamino)pyridine (9.9 mg, 0.081 mmol) in dry CH_2Cl_2 (4 mL) was added either the (*R*)- or the (*S*)-MTPA acid (30.7 mg, 0.13 mmol). After stirring under N_2 at room temperature for 15 h the resulting white suspension was filtered to remove the *N*,*N*′-dicyclohexylurea and the mother liqueurs were diluted with CH_2Cl_2 (6 mL) and washed with water (3 × 2 mL). The organic layer was washed once with 3 mL each of 0.1 N HCl, water, and saturated $NaHCO_3$ solution and then concentrated to an oil, which, after purification on Si gel (hexane/EtOAc, 9.5:0.5), afforded either the (*R*)-MTPA ester **5a** (4.7 mg, 31.2%) or the (*S*)-MTPA ester **5b** (4.9 mg, 32.5%) as a colorless oil. The $\Delta\delta$ values, where $\Delta\delta = \delta_S - \delta_R$, were measured: H-2 −0.05; H-2′ −0.04; H-3 +0.02; H-4 +0.08; H_2-5 +0.10; H_2-8 +0.07; H_2-15 +0.01; H_3-16 +0.04; H_2-17 +0.03; H_3-18 +0.01; H_2-19 −0.03; H_3-20 −0.01; $-CO_2CH_3$ −0.04.

Ozonolysis of Compound 3a. A stream of O_3 was bubbled into MeOH (5 mL) at −78 °C until a blue-colored solution resulted. A portion of this solution (3 mL) was added to a solution of **3a** (18.6 mg, 0.053 mmol) in MeOH (5 mL) kept under N_2 at −78 °C. After stirring for 7 min excess O_3 was removed upon bubbling N_2 for 5 min and the colorless solution was treated with excess $NaBH_4$ and stirred at −78 °C for 20 min. After allowing the reaction mixture to warm to room temperature it was treated with AcOH (1 mL) and concentrated to dryness, and the residue obtained was partitioned between $CHCl_3$ and water (3 × 4 mL). Evaporation of the solvent gave a residue (15.9 mg) that was purified on Si gel (hexane/EtOAc, 4:1) to yield a mixture of alcohols **6** (11.1 mg, 64%) and **7** (3.0 mg, 23%).

Compound 6: colorless oil; $[\alpha]_D^{20}$ −146.8° (*c* 1.3, $CHCl_3$); IR (film) 3455 (br), 3031, 2960, 2925, 2880, 1739, 1463, 1434, 1160, 1046, 971 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.58 (dt, 1H, *J* = 6.4, 16.1 Hz, H-8), 5.51 (d, 1H, *J* = 16.1 Hz, H-7), 4.46 (m, 1H, H-3), 3.70 (s, 3H, $-CO_2CH_3$), 3.56 (dd, 2H, *J* = 1.7, 5.6 Hz, H_2-11), 3.02 (dd, 1H, *J* = 9.7, 15.5 Hz, H-2), 2.37 (dd, 1H, *J* = 3.5, 15.5 Hz, H-2′), 2.18−2.11 (br envelope), 1.77 (dd, 1H, *J* = 4.2, 13.5 Hz, H-5), 1.62−1.15 (br envelope), 0.91 (t, 6H, *J* = 7.3 Hz, Me-13 and Me-17), 0.83 (t, 3H, *J* = 7.5 Hz, Me-15); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.1 (s, C-1), 133.6 (d, C-7), 129.8 (d, C-8), 83.4 (s, C-6), 78.9 (d, C-3), 65.3 (t, C-11), 51.9 (q, $-CO_2CH_3$), 42.3 (d, C-10), 35.1 (d, C-4), 34.2 (t, C-9), 33.2 (t, C-14), 32.7 (t, C-5), 31.4 (t, C-2), 25.0 (t, C-16), 23.3 (t, C-12), 11.3 (q, C-17), 11.0 (q, C-13), 7.1 (q, C-15); EIMS *m/z* 215 $[M - C_7H_{13}O]^+$ (1), 197 (1), 169 (1), 141 (16), 101 (7), 85 (12), 71 (8), 57 (100).

