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THE STRUCTURES AND CYTOTOXIC PROPERTIES OF POLYKETIDE PEROXIDES FROM A *PLAKORTIS* SPONGE

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ABSTRACT.—A morphologically distinct Fijian sponge, *Plakortis* sp., has yielded two new peroxides, plakortolide E [**5**] and plakoric acid [**12**]. After standing for approximately one year, plakortolide E rearranged to plakortolide ether [**10**]. The structures of plakortolide E [**5**] and plakortolide ether [**10**] were established from 2D nmr data and by analogy to a known compound, plakortolide [**3**]. The stereochemistry of the bicyclic ring substituents of **5** was established using nOe and NOESY nmr data along with comparisons to **3**. The absolute stereochemistry at the three chiral sites of **5** was assigned by preparing acyclic compounds **6–9**, and both **8** and **9** were investigated using the modified Mosher's method. This represents the first absolute stereochemistry determination for a sponge-derived polyketide peroxide. The characterization of plakoric acid [**12**] was based on spectral analogies to known polyketides such as plakortin. Plakortolide E [**5**] exhibited selective potency against the melanoma and breast tumor cell lines in the in vitro 60-cell line panel of the National Cancer Institute.

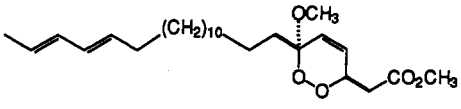
A number of years ago we reported the structures and properties of polyketide six-membered unsaturated ring peroxides, the xestins[1] [**1**] (1). Cyclic peroxides (2–11), or their ring opened analogues (11), continue to emerge from the chemical study of *Plakortis* sponges (family Plakinidae), which are prominent members of both Caribbean and Indo-Pacific coral reefs (12,13). Many *Plakortis*-derived peroxides have a 1,2-dioxane ring with such groups as an acetic acid moiety at C-3 and an aliphatic chain (sometimes terminating with a phenyl ring) at C-6. Plakortin, from the Caribbean sponge *Plakortis halichondriodes*, was the first compound reported as having these structural features (5). Several related metabolites have also been described and a few examples are an unstable aromatic compound (7), plakinic acid A from a non-*Plakortis* sponge in the family Plakinidae (8), an unnamed acid (11), and an unnamed bis cyclic peroxide (4). Several slightly different polyketide peroxides have terminal butenolide moieties. Examples include compound **2**, isolated from a Caribbean *Plakortis* sp., as the initial member of this group to be described (7) and plakortolide [**3**] from a Pacific *Plakortis* sp. (10). The isolation of additional plakortolides B [**4**], C, and D from a Caribbean sponge, *Plakinastrella* cf. *onkodes*, further extends this structural pattern (14).

We have maintained a continuing interest in these compounds because our sample of xestin A [**1**] showed significant cytotoxicity in the NCI in vitro "disease oriented screen" (15). The present study began with the goal of isolating other peroxides from the fractions of a Fijian *Plakortis* sponge which were active in vitro against melanoma cancer cell lines. We now describe the structures of two new peroxy-containing metabolites, plakortolide E [**5**] and plakoric acid [**12**], and report on the cytotoxic properties of plakortolide E.

RESULTS AND DISCUSSION

To date we have only found the *Plakortis* sponge in question from the Fijian habitat known as "the great white wall" of Taveuni. The sponge was collected, preserved, and

[1]Our sponge material which provided the xestins and a simple butenolide was misidentified as a *Xestospongia* (1). Re-examination of the voucher material shows it to be a *Plakortis* sp., making our results consistent with the taxonomy and parallel chemistry reported by others.



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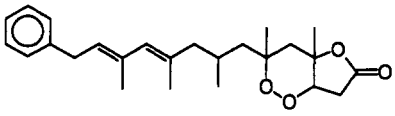

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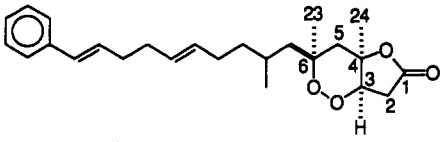

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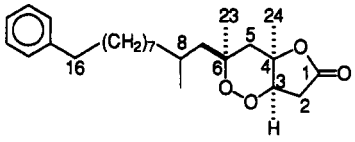

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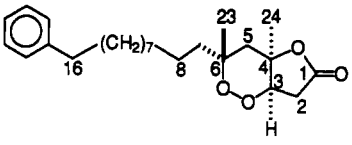

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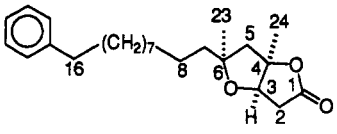

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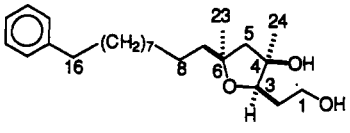
*Absolute stereochemistry not implied.

returned to our laboratory as described in the Experimental. Subsequent extraction with MeOH, followed by a series of solvent partitions, yielded an oil (0.99 g) which, after additional purification, afforded plakortolide E [**5**] (76.7 mg) and plakoric acid [**12**] (19.3 mg). It was found that some samples of plakortolide E [**5**], stored as neat oils for a period of approximately one year, underwent a rearrangement and afforded plakortolide ether [**10**].

