

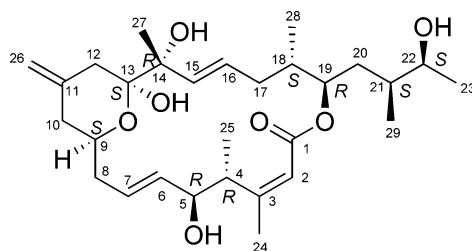
## Iriomoteolide-1a, a Potent Cytotoxic 20-Membered Macrolide from a Benthic Dinoflagellate *Amphidinium* Species

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iriomoteolide-1a (**1**)

A potent cytotoxic 20-membered macrolide, iriomoteolide-1a (**1**), has been isolated from a benthic dinoflagellate *Amphidinium* sp. (strain HYA024), and the structure was elucidated on the basis of detailed analyses of 2-D NMR data. The relative and absolute stereochemistries were assigned by the combination of conformational analyses using NMR data and modified Mosher's method of **1**.

### Introduction

Dinoflagellates belonging in the genus *Amphidinium* are known to produce unique polyketide metabolites with interesting bioactivities.<sup>1,2</sup> Cytotoxic macrolides designated as amphidinolides or caribenolide have been isolated from symbiotic or free-swimming dinoflagellates, *Amphidinium* species.<sup>2</sup> These macrolides have a variety of backbone skeletons and different sizes of macrocyclic lactone rings (12- ~ 29-membered rings). Some of the amphidinolides contain biosynthetically unique partial structures such as vicinally located C<sub>1</sub> branches.<sup>2</sup>

Furthermore, some of these macrolides (e.g., amphidinolides B,<sup>3</sup> G,<sup>4</sup> H,<sup>4</sup> and N<sup>5</sup> and caribenolide-I<sup>6</sup>) exhibit potent cytotoxicity against tumor cell lines in vitro and antitumor activity in vivo. Therefore, these macrolides have attracted great interest as challenging targets for total synthesis<sup>7</sup> and biosynthetic studies.<sup>8</sup>

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In our continuing investigation for unique metabolites from the dinoflagellate *Amphidinium* sp., molecular phylogenetic studies of 18S ribosomal DNA sequences have been examined for dinoflagellates *Amphidinium* species with or without a macrolide-producing ability. It was found that the 18S ribosomal DNA sequences of the macrolide-producing *Amphidinium* strains belonged in a different clade from those of the strains without a macrolide-producing ability.<sup>9</sup> On the basis of the DNA sequence homology, a rapid searching strategy for macrolide-producing strains, using specific DNA sequences and one-cell PCR protocol, was developed.<sup>9</sup>

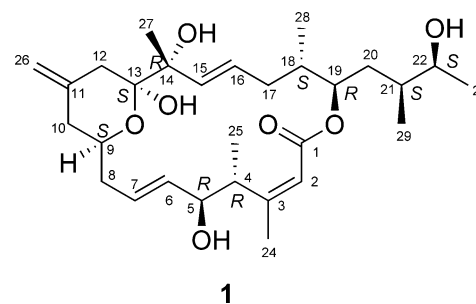
Recently, 250 or more *Amphidinium* strains isolated from benthic sources collected off the South-East Islands, Japan, were tested by one-cell PCR. The extracts of dinoflagellates positive in the PCR studies were further evaluated by cytotoxic screening and metabolomics analyses using 2-D NMR analyses.<sup>10</sup> This led to the discovery of a marine benthic *Amphidinium* strain HYA024 that produced unknown macrolides. Mass cultivation of this strain and examination of the extract resulted in the isolation of a novel, potentially cytotoxic, 20-membered macrolide, iriomoteolide-1a (**1**). Herein, we describe the isolation and structure elucidation of **1**.

## Results and Discussion

The dinoflagellate *Amphidinium* sp. (HYA024 strain) was monoclonally separated from benthic sea sand collected off Iriomote Island, Japan, and mass cultured uniaxially at 23 °C for 2 weeks in a 2% Provasoli's enriched seawater (PES) medium enriched with 3 mM NaHCO<sub>3</sub>. The algal cells (15.3 g, dry weight) obtained from 400 L of the medium were extracted with MeOH/toluene (3:1), and the extracts were partitioned between toluene and H<sub>2</sub>O. The toluene soluble materials were subjected to a SiO<sub>2</sub> gel column to obtain cytotoxic fractions against human B lymphocyte DG-75 cells.

