New Cyclic Polyketide Peroxides from Okinawan Marine Sponge *Plakortis* sp.

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Three new cyclic peroxides (**2**−**4**) have been isolated from the Okinawan marine sponge *Plakortis* sp., and the structures were elucidated by extensive spectroscopic analyses. The absolute stereochemistries of **2** and **3** were determined by chemical conversion, whereas that of the dioxane ring of **4** was assigned by the modified Mosher's method.

Cyclic peroxides are quite common in organic extracts from marine sponges.[2,3] Recently, we have reported the complete characterization of plakortin-like compounds, assigning the absolute configuration of a new cytotoxic polyketide (**1**) isolated from an Okinawan sponge *Plakortis* sp.[4] Here we describe the isolation and structures of three new cyclic polyketide peroxides (**2**−**4**) related either to **1** or to other known peroxides.[5] Furthermore, we assigned the absolute stereochemistry of these new compounds by using both chemical conversion into compounds of known configuration and the MTPA procedure previously applied for **1**.[4,6]

The sponge *Plakortis* sp. was collected off Manzamo, Okinawa, and kept frozen until used. EtOAc-soluble materials of the methanol extract were separated by a Si gel column (hexane−EtOAc) as previously described.[4] Selected fractions were methylated with CH_2N_2 and then purified by reversed-phase HPLC to afford methyl esters (**2a**−**4a**) of three new compounds (**2**−**4**) together with a known peroxide methyl ester (**1a**).

The methyl ester **2a** was obtained as a colorless oil, and its ^{1}H NMR spectrum mainly differed from that of **1a** in lacking signals for the C-11/C-12 double bond.[4] HRFDMS data of **2a** indicated the molecular formula to be $C_{23}H_{42}O_4$, and its ^{13}C NMR (Table 1) spectrum showed two olefin signals at δ 141.3 (C-8) and 127.0 (C-7). The ^{1}H NMR (Table 1) spectrum contained signals typical for a dioxane ring having 3,4-diequatorial substituents.[4,7] In particular, the resonances at δ 1.55 (H-4), 4.20 (H-3), 2.62, and 2.26 (H_2-2) were very close to those of **1** and defined the relative stereochemistry for the cyclic peroxide ring system as the same as that of **1**.[4,6] The structure of **2a** was fully supported by 2D NMR experiments (^{1}H−^{1}H COSY, HMQC, and HMBC). The NOESY spectrum revealed a NOE pattern identical to that of **1**, with cross peaks from H-7 (δ 5.16) both to the methylene protons at C-9 (δ 1.99 and 1.89) and to the axial proton at C-4 (δ 1.55). This indicated the *E* geometry of the double bond and the axial orientation of the major alkyl chain on C-6. To determine the absolute stereochemistry, **2a** was reduced by catalytic hydrogenation with 10% Pd/C to give the diol **5**. As the same diol **5** ($[\alpha]^{23}_D$ −43°) was obtained from **1a** under the same conditions,[4] the configuration of **2a** was assigned to be 3*S*,4*R*,6*S*,10*R*.

The methyl esters **3a** and **4a** were closely related to plakortide H (**6**), recently isolated from the sponge *Plakortis halichondrioides* as an activator of cardiac SR−Ca^{2+} ATPase.[5] Compound **3a** had the molecular formula $C_{22}H_{40}O_4$ deduced by HRFDMS data. The ^{1}H NMR (Table 1) spectrum revealed a sharp methyl singlet at δ 1.66 (CH_3-19) and four methyl triplets at δ 0.84 (CH_3-18 and CH_3-