The major structural features of plakortolide E [**5**] were defined from ^{13}C-nmr resonances (Table 1). The primary functionality consisted of a monosubstituted benzene ring, a peroxide [δ 73.8 d (C-3), 72.9 s (C-6)], and an ester [δ 175.2 s (C-1), 90.1 s (C-4)]. From the outset, the highest hreims spectral peak at *m/z* 372.2664, assigned as $C_{24}H_{36}O_5$ (Δ 0.1 mmu of calcd), was suspected to be from an $M^+ - O$ fragmentation (16). The benzene ring carbons (C_6H_5) plus the additional ^{13}C-nmr APT count of $C_{18}H_{31}$ indicated that no heteroatom protons were present. A bicyclic skeleton like that in compounds **2–4** accounted for the remaining elements of unsaturation. This was consistent with a ^{1}H-nmr (Table 2) AMX multiplet pattern for H-3 (δ 4.19), H-2 (δ 2.93), and H-2′ (δ 2.55) (^3J=1.8 and 6.7 Hz and ^2J=18.3 Hz); the AB pattern for H-



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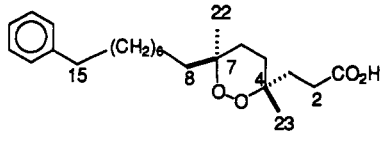

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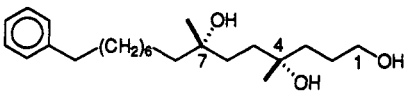

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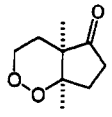


14*

*Absolute stereochemistry not implied.

TABLE 1. ^{13}C-Nmr Shifts of Selected Peaks in $CDCl_3$ and C_6D_6 for Compounds **2–5** and **10**.

Carbon	Compound					
	2[a]	**3**[b]	**4**[c]	**5**	**10**	**10**
	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)	(C_6D_6)	($CDCl_3$)
2		34.2	34.2	38.1	36.4	36.5
3	81.3	80.9	81.6	73.8	81.1	81.0
4	82.5	82.8	82.8	90.1	93.1	94.5
5		42.0	42.1	43.9	49.3	49.1
6	80.4	80.7	80.7	72.9	84.2	84.8
Me-23		24.9	24.9	29.9	23.1	23.6
Me-24		25.9	25.9	26.9	25.9	26.1
16	NA	NA	NA	35.9	36.3	35.9

[a]Data taken from Stierle and Faulkner (7).
[b]Data taken from Davidson (10).
[c]Data taken from Horton *et al.* (14).

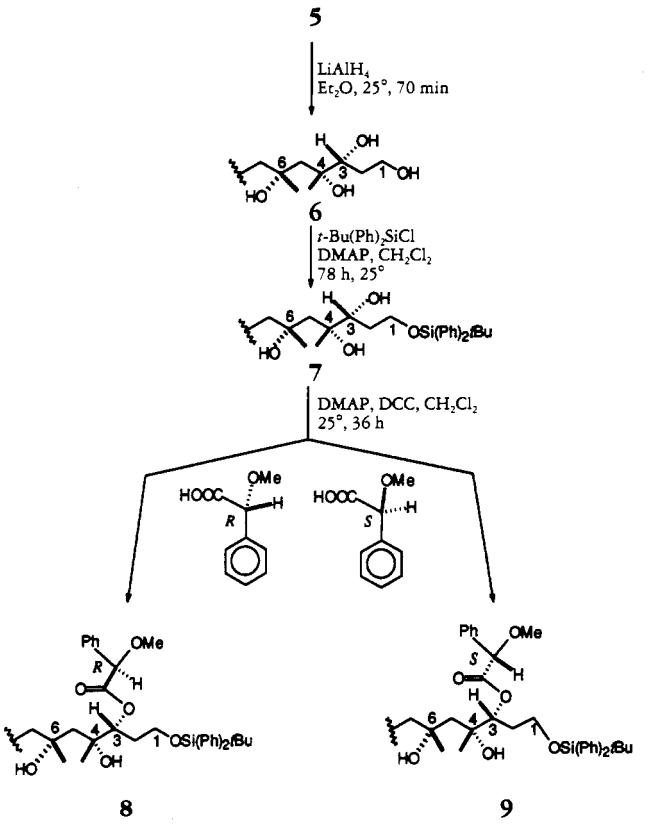
5 (δ 2.15) and H-5′ (δ 2.09); and long-range ^{1}H-^{13}C COSY correlations from H-5/-5′ to C-23 and C-24. A combination of ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY (J=140 Hz and J=9 Hz) correlations justified the assignment of all ^{1}H- and ^{13}C-nmr resonances, except those which were overlapping for atoms 9 to 14. The smooth conversion of **5** to tetraol **6** by $LiAlH_4$ reduction, shown in Scheme 1, confirmed the presence of the peroxide. The assignment of four oxygens in **6** was justified by the lrfabms peak at *m/z* 395 ($[M+1]^+$, $C_{24}H_{42}O_4$) and four characteristic ^{13}C-nmr signals [δ 78.5 (d), 75.3 (s), 74.1 (s), 61.4 (t)]. The upfield shift of C-4 from δ 90.1 in **5** to δ 75.3 or 74.1 in **6** was consistent with the loss of a β-substituent increment additivity effect accompanying the opening of the peroxide.

The relative stereochemistry of **5** was determined by nOe and NOESY data and comparisons to the literature data of the other plakortolides (7,10,14). The cis junction of the ring system was determined by the strong NOESY cross-peaks between H-3 and Me-24 in either $CDCl_3$ or C_6D_6. A comparison of the ^{13}C-nmr shifts of C-6, C-4, C-3, Me-23, and Me-24 of compounds **2–4** (Table 1) against the ^{13}C-nmr shifts of the same nuclei of plakortolide E [**5**] (Table 1) indicated that the bicyclic ring of **5** must have a diastereomeric configuration relative to that of **2–4**. Furthermore, the downfield shift of Me-23 from δ 24.9 of **3** and **4** to δ 29.9 of **5** indicated that this group had moved from an axial to an equatorial position (17), and justified the relative stereochemistry shown here.