Metabolomics analyses of these cytotoxic fractions were carried out using HMQC experiments.<sup>10</sup> HMQC spectra showed characteristic correlations for  $\delta$  ca. 70/5.4 ~ 5.2 and ca. 110/4.8 ( $F_1/F_2$ ), the former of which was considered to be a marker correlation for ester functionality, while the latter was a marker of the exomethylene functional group. One of the cytotoxic

CHART 1



fractions was purified with C<sub>18</sub> and NH<sub>2</sub>-SiO<sub>2</sub> column chromatographies followed by C<sub>18</sub> HPLC to afford iriomoteolide-1a (**1**, 0.028% from dry weight) (Chart 1) as one of cytotoxic components. No macrolides, such as amphidinolides or carbeneolides, have been isolated previously from strain HYA024.

Iriomoteolide-1a {**1**, [ $\alpha$ ]<sub>D</sub><sup>20</sup> +31° (c 0.35, CHCl<sub>3</sub>)} showed a pseudo-molecular ion peak at  $m/z$  529 (M + Na)<sup>+</sup> in the ESIMS spectrum, and the molecular formula of C<sub>29</sub>H<sub>46</sub>O<sub>7</sub> was revealed by HRESIMS data [ $m/z$  529.3148 (M + Na)<sup>+</sup>,  $\Delta$  +0.7 mmu]. <sup>1</sup>H and <sup>13</sup>C NMR data (Table 1) in CDCl<sub>3</sub> assigned by using HMQC and CH and CH<sub>2</sub> selected E-HSQC spectra<sup>11</sup> revealed the presence of a total of 29 carbon atoms due to an ester carbonyl, two quaternary sp<sup>2</sup> carbons, five sp<sup>2</sup> methines, an sp<sup>2</sup> methylene, two quaternary sp<sup>3</sup> carbons, one of which is a hemiketal carbon, seven sp<sup>3</sup> methines including four oxygenated ones, five sp<sup>3</sup> methylenes, and six methyls. Since five out of seven degrees of unsaturation were accounted for, iriomoteolide-1a (**1**) was inferred to possess two rings. The <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> included a sharp singlet D<sub>2</sub>O-exchangeable proton ( $\delta$ <sub>H</sub> 3.52, OH-13), indicating the presence of intramolecular hydrogen bonding.

The planar structure of **1** was mainly elucidated on the basis of 2-D NMR data measured in CDCl<sub>3</sub>. Analyses of <sup>1</sup>H-<sup>1</sup>H COSY and TOCSY spectra disclosed three proton-proton networks from H-2 to H<sub>3</sub>-24, from H-4 to H<sub>2</sub>-10 and H<sub>3</sub>-25, and from H-15 to H<sub>3</sub>-23, H<sub>3</sub>-28, and H<sub>3</sub>-29 (Figure 1). The presence of two disubstituted E-double bonds at C-6-C-7 and C-15-C-16 were implied by  $J_{HH}$  values [H-6 ( $\delta$ <sub>H</sub> 5.57)/H-7 ( $\delta$ <sub>H</sub> 5.68): 15.7 Hz, H-15 ( $\delta$ <sub>H</sub> 5.68)/H-16 ( $\delta$ <sub>H</sub> 5.76): 15.5 Hz], while a trisubstituted double bond at C-2-C-3 was indicated to possess Z-geometry by the chemical shift ( $\delta$ <sub>H</sub> 2.12,  $\delta$ <sub>C</sub> 23.8) of C-24 and the ROESY correlation for H-2 ( $\delta$ <sub>H</sub> 6.02)/H<sub>3</sub>-24 ( $\delta$ <sub>H</sub> 2.10) in C<sub>6</sub>D<sub>6</sub>. The double bond at C-2-C-3 was shown to be adjacent to C-4 by HMBC correlations for H-2 ( $\delta$ <sub>H</sub> 5.72)/C-3 ( $\delta$ <sub>C</sub> 162.0), H<sub>3</sub>-24/C-4 ( $\delta$ <sub>C</sub> 47.9), and H<sub>3</sub>-25 ( $\delta$ <sub>H</sub> 1.24)/C-3 in CDCl<sub>3</sub>. HMBC correlations for H<sub>2</sub>-26 ( $\delta$ <sub>H</sub> 4.82 and 4.81)/C-10 ( $\delta$ <sub>C</sub> 40.7), H<sub>2</sub>-26/C-11 ( $\delta$ <sub>C</sub> 141.7), and H<sub>2</sub>-26/C-12 ( $\delta$ <sub>C</sub> 36.88) suggested that C-10 was attached to C-12 through an exomethylene unit (C-11-C-26). The connection between C-12 and C-15 via two sp<sup>3</sup> oxygenated quaternary carbons (C-13 and C-14) was revealed by HMBC correlations for H<sub>2</sub>-12 ( $\delta$ <sub>H</sub> 2.40 and 2.26)/C-13 ( $\delta$ <sub>C</sub> 99.7), 13-OH ( $\delta$ <sub>H</sub> 3.52)/C-12, 13-OH/C-13, 13-OH/C-14 ( $\delta$ <sub>C</sub> 77.2), H<sub>3</sub>-27 ( $\delta$ <sub>H</sub> 1.25)/C-13, H<sub>3</sub>-27/C-14, and H<sub>3</sub>-27/C-15 ( $\delta$ <sub>C</sub> 134.9). The existence of a six-membered hemiacetal ring was deduced from the ROESY correlation for H-9 ( $\delta$ <sub>H</sub> 3.81)/13-OH. The relatively low-field resonance of H-19 ( $\delta$ <sub>H</sub> 5.11) suggested that C-19 was involved in an ester