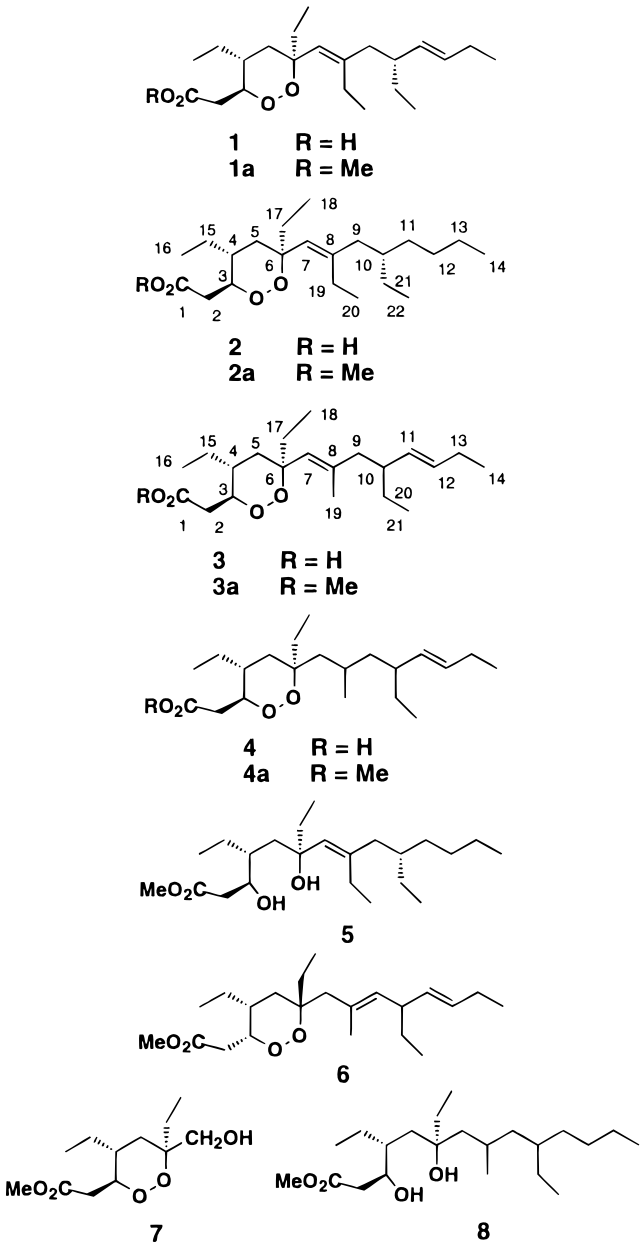

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Table 1. ^{1}H and ^{13}C NMR Data for the Methyl Esters (2a−4a) of Cyclic Peroxides (2−4) in $CDCl_3$

	2a		**3a**		**4a**	
position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		171.2 (s)		171.3 (s)		171.3 (s)
2	2.62 (dd, 15.8, 3.7) 2.26 (m)	36.3 (t)	2.62 (dd, 15.9, 3.2) 2.27 (m)	36.2 (t)	2.62 (dd, 15.7, 3.2) 2.42 (m)	36.6 (t)
3	4.20 (ddd, 9.6, 9.6, 3.2)	81.9 (d)	4.20 (ddd, 12.5, 9.6, 3.1)	81.9 (d)	4.11 (ddd, 9.5, 9.5, 3.2)	81.9 (d)
4	1.55 (m)	37.4 (d)	1.54 (m)	37.2 (d)	1.64 (m)	36.7 (d)
5	1.99 (m) 1.28 (m)	39.4 (t)	2.04 (m) 1.26 (m)	37.3 (d)	1.79 (dd, 13.4, 6.4) 1.20 (bt, 13.4)	37.0 (t)
6		84.9 (s)		84.9 (s)		83.9 (s)
7	5.16 (s)	127.0 (d)	5.20 (s)	127.6 (d)	1.77 (m) 1.46 (m)	39.6 (t)
8		141.3 (s)		136.2 (s)	1.58 (m)	25.6 (d)
9	1.99 (m) 1.89 (dd, 13.0, 7.2)	41.0 (t)	2.07 (m)[b] 1.95 (m)[b]	47.3 (t)	1.42 (m) 1.07 (m)	43.5 (t)
10	1.40 (m)	36.8 (d)	2.02 (m)	42.4 (d)	1.85 (m)	42.5 (t)
11	1.26 (m) 1.22 (m)	32.8 (t)	5.13 (dd, 15.3, 8.1)	133.1 (d)	5.14 (dd, 15.2, 8.1)	133.5 (d)
12	1.26 (m)	23.2 (t)	5.37 (dt, 15.3, 6.3)	131.9 (d)	5.38 (dt, 15.2, 6.5)	132.0 (d)
13	1.26 (m)	28.7 (t)	1.97 (m)	25.1 (t)	2.00 (ddt, 14.9, 7.4, 1.2)	25.8 (t)
14	0.85 (t, 7.4)[a]	10.7 (q)	0.97 (t, 7.5)	14.0 (q)	0.96 (t, 7.5)	14.3 (q)
15	1.42 (m) 1.09 (m)	23.9 (t)	1.43 (m) 1.09 (m)	23.4 (t)	1.44 (m) 1.09 (m)	24.4 (t)
16	0.90 (t, 7.5)	10.6 (q)	0.89 (t, 7.5)	10.3 (q)	0.90 (t, 7.5)	10.7 (q)
17	1.61 (q, 7.5)	32.4 (t)	1.58 (q, 7.5)	32.0 (t)	1.58 (m)	32.2 (t)
18	0.86 (t, 7.5)	7.9 (q)	0.84 (t, 7.4)	7.7 (q)	0.85 (t, 7.5)	7.5 (q)
19	2.15 (m)	22.5 (t)	1.66 (s)	16.9 (q)	0.87 (d, 6.7)	21.4 (q)
20	0.97 (t, 7.5)	12.2 (q)	1.40 (m)[b] 1.17 (q, 7.4)	nd	1.31 (m) 1.17 (m)	29.0 (t)
21	1.26 (m)	25.7 (t)	0.84 (t, 7.4)	11.5 (q)	0.82 (t, 7.5)	11.8 (q)
22	0.89 (m)[a]	14.1 (q)				
Me	3.71 (s)	51.9 (q)	3.70 (s)	51.9 (q)	3.72 (s)	51.8 (q)