TABLE 2. ^{1}H-Nmr Shifts of Selected Peaks in $CDCl_3$ for Compounds **2–5** and **10**.

Proton	Compound				
	2[a]	**3**[b]	**4**[c]	**5**	**10**
2	2.90	2.89	2.90	2.93	2.78
2′	2.58	2.58	2.60	2.55	2.65
3	4.45	4.44	4.45	4.19	4.35
5	2.21	2.19	2.12	2.15	2.40
5′	1.70	1.67	1.70	2.09	1.87
Me-23	1.25	1.22	1.25	1.35	1.24
Me-24	1.36	1.35	1.37	1.44	1.51
16	NA	NA	NA	2.6	2.6

[a]Data taken from Stierle and Faulkner (7).
[b]Data taken from Davidson (10).
[c]Data taken from Horton *et al.* (14).

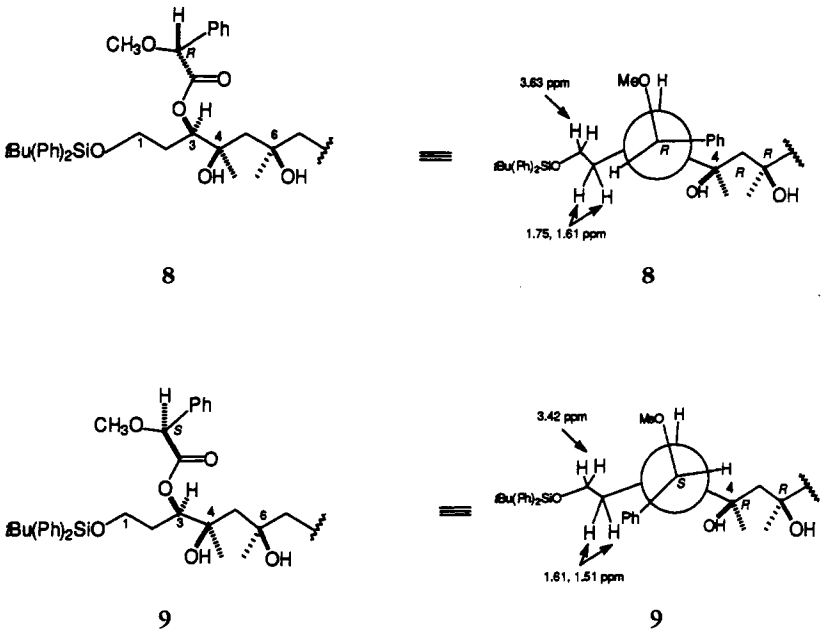


SCHEME 1. Preparation of *O*-Me mandelate derivatives.

The modified Mosher's empirical shift correlation method using *O*-methyl mandelate derivatives was next employed to determine the absolute stereochemistry of **5** (18–20). The progression of the synthetic work is shown in Scheme 1 and it afforded secondary esters of (*R*) and (*S*) *O*-methoxy mandelic acids as compounds **8** and **9**. In order to eliminate the concern of more than one methoxy mandelate ester forming from tetraol **6** this compound was first protected as the *t*-butyldiphenylsilyl ether **7**. Further treatment of **7** with either the *R*(+) or *S*(−) enantiomers of *O*-methyl mandelic acids gave the diastereomers **8** or **9**, respectively (Scheme 1).

The diagnostic resonances shown in Figure 1 include those of the (*R*) mandelate **8** – δ 3.63 (H_2-1) and δ 1.75/1.61 (H-2/-2′); and the (*S*) mandelate **9** – δ 3.42 (H_2-1) and δ 1.61/1.51 (H-2/-2′). The greater shielding of the H_2-1 and H_2-2 protons in the *S*(−) mandelate **9** indicates that the phenyl group of the mandelate is positioned over that side of the molecule which supports assignment of absolute stereochemistry of (*R*)-3 in both **8** and **9** and, employing the relative stereochemistry assigned above, the additional stereochemistry of (*R*)-4 and (*R*)-6 is indicated for **5** and **6–9**.

A particularly perplexing aspect of this work arose when a sample of plakortolide E [**5**] was examined after it had been stored for a prolonged period. That this compound had changed was evident by comparing the ^{13}C-nmr shifts of C-6, C-4, C-3, Me-23, and Me-24 of the original sample (Table 1, **5**) versus the aged sample (Table 1, **10**). The molecular formula, $C_{24}H_{36}O_3$, of this new compound designated as plakortolide ether [**10**] was established by the hrfabms, *m/z* 373.2702 $[M+1]^+$ (Δ 4.1 mmu of calcd). Further chemical investigation of this new compound began with $LiAlH_4$ reduction of



8 8

9 9

FIGURE 1. Results of modified Mosher's method with *O*-methoxy mandelates to determine the absolute stereochemistry of plakortolide E [**5**].

it to generate an ether diol [**11**] of the formula $C_{24}H_{40}O_3$, also established by hrfabms *m/z* 377.3047 $[M+1]^+$ (Δ 0.9 mmu of calcd). Additional confirmation of this formula was provided by observing a mass shift by generating the sodium adduct of **11**, $C_{24}H_{40}O_3Na$ (hrfabms *m/z* 399.2874 $[M+Na]^+$, Δ 0.1 mmu of calcd). The stereochemistry of **10** was determined from the correlation peaks observed from H-3 to both Me-23 and Me-24 in a ROESY nmr experiment. Previous examples of cyclic peroxides undergoing a transformation to cyclic ethers by oxygen rearrangement have been reported (21), but the peroxide oxygens are generally retained as a cyclic ketal or hemi-ketal moiety. Conversion of peroxides to ethers can also be accomplished by using reducing agents (22). In this case, however, the transformation of **5** to **10** by oxygen extrusion does not parallel either of these or any other literature precedents. It appears that the most stable of the C-6 epimers of **10** was formed in the rearrangement; the minimized calculated energies revealed that **10** with the C-6 configuration as shown is 0.64 kcal lower in energy than its C-6 epimer.