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TABLE 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for Iriomoteolide-1a (1)

position	$^1\text{H}$				$^{13}\text{C}$	
	$\text{CDCl}_3$		$\text{C}_6\text{D}_6$		$\text{CDCl}_3$	
1					167.4	C
2	5.72	brs	6.02	brs	115.8	CH
3					162.0	C
4	2.46	dq, 2.9, 7.3	1.90	m	47.9	CH
5	4.28	m	3.89	m	72.3	CH
6	5.57	dd, 4.1, 15.7	5.25	dd, 4.6, 15.3	132.0	CH
7	5.68	m	5.80	ddd, 4.6, 10.0, 14.6	126.8	CH
8a	2.18 <sup>a</sup>	m	2.19	m	39.5	CH <sub>2</sub>
8b			2.00	m		
9	3.81	brt, 11.5	4.00	tt, 2.0, 11.9	71.8	CH
10a	2.21 <sup>b</sup>	brd, 12.7	2.15	m	40.7	CH <sub>2</sub>
10b	1.90 <sup>b</sup>	brt, 12.3	1.90	dd, 11.9, 13.0		
11					141.7	C
12a	2.40	d, 13.6	2.73	d, 13.3	36.88	CH <sub>2</sub>
12b	2.26	brd, 13.6	2.45	brd, 13.3		
13	3.52 <sup>c</sup>	brd, 1.9	3.82 <sup>c</sup>	brs	99.7	C
14					77.2	C
15	5.68	brd, 15.5	6.07	d, 14.6	134.9	CH
16	5.76	ddd, 3.1, 10.8, 15.5	5.97	ddd, 4.6, 10.0, 14.6	128.8	CH
17a	2.15	m	2.08	m	38.2	CH <sub>2</sub>
17b	1.96	dt, 14.1, 11.6	2.14	m		
18	1.82	m	1.63	m	36.94	CH
19	5.11	m	5.38	ddd, 2.7, 4.6, 8.0	70.8	CH
20a	1.80	ddd, 4.4, 8.7, 13.8	1.94	ddd, 4.0, 8.0, 13.9	36.5	CH <sub>2</sub>
20b	1.15	ddd, 4.4, 8.8, 13.8	1.14	ddd, 4.6, 8.6, 13.9		
21	1.40	m	1.40	m	36.5	CH
22	3.58	quint, 6.3	3.41	dq, 6.0, 6.6	72.2	CH
23	1.11 <sup>d</sup>	d, 6.3	0.98 <sup>d</sup>	d, 6.6	19.8	CH <sub>3</sub>
24	2.12 <sup>d</sup>	s	2.10 <sup>d</sup>	s	23.8	CH <sub>3</sub>
25	1.24 <sup>d</sup>	d, 7.3	1.08 <sup>d</sup>	d, 6.6	15.6	CH <sub>3</sub>
26a	4.82 <sup>a</sup>	brs	5.01	brs	110.6	CH <sub>2</sub>
26b			4.97	brs		
27	1.25 <sup>d</sup>	s	1.54 <sup>d</sup>	s	23.1	CH <sub>3</sub>
28	0.99 <sup>d</sup>	d, 6.8	0.94 <sup>d</sup>	d, 6.8	14.2	CH <sub>3</sub>
29	0.91 <sup>d</sup>	d, 6.7	0.97 <sup>d</sup>	d, 6.7	15.5	CH <sub>3</sub>