[a,b] Assignments with the same superscripted letter are interchangeable.

21), 0.97 (CH_3-14), and 0.89 (CH_3-16). The remaining signals, having chemical shifts very similar to those of **1**, were assigned by 2D NMR experiments. The *E* geometry for both double bonds was deduced from the coupling constant (J = 15.3 Hz) between H-11 and H-12 and NOE between H-7 and the methylene protons on C-9 in the NOESY spectrum. On the other hand, relative configuration of the dioxane ring was inferred by comparison of NMR data of **3a** with those of **1**[4] and **6**[5] and confirmed by oxidation of **3a** into the peroxyalcohol **7**. Although it has been reported that the trisubstituted double bond in this class of compounds is quite stable to electrophilic addition,[4,7] we found that a more drastic ozonolysis of **1a** followed by reduction with $NaBH_4$ afforded the desired alcohol **7** without affecting the peroxide linkage. Treatment of **3a** under the same conditions gave the same peroxyalcohol **7**, thus confirming the same relative stereochemistry of the dioxane ring.

The methyl ester **4a** was obtained as colorless oil, and the molecular formula $C_{22}H_{40}O_4$ was established from HRFDMS and ^{13}C NMR data (Table 1). The ^{1}H NMR (Table 1) spectrum differed slightly from those of **1**, **2a**, and **3a** in the presence of four methyl triplets (δ 0.96, 0.90, 0.85, and 0.82) and one methyl doublet (δ 0.87). Coupling constant analysis and NOESY data were in agreement both with *E* geometry (J = 15.2 Hz) of the Δ^{11}-double bond and with a trans diequatorial relationship between substituents at C-3 and C-4 of the peroxide ring. The relative configuration at C-6 was assigned on the basis of the chemical shifts of H_2-17 (δ 1.58 and 1.41), which would have to be further shifted to downfield if the ethyl group were axial.[4] In agreement with this geometry, it may be noted that the chemical shift of these methylene protons is more similar to those in **1** (H-17, δ 1.59) and **2a** (H-17, δ 1.61) than those in plakortide F (**6**) (H-15, δ 2.02 and 1.50). The absolute stereochemistry of the dioxane ring in **4a** was determined by modified Mosher's method applied for the acyclic derivative **8**, which was prepared according to the previously reported procedure.[4] Chemical shift differences between the diastereomeric couple of the MTPA esters (Figure 1) assigned *S* configuration at C-3 of **8**, thus establishing the absolute stereochemistry of **4a** as 3*S*, 4*R*, and 6*S*.