The second new metabolite, plakoric acid A [**12**], was isolated as an amorphous brown solid. Analogous to the situation in **5**, the highest hrfabms peak at *m/z* 361.2819, which required the formula of $C_{23}H_{37}O_3$ (Δ 7.6 mmu of calcd), was assumed to represent the $[M+1-O]^+$ ion. The nmr spectra contained evidence for the three elements of functionality consisting of a monosubstituted benzene ring, a peroxide [δ 84.3 s (C-7), 69.3 s (C-4)], and an acid [δ 170.1 s (C-1)]. The two ^{1}H-nmr singlet methyls were assumed to be attached to the peroxide ring and this was confirmed by HMBC correlations from each of these protons to the peroxide carbons. Additional support for the carboxylic acid group was provided by the sharp, intense ir absorption at 1713 cm^{-1} and a broad absorption from 3200–3400 cm^{-1}. The long methylene chain was characterized by broad and intense overlapping nmr signals observed in the ^{1}H- and ^{13}C-nmr spectra. Consistent with the C-2–C-3 and C-5–C-6 groups in **12** were the two isolated spin-systems observed in the ^{1}H-^{1}H COSY spectrum. One spin-system consisted of a

double doublet at δ 2.64 (J=2.5 and 17.5 Hz, H-6) and a doublet at δ 2.40 (J=17.5 Hz, H-6′) which were coupled to another double-doublet at δ 1.93 (J=2.5 and 14.5 Hz, H-5) and a doublet at δ 1.78 (J=14.5 Hz, H-5′). The other spin-system appeared as overlapped multiplets centered at δ 1.59 (H_2-3 and H_2-2). The HMBC correlations from C-6 and C-7 to H_3-22 indicated the position of Me-22. Likewise, HMBC correlations from C-3, C-4, and C-5 to Me-23 allowed the connection of Me-23 to C-4. Overall, these and other HMBC correlations established the connectivities from C-1 to C-8. Reduction