<sup>a</sup> 2H. <sup>b</sup> Coupling constants for H-10a are small and large values for H-9 and H-10b, respectively. On the other hand, coupling constants for H-10b are both large values for H-9 and H-10a. <sup>c</sup> 13-OH. <sup>d</sup> 3H.

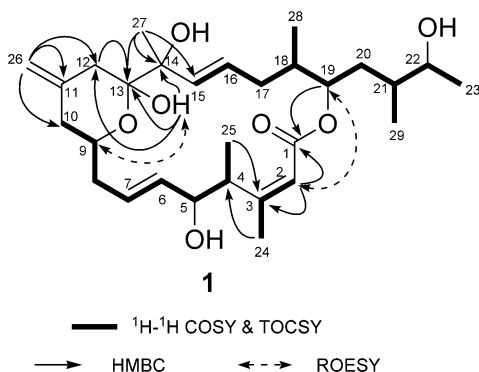


FIGURE 1. Selected 2-D NMR correlations for iriomoteolide-1a (1).

linkage with C-1, which was also supported by HMBC correlations for H-2/C-1 ( $\delta_{\text{C}}$  167.4) and H-19/C-1 and ROESY correlation for H-2/H-19. Thus, the planar structure of iriomoteolide-1a was concluded to be **1**.

The relative stereochemistry of **1** was deduced from detailed analyses of bond rotations<sup>12</sup> based on  $^1\text{H}$ - $^1\text{H}$  and  $^{13}\text{C}$ - $^1\text{H}$  coupling constants and ROESY data in  $\text{CDCl}_3$  and  $\text{C}_6\text{D}_6$ .  $^1\text{H}$ - $^1\text{H}$  coupling constants were obtained from the resolution

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enhanced  $^1\text{H}$  NMR spectrum and differential homonuclear decoupling experiments, while long-range  $^{13}\text{C}$ - $^1\text{H}$  coupling constants were obtained by analyses of HETLOC<sup>13</sup> and J-IMPEACH-MBC<sup>14</sup> spectra. Coupling constants and ROESY correlations in  $\text{CDCl}_3$  and  $\text{C}_6\text{D}_6$  were close to each other, thus suggesting that the solution conformations of **1** in both solvents were similar to each other. For typical vicinally located oxythine-methylated methine bonds such as C-4–C-5, C-18–C-19, and C-21–C-22, the relative configurations were concluded to be erythro, erythro, and threo, respectively, by *J*-based configuration analysis<sup>15</sup> as described next (Figure 2).

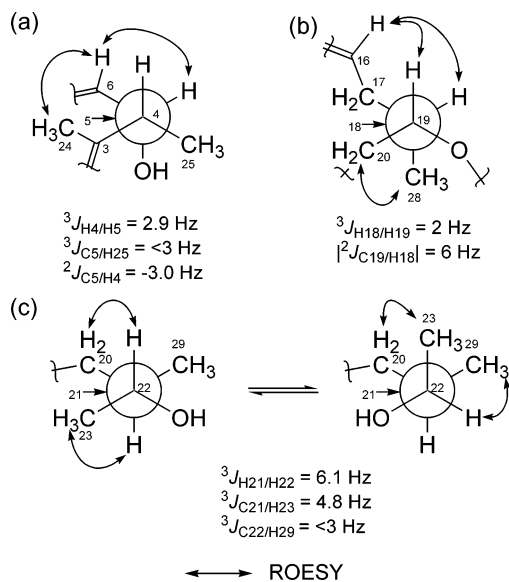
For the C-1–C-14 portion (Figure 3), a gauche configuration for H-4–H-5 was suggested by the  $J(\text{H-4}/\text{H-5})$  value (2.9 Hz), while relatively small  $J(\text{C-5}/\text{H}_3\text{-25})$  and  $J(\text{C-5}/\text{H-4})$  values<sup>16</sup> (<3 and 3.0 Hz, respectively) indicated gauche alignment for C-25–