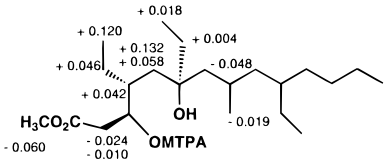

Figure 1. ^{1}H NMR chemical shift differences ($\Delta\delta$) for MTPA esters of **8**; $\Delta\delta$ (ppm) = δ[(*S*)-MTPA ester] − δ [(*R*)-MTPA ester].

Marine sponges of the genus *Plakortis* frequently contain peroxidic compounds with five- or six-membered rings. The co-occurrence of different polyketide skeletons in the same organism seems to indicate the existence of a unique biosynthetic pathway, which may have different precursors as their substrates.[7]

Experimental Section

General Methods. Optical rotations were determined on a JASCO DIP-370 polarimeter. 1D and 2D NMR spectra were recorded on Bruker ARX-500 and Bruker AMX-600 spectrometers. The 7.26-ppm resonance of residual $CHCl_3$ and 77.0 ppm of $CDCl_3$ were used as internal references. FDMS spectra were obtained on a JEOL JMS−SX102A spectrometer operating at 70 eV. IR data were recorded by a JASCO FT/IR-230 spectrometer.

Collection, Extraction, and Purification. The sponge *Plakortis* sp. (SS-11) collected off Manzamo, Okinawa, was the same as that previously reported.[4] The frozen material (0.5 kg, wet wt) was extracted with MeOH (2 × 1.0 L). After removing the volatile solvent, the aqueous residue was diluted

by freshwater (200 mL) and extracted first with EtOAc (3 × 200 mL) and later with *n*-BuOH (3 × 150 mL) to give 356 mg of EtOAc extract and 1.98 g of *n*-BuOH extract. The EtOAc-soluble material was concentrated to dryness and separated by a Silica gel column with hexane−EtOAc (3:1). The earlier fractions (ca. 80 mg) were evaporated to dryness and methylated by a saturated solution of CH_2N_2 in Et_2O. After removing the organic solvent and the excess of CH_2N_2, the residue was purified by reversed-phase HPLC (Capcell Pack C-18 column; 10 × 250 mm; CH_3CN-H_2O, 92:8; flow rate, 2.0 mL/min, detection at 205 nm) to give, in order of elution, **3a** (0.6 mg, 0.00012% wet wt), **4a** (1.0 mg, 0.0002%), **1a** (26 mg, 0.0052%), and **2a** (4.5 mg, 0.0009%).

Compound 2a: pale yellow oil, $[\alpha]^{23}_D$ +55° (*c* 0.56, $CHCl_3$); IR (film) 2960, 2930, 2875, 1745, 1460, 1440, and 1180 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$), see Table 1; FDMS *m*/*z* 382 (M^+, 100), 353 (20), 336 (45), and 282 (50); HRFDMS *m*/*z* 382.3062 (calcd for $C_{23}H_{42}O_4$, 382.3084); HMBC ($CDCl_3$, H/C) 2/1, 2/3, 3/1, 3/2, 5/2, 5/3, 5/6, 5/7, 7/6, 7/8, 7/9, 7/19, 9/7, 9/8, 9/10, 9/19, 9/21, 13/14, 17/5, 17/6, 17/7, 17/18, 18/6, 19/7, 19/8, 19/9, 19/20, and 20/8; NOESY ($CDCl_3$, H/H) 2/3, 2/4, 2/15, 3/5, 3/15, 5/16, 7/9, 9/19, 9/20, and 10/19.

Compound 3a: pale yellow oil, $[\alpha]^{23}_D$ +19° (*c* 0.13, $CHCl_3$); IR (film) 2960, 2925, 2875, 1745, 1460, 1440, and 1180 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$), see Table 1; FDMS *m*/*z* 366 (M^+, 5), 337 (20), 279 (30), and 55 (100); HRFDMS *m*/*z* 366.2790 (calcd for $C_{22}H_{38}O_4$, 366.2770).