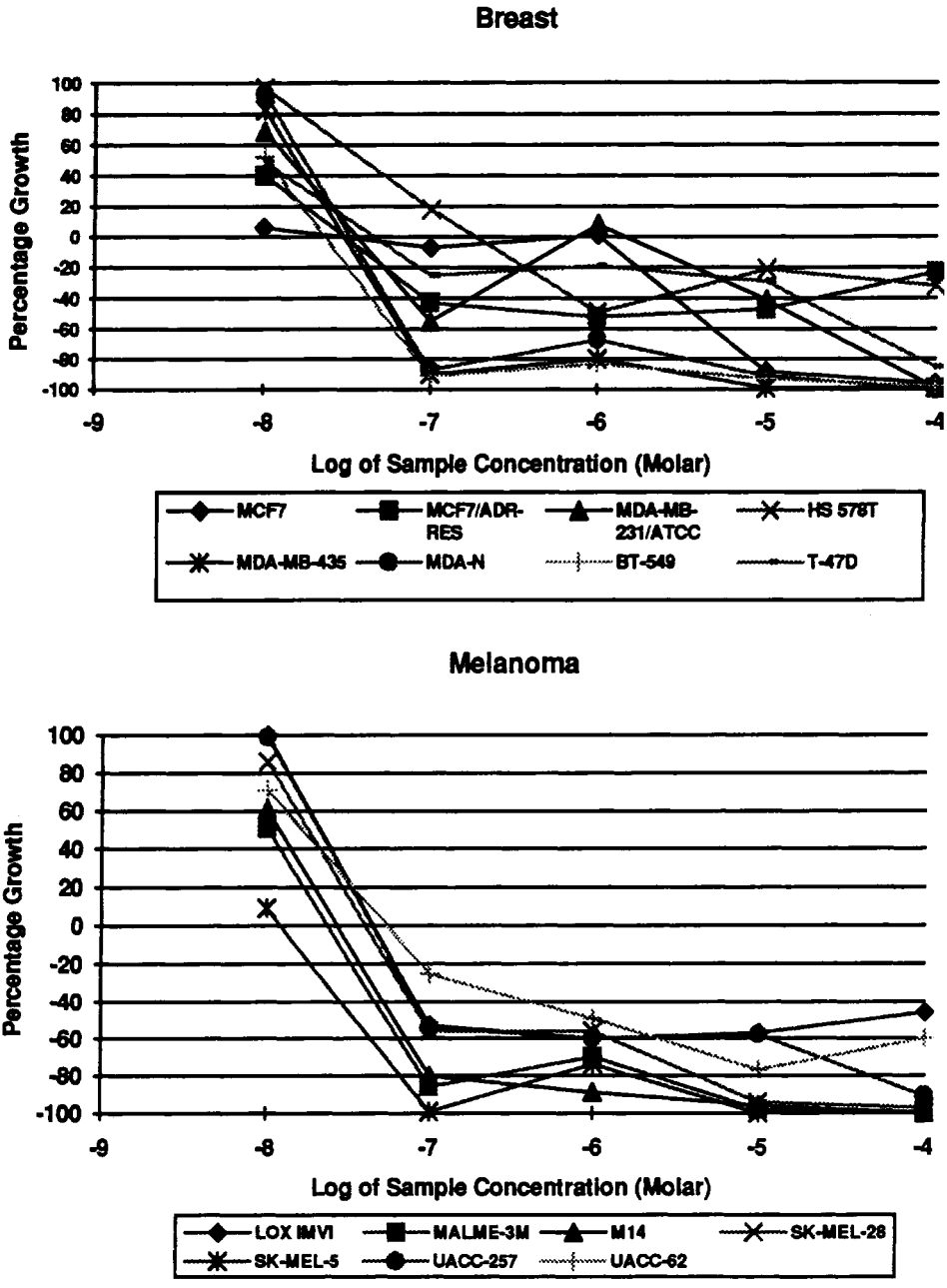


FIGURE 2. Dose-response curves of plakortolide E [**5**] against the NCI library of melanoma and breast cancer cell lines.

of **12** with $LiAlH_4$ afforded the expected triol [**13**], $C_{23}H_{40}O_3$ (hrfabms *m/z* 365.3037 $[M+1]^+$, Δ 1.8 mmu of calcd). In addition, one of the quaternary carbons bearing oxygens shifted upfield in the conversion of **12** to **13** (to δ 75.0 s or 74.5 s). The low-field ^{13}C-nmr resonances of the methyls [δ31.0 q (C-23) and 28.0 q (C-22)] suggests that both of these methyls are in the equatorial position (23).

There are several properties of compounds **5** and **12** that deserve further comment. The presence of the three-carbon chain off the peroxide ring in plakoric acid [**12**] is unprecedented. In a less dramatic fashion, plakortolide E [**5**] extends the structural theme represented in compounds **2–4**. While there have been many prior reports of polyketide cyclic peroxides from sponges, our work provides the first report of their absolute stereochemical features. Surprisingly, the closest synthetic analogy to the bicyclic moiety of peroxides **2–5** is the functionality represented in **14**, a compound prepared by Yoshida *et al.* (24). Plakortolide E [**5**] (NSC 660654) has an interesting in vitro cytotoxicity profile in the NCI in vitro 60-cell line panel whereas plakoric acid [**12**] (NSC 664242) and plakortolide ether [**10**] are completely inactive. Figure 2 shows the behavior of **5** in the melanoma and breast cancer cell lines that are most sensitive to this compound. The dose-response curves in Figure 2 show that **5** is very potent (less than 1 μM for the LC_{50} values) against most of the cells in the melanoma lines and three out of eight cells in the breast lines, while it was not very active in the leukemia panel. Aged samples of **5**, which had previously rearranged to **10**, were inactive in the in vitro 60-cell line panel.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The nmr spectra were recorded at 250, 300, or 500 MHz for ^1H and 62.9, 75.5, or 125.7 MHz for ^{13}C. Multiplicities of ^{13}C-nmr resonances were determined from APT data, DEPT data, or ^1H-^{13}C COSY experiments (300 MHz). Low- and high-resolution eims and fabms data were obtained on a Magnetic sector instrument. Hplc was done with 10 μm C_{18} silica columns. Molecular modeling was performed with PCMODEL 4.0 running on a Personal Iris workstation.

ANIMAL MATERIAL.—The sponge (2.4 kg wet wt), collected from Fiji (coll. no. 92123) was identified as a *Plakortis* sp. (order Homosclerophorida, family Plakinidae) by Ms. M.C. Diaz (U.C. Santa Cruz) and Dr. R.W.M. van Soest (Institute of Taxonomic Zoology, University of Amsterdam). A voucher sample (92123) as well as an underwater photograph are available from the U.C. Santa Cruz archives. The collection was made at a depth of 10–30 m.

Description.—Shape.—Massively encrusting globs 2–3 cm high, oscules had a membrane; *color*—live specimens ranged in color from brown-green to a dark brown, dry specimens were tan; *surface*—smooth; *consistency*—dense and easy to break; *ectosome*—non-detachable; *choanosome*—cavernous with a packed arrangement of spicules; *spicules*—monoaxonic diactineas (100–170, 140, 40, 35 μm)×(4, 1, 2 μm), tetraxon-rays (22×4 μm), only two observed.

EXTRACTION AND ISOLATION.—The sponge was initially preserved by overnight soaking in EtOH-H_2O (1:1). The solution was then discarded. The damp sponge was transported at room temperatures to U.C. Santa Cruz where it was soaked in MeOH (3 liters three times for 72 h). The combined MeOH extracts were concentrated and the resultant oil was partitioned between CH_2Cl_2-H_2O (1:1). The CH_2Cl_2-soluble fraction yielded 5.4 grams of viscous oil which was further partitioned between 10% aqueous MeOH and hexanes. The aqueous MeOH fraction was adjusted to 20% aqueous MeOH and was extracted with CCl_4. The CCl_4-soluble fraction was then evaporated to produce a pale yellow viscous oil (0.99 g).

The CCl_4-soluble fraction was applied to a Sephadex LH-20 column (MeOH) and some of the fractions, further purified over a C_{18} silica flash column (eluted with 15% aqueous MeOH, 100% MeOH, then CH_3CN), followed by C_{18} silica hplc (eluted with 25% aqueous CH_3CN), yielded compounds **5** and **12**. Nmr assignments were made with ^1H-^1H COSY, ^1H-^{13}C COSY, APT, and DEPT experiments.