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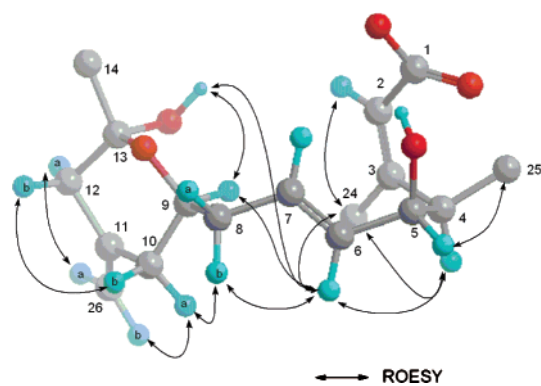
(14) Williamson, R. T.; Marquez, B. L.; Gerwick, W. H.; Martin, G. E.; Krishnamurthy, V. V. *Magn. Reson. Chem.* **2001**, *39*, 127–132.

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(16) When the dihedral angles between  $^{13}\text{C}$ -attached oxygen and the proton are 180 and 60°, typical  $^2J_{\text{CH}}$  values were 0 to approximately –2 and –5 to approximately –7 Hz, respectively. On the other hand, when the dihedral angles between carbon and proton through three bonds are 180 and 60°, typical  $^3J_{\text{CH}}$  values were 6 to ~8 and 1 to ~3 Hz, respectively. For review, see: Hansen, P. E. *Prog. NMR Spectrosc.* **1981**, *14*, 175–296.

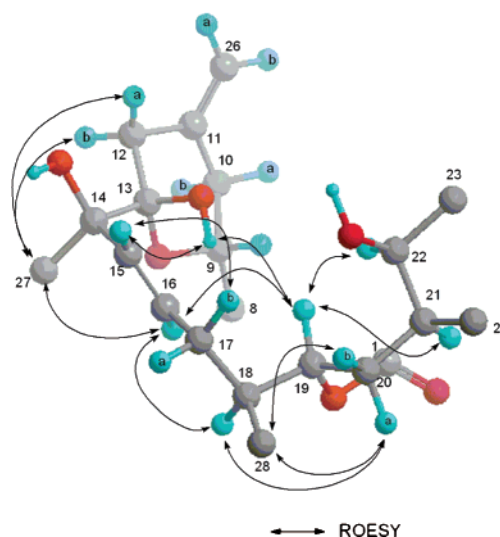


**FIGURE 2.** Rotation models for (a) C-4=C-5, (b) C-18-C-19, and (c) C-21-C-22 bonds of iriomoteolide-1a (**1**).



**FIGURE 3.** ROESY correlations and relative stereochemistry for C-1-C-14 portion in iriomoteolide-1a (**1**).

H-5 and anti for H-4-OH-5. Thus, the C-4-C-5 bond was revealed to be in the erythro configuration (Figure 2a). ROESY correlations for H-4 ( $\delta_H$  1.90)/H<sub>3</sub>-24 ( $\delta_H$  2.10), H-4/H-6 ( $\delta_H$  5.25), H-6/H-8b ( $\delta_H$  2.00), and H-6/H<sub>3</sub>-24 in C<sub>6</sub>D<sub>6</sub> indicated that H-4, H-6, H-8b, and C-24 were oriented to the same side of the macrocyclic ring. Gauche and anti relations for H-7-H-8a and H-7-H-8b, respectively, were deduced from  $J$ (H-7/H-8a) and  $J$ (H-7/H-8b) values (4.6 and 10.0 Hz, respectively).  $J$ (H-8a/H-9) and  $J$ (H-8b/H-9) values were relatively large and small (11.9 and 2.0 Hz, respectively), indicating that relationships for H-8a-H-9 and H-8b-H-9 were anti and gauche, respectively. On the other hand, gauche and anti relations for H-9-H-10a and H-9-H-10b, respectively, were implied by  $J$ (H-9/H-10a) and  $J$ (H-9/H-10b) values (2.0 and 11.9 Hz, respectively). From the ROESY correlation for H-8b/H-10a, the orientations of H-8b and H-10a were shown to be the same side of the macrocyclic ring. ROESY correlations were observed for H-10a ( $\delta_H$  2.21)/H-26b ( $\delta_H$  4.97), H-10b ( $\delta_H$  1.90)/H-12b ( $\delta_H$  2.45), and H-12a ( $\delta_H$  2.73)/H-26a ( $\delta_H$  5.01), suggesting that H-10a and H-12a were both equatorial configurations, while H-10b and H-12b were axial. The syn configuration for H-9-13-OH in the tetrahydropyran ring was deduced from ROESY correlations for H-9 ( $\delta_H$  3.81)/13-OH ( $\delta_H$  3.52) observed in CDCl<sub>3</sub>.