Compound 7: colorless oil; IR (film) 3380 (br), 2962, 1741 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.53 (ddd, 1H, *J* = 3.6, 5.4, 9.7 Hz), 4.14 (br d, 1H, *J* = 11.7 Hz), 3.73 (s, 3H, $-CO_2CH_3$), 3.40 (br d, 1H, *J* = 11.7 Hz), 3.04 (dd, 1H, *J* = 9.8, 15.6 Hz), 2.41 (dd, 1H, *J* = 3.5, 15.6 Hz), 2.14 (m, 1H), 1.67−1.09 (br envelope, 7H), 0.92 (t, 3H, *J* = 7.1 Hz), 0.90 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.0 (s), 82.6 (s), 78.7 (d), 61.7 (t), 52.0 (q), 34.6 (d), 31.1 (t), 29.5 (t), 27.6 (t), 25.4 (t), 11.0 (q), 7.0 (q); EIMS *m/z* 215 $[M - CH_3O]^+$ (1), 141 (13), 109 (4), 101 (6), 85 (10), 57 (100).

Absolute Stereochemistry of Compound 3a at C-10. To a solution of alcohol **6** (10.4 mg, 0.032 mmol), dicyclohexylcarbodiimide (33 mg, 0.16 mmol), and (dimethylamino)pyridine (12.1 mg, 0.099 mmol) in dry CH_2Cl_2 (4 mL) was added either the (*R*)- or the (*S*)-MTPA acid (37.5 mg, 0.16 mmol). After stirring under N_2 at room temperature for 15 h the resulting white suspension was filtered to remove the *N*,*N*′-dicyclohexylurea and the mother liqueurs were diluted with CH_2Cl_2 (8 mL) and washed with water (2 × 4 mL). The organic layer was then washed once with 4 mL each of 0.1 N HCl, water, and saturated $NaHCO_3$ solution and concentrated to an oil, which, after purification on Si gel ($CHCl_3$), afforded either the (*R*)-MTPA ester **6a** (8.7 mg, 50.4%) or the (*S*)-MTPA ester **6b** (15.8 mg, 91.6%) as a colorless oil. Partial ^1H NMR ($CDCl_3$, 300 MHz) data for **6a**: δ 4.32 (dd, 1H, *J* = 5.6, 10.8 Hz, H-11), 4.16 (dd, 1H, *J* = 5.4, 10.8 Hz, H-11′), 1.73 (m, 1H, H-10), 1.18 (m, 2H, H-16), 0.88 (t, 3H, *J* = 7.5 Hz, Me-17). Partial ^1H NMR ($CDCl_3$, 300 MHz) data for **6b**: δ 4.26 (dd, 1H, *J* = 5.4, 10.9 Hz, H-11), 4.21 (dd, 1H, *J* = 5.6, 10.9 Hz, H-11′), 1.74 (m, 1H, H-10), 1.18 (m, 2H, H-16), 0.90 (t, 3H, *J* = 7.4 Hz, Me-17).

Plakortide N (4) [(3*S*,4*S*,6*R*,7*E*,10*R*,11*E*)-3,6-epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid]: colorless oil; $[\alpha]_D^{24}$ −275.0° (*c* 0.52, $CHCl_3$); IR (film) 3400−3000 (br), 2961, 2932, 2879, 2853, 1711, 1459, 1289, 1193, 968 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 2); HRFAB-MS (3-NBA) *m/z* $[M + Na]^+$ calcd for $C_{22}H_{38}O_4Na$ 389.2668, found 389.2678.

Methylation of Plakortide N (4). To a solution of **4** (20.3 mg, 0.055 mmol) in $CHCl_3$ (10 mL) was added a solution of diazomethane in ether (15 mL). After the mixture was stirred at 25 °C for 1 h, excess reagent and solvents were removed by rotoevaporation. The oily residue left over was purified on a Si gel (0.7 g) column with 1% acetone in hexane to afford methyl 3,6-epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoate (**4a**): colorless oil; $[\alpha]_D^{20}$ −151.3° (*c* 1.4, $CHCl_3$). The ^1H ($CDCl_3$, 300 MHz) and ^{13}C NMR (C_6D_6, 75 MHz) spectra of methyl ester **4a** were similar in all respects to those previously reported for cyclic peroxide ester **10**.[3]

Reduction of Plakortide F (2). A solution of **2** (6.6 mg, 0.018 mmol) in MeOH (3 mL) was stirred under H_2 and 10% Pd on charcoal at room temperature for 10 h. The filtered solution was dried under vacuum to afford a pure colorless oil (7.7 mg, 76%). The optical rotation, GLC and TLC retention times, EIMS, and the ^1H ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra of this product were identical in all respects to those already described for diol **5**.

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