Plakortolide E [**5**].—A brown waxy solid (76.7 mg, 3%): $[\alpha]_D$ +10.0° (*c*=0.09, CH_2Cl_2); ir (dry film) ν max 2930 (phenyl), 2855 (phenyl), 1763 (C=O, lactone), 1735, 1463, 1281, 1174, 942 cm^{-1}; hreims *m/z* 372.2664 [M^+−O, $C_{24}H_{36}O_3$, Δ 0.1 of calcd]; lreims *m/z* 372 [$M-O_2$]$^+$ (100), 357 (26), 340 (12), 327 (15), 313 (15); ^1H nmr ($CDCl_3$) δ 7.26 (2H, m, H-19, H-21), 7.18 (3H, m, H-18, H-22, H-20), 4.19 (1H, dd, *J*=1.8 and 6.7 Hz, H-3), 2.15 and 2.09 (2H, AB, *J*=15.0 Hz, H-5/H-5′), 2.93 (1H, dd, *J*=6.7 and 18.3

Hz, H-2), 2.55 (1H, dd, J=1.8 and 18.3 Hz, H-2′), 2.60 (1H, t, J=7.4 Hz, H-16), 1.61 (1H, overlapped m, H-7), 1.55 (2H, overlapped m, H_2-15), 1.44 (3H, s, H_3-24), 1.35 (3H, s, H_3-23), 1.29 (br signal for H-7′, and H_2-8 to H_2-14); ^{13}C nmr ($CDCl_3$) δ 175.2 (C-1), 142.9 (C-17), 128.3 (C-19, -21), 128.2 (C-18, -22), 125.5 (C-20), 90.1 (C-4), 73.8 (C-3), 72.9 (C-6), 43.9 (C-5), 43.6 (C-7), 38.1 (C-2), 35.9 (C-16), 31.5 (C-15), 30.0 (C-23), 30.0–29.4 (C-9 to C-14), 26.9 (C-24), 24.4 (C-8). Long-range ^{1}H-^{13}C COSY correlations included correlations from H_3-23 to C-6 and to C-7, from both H_3-23 and H_3-24 to C-5, from H_3-24 to C-4 and to C-3, and from H-2′ to C-1.

Plakortolide ether [**10**].—A brown waxy solid: [α]D +59.9° (c=0.2 CH_2Cl_2); ir (dry film) ν max 2929 (phenyl), 2855 (phenyl), 1777 (C=O, lactone), 1712, 1521, 1264, 1155, 1073, 895, 704 cm^{-1}; hrfabms *m/z* 373.2702 [M$^+$, $C_{24}H_{37}O_3$, Δ 4.1 of calcd]; lrfabms (dithiothreiotol-dithioerythritol, 3:1) *m/z* 373 (M$^+$, 100), 355 (75), 337 (45), 313 (85); ^{1}H nmr ($CDCl_3$) δ 7.26 (2H, m, H-19, H-21), 7.18 (3H, m, H-18, H-22, H-20), 4.35 (1H, d, J=4.2 Hz, H-3), 2.73 (1H, d, J=4.2 Hz, H-2), 2.70 (1H, s, H-2′), 2.60 (2H, t, J=7.7 Hz, H_2-16), 2.40 (1H, d, J=14.3 Hz, H-5), 1.87 (1H, d, J=14.3 Hz, H-5′), 1.60 (2H, m, H_2-15), 1.58 (2H, m, H_2-7), 1.51 (3H, s, H_3-24), 1.26 (br m, H_2-8 to H_2-14), 1.24 (3H, s, H_3-23); ^{13}C nmr ($CDCl_3$) δ 176.0 (C-1), 143.1 (C-17), 128.3 (C-19, -21), 128.2 (C-18, -22), 125.5 (C-20), 94.5 (C-4), 84.8 (C-6), 81.0 (C-3), 49.1 (C-5), 41.7 (C-7), 36.5 (C-2), 35.9 (C-16), 31.5 (C-15), 30.0–29.4 (C-9 to C-14), 26.1 (C-23), 24.5 (C-8), 23.6 (C-24); ^{1}H nmr (C_6D_6) δ 7.24 (2H, m, H-19, H-21), 7.15 (3H, m, H-18, H-22, H-20), 3.70 (1H, d, J=4.9 Hz, H-3), 2.56 (2H, t, J=8.0 Hz, H_2-16), 2.50 (1H, d, J=17.7 Hz, H-2), 2.17 (1H, d, J=13.8 Hz, H-5), 2.16 (1H, dd, J=4.9 and 17.7 Hz, H-2′), 1.61 (2H, overlapped m, H_2-15), 1.58 (2H, m, H_2-7), 1.54 (1H, overlapped d, J=13.8 Hz, H-5′), 1.29 (br m, H_2-8 to H_2-14), 0.99 (3H, s, H_3-23), 0.94 (3H, s, H_3-24); ^{13}C nmr (C_6D_6) δ 173.0 (C-1), 142.0 (C-17), 127.2 (C-19, -21), 127.2 (C-18, -22), 125.0 (C-20), 93.1 (C-4), 84.2 (C-6), 81.1 (C-3), 49.3 (C-5), 42.0 (C-7), 36.4 (C-2), 36.3 (C-16), 31.9 (C-15), 30.0–29.4 (C-9 to C-14), 25.9 (C-23), 24.8 (C-8), 23.1 (C-24).