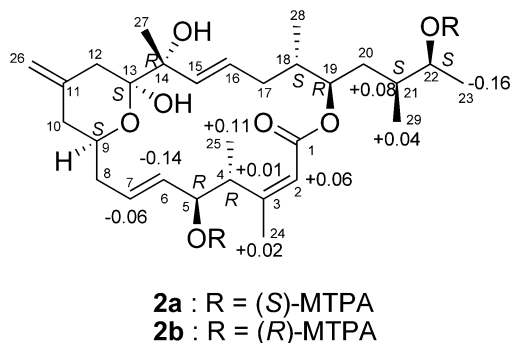


**FIGURE 4.** ROESY correlations and relative stereochemistry for C-8-C-20 portion in iriomoteolide-1a (**1**). The rotation of C-21-C-22 bond represented as one of two rotamers shown in Figure 2c.

For the C-13-C-20 portion (Figure 4), the integrated value of the ROESY correlation for H-12b ( $\delta_H$  2.45)/H<sub>3</sub>-27 ( $\delta_H$  1.54) in C<sub>6</sub>D<sub>6</sub> was larger than that of H-12a ( $\delta_H$  2.73)/H<sub>3</sub>-27, and the ROESY correlations were observed for H-16 ( $\delta_H$  5.97)/H<sub>3</sub>-27 (in C<sub>6</sub>D<sub>6</sub>) and 13-OH/H-15 ( $\delta_H$  5.68) (in CDCl<sub>3</sub>). These indicated that H-12b, H-16, and H<sub>3</sub>-27 were oriented to the same side of the macrocyclic ring. Both anti relations to H-16-H-17b and H-17b-H-18 were elucidated by relatively large  $J$ (H-16/H-17b) and  $J$ (H-17b/H-18) values (10.0 and 11.2 Hz, respectively) and ROESY correlations for H-15/H-17b ( $\delta_H$  2.14) and H-16/H-18 ( $\delta_H$  1.63) in C<sub>6</sub>D<sub>6</sub>. The  $J$ (H-18/H-19) (2.0 Hz) and  $|J$ (C-19/H-18)| values (6 Hz)<sup>17</sup> suggested both gauche relations for H-18-H-19 and H-18-19-O. Considering the ROESY correlations for H-16/H-19 ( $\delta_H$  5.38) and H<sub>2</sub>-20 ( $\delta_H$  1.94 and 1.14)/H<sub>3</sub>-28 ( $\delta_H$  0.94), the C-18-C-19 bond was revealed to be in the erythro configuration (Figure 2b), thus suggesting both equatorial orientations for the methyl group (C-28) on C-18 and the side chain (C-20 to ~C-23) on C-19. Antiperiplanar relations for H-19-H-20a and H-20b-H-21 were deduced from the following coupling constants:  $J$ (H-19/H-20a) 8.0 Hz,  $J$ (H-19/H-20b) 4.6 Hz,  $J$ (H-20a/H-21) 4.0 Hz,  $J$ (H-20b/H-21) 8.6 Hz and ROESY correlations: H-18/H-20a, H-19/H-21 ( $\delta_H$  1.40).

For the C-21-C-22 bond,  $J$ (H-21/H-22) and  $J$ (C-21/H<sub>3</sub>-23) were typical medium values (6.1 and 4.8 Hz, respectively) for  $^3J_{HH}$  and  $^3J_{CH}$ , suggesting that this bond underwent a conformational change between anti and gauche. On the other hand, the gauche configuration for H-22-C-29 was deduced from the relatively small  $J$ (C-22/H<sub>3</sub>-29) value (<3 Hz). Of the six possible rotamers arising from erythro and threo, one pair of the threo relations shown in Figure 2c satisfied the ROESY correlations for H<sub>2</sub>-20/H-22 ( $\delta_H$  3.41), H-21/H<sub>3</sub>-23 ( $\delta_H$  0.98), and H-22/H<sub>3</sub>-29 ( $\delta_H$  0.97). Therefore, the total relative configurations of nine chiral centers were concluded as shown in Figures 3 and 4.