Compound 4a: pale yellow oil, $[\alpha]^{23}_D$ + 40° (*c* 0.17, $CHCl_3$); IR (film) 2960, 2925, 2875, 1740, 1460, and 1180 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$), see Table 1; FDMS *m*/*z* 368 (M^+, 20), 339 (25), and 278 (100); HRFDMS *m*/*z* 368.2948 (calcd for $C_{22}H_{40}O_4$, 368.2927).

Absolute Stereochemistry of 2a. A solution of compound **1a** (3.7 mg) in EtOH (1 mL) was stirred under H_2 on 10% Pd/C for 3 h. The filtered solution was dried under vacuum to give **5** (2.6 mg). Under the same conditions, compound **2a** also gave **5**.

Compound 5: colorless solid, $[\alpha]^{23}_D$ −36° (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.89 (1H, s), 3.85 (1H,ddd, J = 7.5, 3.1, 1.5 Hz), 3.72 (3H, s), 2.62 (1H, dd, J = 16.2 and 2.5 Hz), 2.38 (1H, dd, J = 16.2, 10.0 Hz), 2.36 (1H, m), 2.24 (1H, m), 1.94 (1H, dd, J = 13.7, 6.8 Hz), 1.88 (1H, dd, J = 13.7, 6.8 Hz), 1.67 (1H, m), 1.40 (1H, m), 1.26 (m), 0.99 (3H, t, J = 7.5 Hz), 0.91 (3H, t, J = 7.5 Hz), 0.89 (3H, t, J = 6.6 Hz), 0.87 (3H, t, J = 7.5 Hz), and 0.84 (3H, t, J = 7.5 Hz); EIMS *m*/*z* 366 (5, M − H_2O), 355 (10), 337 (100), 225 (80), and 113 (90).

Absolute Stereochemistry of 3a. A solution of **1a** (3.0 mg) in MeOH (2.5 mL) was stirred at −78 °C for 10 min and then bubbled with a stream of O_3 for 3 min. The reaction mixture was allowed to warm to 0 °C, and then the excess of ozone was removed by nitrogen. The colorless solution was treated with excess $NaBH_4$, and the resulting suspension was stirred at 0 °C for 20 min. Then, the excess of $NaBH_4$ was destroyed by HOAc, and the solution was evaporated to dryness. The residue was partitioned between brine and EtOAc (3 × 5 mL). The organic extracts were combined, dried on Na_2SO_4, and evaporated to dryness to give 2.2 mg of **7**. Under the same conditions, compound **3a** also gave **7**.

Compound 7: colorless solid, ^1H NMR ($CDCl_3$) δ 4.21 (1H, br dd, J = 10.1, 3.2 Hz), 4.05 (1H, d, J = 11.8 Hz), 3.71 (3H, s), 3.46 (1H, d, J = 11.8 Hz), 2.67 (1H, dd, J = 15.9, 3.2 Hz), 2.35 (1H, dd, J = 15.9, 8.9 Hz), 1.80 (1H, dd, J = 13.9, 4.9 Hz), 1.67 (1H, m), 1.50 (1H, m), 1.39 (1H, m), 1.09 (1H, m), 0.91 (3H, t, J = 7.5 Hz), and 0.90 (3H, t, J = 7.5 Hz); EIMS *m*/*z* 215 ($M - CH_2OH$)$^+$ and 141.

Absolute Stereochemistry of 4. A solution of **4a** (0.7 mg) in EtOH (1 mL) was stirred under H_2 on 10% Pd/C for 2 h. The filtered solution was dried under vacuum to give **8**. This product was disolved in $CHCl_3-MeOH$ and divided in two aliquots, which were separately esterified with 4 μL of (*R*)- and (*S*)-MTPACl in dry pyridine (0.5 mL) under argon.[4] After usual workup, the yellow oily residue was purified by a Si gel column ($CHCl_3-MeOH$, 99:1) to give (*S*)- and (*R*)-MTPA esters of **8**, respectively.

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