Plakoric Acid [**12**].—A brown waxy solid (19.3 mg, 0.8%): [α]D −18.7° (c=1.0, CH_2Cl_2); ir (dry film) ν max 3200–3400 (acid OH), 1713 cm^{-1} (C=O, carboxylic acid); hrfabms *m/z* 361.2819 ([M+1−O]$^+$, $C_{23}H_{37}O_3$, Δ 7.6 mmu of calcd); lrfabms (NBA) *m/z* 361 (31), 351 (10), 343 (100), 325 (26), 313 (11); ^{1}H nmr ($CDCl_3$) δ 7.26 (2H, m, H-18, H-20), 7.18 (3H, m, H-17, H-19, H-21), 2.64 (1H, dd, J=2.5 and 17.5 Hz, H-6), 2.61 (2H, t, J=8.0 Hz, H_2-15), 2.40 (1H, d, J=17.5 Hz, H-6′), 1.93 (1H, dd, J=2.5 and 14.5 Hz, H-5), 1.78 (1H, d, J=14.5 Hz, H-5′), 1.59 (4H, overlapped m, H_2-3 and H_2-2), 1.54 (3H, s, H_3-22), 1.38 (3H, s, H_3-23), 1.27 (overlapped m, H_2-9 to H_2-14); ^{13}C nmr ($CDCl_3$) δ 170.1 (C-1), 142.9 (C-16), 128.4 (C-18, -20), 128.2 (C-17, -21), 125.6 (C-19), 84.3 (C-7), 69.3 (C-4), 44.6 (C-3), 44.2 (C-6), 43.7 (C-5), 36.0 (C-15), 31.5 (C-2), 31.0 (C-23), 29.8–29.3 (C-8, C-10 to C-14), 28.0 (C-22), 23.4 (C-9).

Preparation of **6**.—Compound **5** (20 mg), dissolved in dry THF, was placed in a flame-dried flask and $LiAlH_4$ (spatula tip amount) was added. After 70 min at room temperature the reaction was quenched with NH_4Cl (10%) and the THF was evaporated. The resulting solution was filtered through Celite. The solid residue was washed with NaCl (saturated aqueous) solution and then with CH_2Cl_2. The combined aqueous phases were extracted with CH_2Cl_2 (3×0.5 ml). All of the CH_2Cl_2 fractions were combined and evaporated to yield a pale brown waxy solid (18 mg, 90% yield): lrfabms (NBA) *m/z* 395 ([M+1]$^+$, 23), 377 (23), 359 (100), 341 (15), 329 (6), 307 (97); ^{1}H nmr ($CDCl_3$) δ 7.26 (2H, m, H-19, H-21), 7.18 (3H, m, H-18, H-20, H-22), 3.84 (2H, m, H_2-1), 3.67 (1H, d, J=5.3 Hz, H-3), 3.65 (1H, d, J=5.3 Hz, H-3′), 2.60 (4H, overlapped t, J=7.5 Hz, H_2-2 and H_2-16), 1.74 (1H, overlapped H-5), 1.69 (2H, overlapped H_2-8), 1.64 (2H, overlapped H_2-9), 1.56 (1H, overlapped H-5′), 1.56 (1H, overlapped H-7), 1.37 (3H, s, H_3-23 or H_3-24), 1.26 (overlapped H-7′, H_2-10 to H_2-15), 1.26 (3H, s, H_3-24 or H_3-23); ^{13}C nmr ($CDCl_3$) δ 142.9 (C-17), 128.4 (C-19, -21), 128.2 (C-18, -22), 125.6 (C-20), 78.5 (C-3), 75.3 (C-4 or C-6), 74.1 (C-6 or C-4), 61.4 (C-1), 46.8 (C-5), 45.8 (C-7), 36.0 (C-2, -16), 32.5 (C-9), 31.6 (C-15), 30.2–29.4 (C-10 to C-14), 27.9 (C-23 or C-24), 24.0 (C-8), 23.8 (C-23 or C-24).

Preparation of **7**.—Compound **6** (8 mg) dissolved in dry CH_2Cl_2 was mixed with *t*-butyl diphenyl silyl chloride (1 drop) and a trace of DMAP. The reaction mixture was stirred for 78 h at room temperature then washed with 5 ml HCl (1%). The aqueous layer was extracted three times with CH_2Cl_2. The CH_2Cl_2 fractions were combined, dried over $MgSO_4$, and then filtered through Celite. The evaporated filtrate yielded a pale yellow oil which was further purified on a silica column eluted with hexanes-EtOAc (9:1) to afford **7** (4 mg, 30% yield): ^{1}H nmr ($CDCl_3$) δ 7.67 (m, Si-phenyl), 7.44 (m, Si-phenyl), 7.26 (2H, m, H-19 and H-21), 7.17 (3H, m, H-18, H-20, H-22), 3.91 (1H, m, H-3), 3.74 (1H, d, J=2.8 Hz, H-1), 3.71 (1H, d, J=2.8 Hz, H-1′), 2.66 (2H, t, J=7.44 Hz, H_2-16), 1.66 (2H, AB, J=14.5 Hz, H_2-5), 1.61 (3H, overlapped m, H_2-8, H-7), 1.35 (3H, s, H_3-23 or H_3-24), 1.27 (overlapped m, H-7′, H_2-9 to H_2-15, H_3-23 or H_3-24), 1.06 (s, H_9-25); ^{13}C nmr ($CDCl_3$) δ 143.0 (C-17), 135.6, 132.9, 130.0, 128.0, 127.9 (Si-phenyl), 128.4 (C-19, -21), 128.2 (C-18, -22), 125.6 (C-20), 78.8 (C-3), 75.2 (C-6 or 4), 73.4 (C-4 or 6), 63.6 (C-1), 46.9 (C-5), 45.7 (C-7), 36.0 (C-16), 32.7 (C-2), 31.6 (C-9), 30.3–29.3 (C-10 to C-14), 28.2 (C-23 or C-24), 26.9 (C-25), 24.1 (C-24 or C-23), 24.0 (C-8), 19.1 (C-26).