(17) Although the modulus of  $J$ (C-19/H-18) was obtained from the *J*-IMPEACH MBC spectrum, the sign was not determined.



**FIGURE 5.**  $\Delta\delta$  values [ $\Delta\delta$  (in ppm) =  $\delta_S - \delta_R$ ] obtained for the 5,22-bis-(S)- and (R)-MTPA esters (**2a** and **2b**, respectively) of iriomoteolide-1a (**1**).

To determine the absolute stereochemistry of iriomoteolide-1a (**1**), a modified Mosher's method<sup>18</sup> was applied for two hydroxyl groups at C-5 and C-22. The <sup>1</sup>H NMR data for 5,22-bis-(S)- and (R)-MTPA esters (**2a** and **2b**, respectively) of **1** were assigned by analysis of the TOCSY spectra.  $\Delta\delta$  values ( $\Delta\delta = \delta_S - \delta_R$ ) obtained from <sup>1</sup>H NMR data of **2a** and **2b** showed positive signs for H-2 (+0.06), H-4 (+0.01), H-21 (+0.08), H<sub>3</sub>-24 (+0.02), H<sub>3</sub>-25 (+0.11), and H<sub>3</sub>-29 (+0.04) and negative signs for H-6 (-0.14), H-7 (-0.06), and H<sub>3</sub>-23 (-0.16) (Figure 5), thus suggesting that C-5 and C-22 possessed R- and S-configurations, respectively. Therefore, the absolute configurations of **1** were assigned as 4R, 5R, 9S, 13S, 14R, 18S, 19R, 21S, and 22S.

Iriomoteolide-1a (**1**) is a 20-membered macrolide having a novel carbon skeleton with four hydroxyl groups, five methyls, an exomethylene branch, three endogenous double bonds, and a tetrahydropyran ring. Vicinally locating the C<sub>1</sub> branches (C-24 and C-25) on a C-4–C-5 portion were characteristic for the macrolides from the dinoflagellate *Amphidinium* species.<sup>19</sup> Furthermore, iriomoteolide-1a (**1**) is the first macrolide with a Z-olefin and the first with an overall chain length composed of 23 carbons, in the series of macrolides isolated from the *Amphidinium* dinoflagellates thus far.

Iriomoteolide-1a (**1**) exhibited potent cytotoxicity against human B lymphocyte DG-75 cells (IC<sub>50</sub>: 0.002  $\mu$ g/mL), which was 20 times more potent than that of doxorubicin (IC<sub>50</sub>: 0.04  $\mu$ g/mL). The cytotoxic activity of **1** was almost equal to that of amphidinolide H<sup>4,20</sup> (IC<sub>50</sub>: 0.001  $\mu$ g/mL), which was one of the most potent cytotoxic macrolides from the *Amphidinium* dinoflagellates. Iriomoteolide-1a (**1**) also showed potent cytotoxicity against Epstein–Barr virus (EBV)-infected human B lymphocyte Raji cells (IC<sub>50</sub>: 0.003  $\mu$ g/mL). This cytotoxic activity against EBV-infected cells may be due not to an antiviral effect but rather to a general toxicity effect. In marine organisms, potent cytotoxicity has been associated with macrolides containing a six-membered hemiketal ring, an example

being peloruside A<sup>21</sup> reported from the sponge *Mycale hentscheli*. Recently, the unique mechanism of action for peloruside A has been revealed by chemical biological methods<sup>22</sup> and in silico studies.<sup>23</sup> The mechanism of action for the target molecule for **1** is currently under investigation.