Preparation of mandelate esters **8** *and* **9**.—Both the *R*(+) and *S*(−) mandelic esters **8** and **9** were prepared in the same fashion. Compound **7** (2 mg) was dissolved in CH_2Cl_2 (0.5 ml) and other reagents were added to this solution including DCC (spatula tip amount), DMAP (spatula tip amount), and either the *S*(−)-*O*-methyl mandelic acid or the *R*(+)-*O*-methyl mandelic acid (spatula tip amount). The reaction mixture was stirred for 36 h at room temperature, then filtered and the CH_2Cl_2 was evaporated. The residue was washed 3 times with hexanes, followed by HCl (1 N, 2×0.5 ml), saturated $NaHCO_3$ (2×0.5 ml), and saturated NaCl (2×0.5 ml). The organic phase was next filtered and the solvent was evaporated to yield the mandelate esters mixed with a small amount of **7**. *R*-*O*-Methyl mandelate ester [**8**]: ^{1}H nmr ($CDCl_3$) δ 3.63 (H_2-1), 1.75 (H-2), 1.61 (H-2′), the other signals except for the phenyl signals are overlapped. *S*-*O*-Methyl mandelate ester [**9**]: ^{1}H nmr ($CDCl_3$) δ 3.42 (H_2-1), 1.61 (H-2), 1.51 (H-2′), the other signals except for the phenyl signals are overlapped.

Preparation of **11**.—Into a flame-dried flask was placed compound **10** (5.0 mg) dissolved in dry THF. $LiAlH_4$ (spatula tip amount) was added, and after 70 min at room temperature the reaction was quenched with NH_4Cl (10% aqueous) and the THF was evaporated. The resulting solution was treated, in an analogous manner to the preparaation of **6** described above, to yield **11** as a fine white powder (2.2 mg, 44% yield): hrfabms *m/z* 377.3047 ($[M+1]^+$, $C_{24}H_{41}O_3$, Δ 0.9 calcd), 399.2874 ($[M+Na]^+$, $C_{24}H_{40}O_3Na$, Δ 0.1 mmu of calcd); lrfabms *m/z* 377 (M^+, 14), 359 (100), 341 (22); ^{1}H nmr ($CDCl_3$) δ 7.26 (2H, m, H-19, H-21), 7.18 (3H, m, H-18, H-20, H-22), 3.81 (2H, m, H_2-1), 3.71 (1H, d, J=4.8 Hz, H-3), 2.58 (4H, t, J=7.2 Hz, H_2-16, H_2-2), 1.98 (1H, d, J=14.4 Hz, H-5), 1.87 (2H, m, H_2-15), 1.78 (1H, d, J=14.4 Hz, H-5′), 1.58 (2H, m, H_2-7), 1.29 (m, H_2-8 to H_2-14), 1.22 (3H, s, H_3-24), 1.20 (3H, s, H_3-23); ^{13}C nmr ($CDCl_3$) δ 142.0 (C-17), 128.2 (C-18, -22), 128.0 (C-19, -21), 126.0 (C-20), 84.1 (C-3), 81.5 (C-6), 79.6 (C-4), 61.4 (C-1), 52.6 (C-5), 43.0 (C-7), 36.0 (C-16), 31.5 (C-15), 30.2 (C-2, -8), 29.6 (C-11 to C-14), 29.4 (C-10), 26.2 (C-23), 24.8 (C-9), 23.4 (C-24).

Preparation of **13**.—Into a flame-dried flask was placed compound **12** (4.9 mg) dissolved in dry THF. $LiAlH_4$ (spatula tip amount) was added, and after 70 min at room temperature the reaction was quenched with NH_4Cl (10% aqueous) and the THF was evaporated. The resultant solution was treated as in the above reaction to yield **13** as a fine white powder (4.4 mg, 88% yield): hrfabms (dithiothreiotol-dithioerythritol, 3:1) *m/z* 365.3037 ($[M+1]^+$, $C_{23}H_{40}O_3$, Δ 1.8 mmu of calcd); ^{1}H nmr ($CDCl_3$) δ 7.26 (2H, m, H-19, H-21), 7.18 (3H, m, H-18, H-20, H-22), 3.94 (2H, m, H_2-1), 2.60 (2H, t, J=7.5 Hz, H_2-15), 1.87–1.49 (overlapped H_2-2 to H_2-6, H_2-8), 1.42 (3H, s, H_3-22 or 23), 1.36 (3H, s, H_3-22 or 23), 1.29 (overlapped H_2-9 to H_2-14); ^{13}C nmr ($CDCl_3$) δ 142.9 (C-16), 128.4 (C-18, -20), 128.2 (C-17, -21), 125.6 (C-19), 75.0 (C-7 or C-4), 74.5 (C-4 or C-7), 59.9 (C-1), 49.2 (C-2), 46.2 (C-6), 44.5 (C-3), 36.0 (C-15), 32.8 (C-8), 31.9 (C-14), 30.2–29.4 (C-10, C-11 to C-13), 28.7 (C-22 or C-23), 28.6 (C-23 or C-22), 23.3 (C-9).

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