## Experimental Section

**NMR Measurement.** CH and CH<sub>2</sub> selected HSQC spectra were measured with spectral widths of 5434 Hz for the F<sub>2</sub> dimension and 4545 Hz (ca. 50 to ~20 ppm) for the F<sub>1</sub> dimension, and 16 scans with two dummy scans were accumulated into 1000 data points for each 64 t<sub>1</sub> increments. The repetition delay, BIRD delay, and constant time for J<sub>CH</sub> evaluation were 2 s, 0.3 s, and 3.7 ms, respectively. For CH selection, the editing flip angle and delay  $\tau$  were set to  $\pi$  and 3.7 ms, respectively, while those for CH<sub>2</sub> selection were  $\pi/2$  and 1.35 ms. The measuring times were both ca. 1 h.

The HETLOC spectrum was obtained with the pulse sequence proposed by Woolborn and Leibfritz<sup>13b</sup> with composite pulses<sup>24</sup> for broadband constant rotations (broadwidth  $\pm 0.60$ ). The durations for the trim pulse, the delay in the BIRD pulse, and the constant time for J<sub>CH</sub> evaluation were 2.5, 300, and 3.57 ms, respectively. The MLEV-17 spin-lock period was set to 30 ms for <sup>2,3</sup>J<sub>CH</sub>. For 256 t<sub>1</sub> increments, 384 transients with 16 dummy scans were accumulated in 4000 data points. Zero-filling to 1000 for F<sub>1</sub> and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2-D Fourier transformation. The measuring time was ca. 64 h.

The J-IMPEACH MBC experiment was carried out with spectral widths of 5434 Hz for the F<sub>2</sub> dimension and 27 777 Hz for the F<sub>1</sub> dimension, and 512 scans with two dummy scans were accumulated into 1000 data points for each 256 t<sub>1</sub> increments. The scaling factor and constant time variable delay were set to 20 and 175 ms, respectively. The measuring time was ca. 72 h.

**Cultivation and Isolation.** To benthic sea sands collected off Iriomote Island, Japan, was added seawater medium containing a 1% ES supplement, and the mixture was incubated for several weeks. The dinoflagellate (strain number HYA024) was separated monoclally by micropipetting. The voucher specimen was deposited at the Center for Advanced Marine Core Research, Kochi University.

The dinoflagellate was uniaxially cultured at 23 °C for 2 weeks in seawater medium enriched with 2% PES supplement, 16 h light and 8 h dark. The harvested cells (15.3 g, from 400 L of culture) were extracted with MeOH/toluene (3:1, 500 mL  $\times$  3), and the extract was partitioned between toluene (500 mL  $\times$  3) and water (500 mL). The toluene-soluble fractions (2 g) were subjected to SiO<sub>2</sub> column chromatography (40  $\times$  200 mm) using a stepwise elution of CHCl<sub>3</sub> (200 mL) and CHCl<sub>3</sub>/MeOH (98:2, 200 mL). The fraction eluted with (CHCl<sub>3</sub>/MeOH, 98:2) was chromatographed successively by using a C<sub>18</sub> (CH<sub>3</sub>CN/H<sub>2</sub>O, 7:3) and then a NH<sub>2</sub>-SiO<sub>2</sub> column (hexane/EtOAc, 2:1). A macrolide containing fraction was separated by C<sub>18</sub> HPLC [YMC-Pack Pro C<sub>18</sub>, 5  $\mu$ m, YMC Co., Ltd., 10 mm  $\times$  250 mm; eluent, CH<sub>3</sub>CN/H<sub>2</sub>O (60:40); flow rate, 2

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mL/min; UV detection at 210 nm] to afford iriomoteolide-1a (**1**, 4.3 mg, 0.028%).

**Iriomoteolide-1a (1).** Colorless amorphous;  $[\alpha]_{\text{D}}^{20} +31^{\circ}$  (*c* 0.35, CHCl<sub>3</sub>); IR (neat)  $\nu_{\text{max}}$  3446 (broad), 2920 1686, 1214, and 1008 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data (Table 1); ESIMS (pos) *m/z* 529 (M + Na)<sup>+</sup>; HRESIMS *m/z* 529.3148 [calcd for C<sub>29</sub>H<sub>46</sub>O<sub>7</sub>Na, (M + Na)<sup>+</sup>: 529.3141].

**5,22-Bis-(S)-MTPA Ester (2a) of Iriomoteolide-1a (1).** To a solution of iriomoteolide-1a (**1**, 0.2 mg) in 1% DMAP solution in CH<sub>2</sub>Cl<sub>2</sub> (20  $\mu$ L) were added Et<sub>3</sub>N (2  $\mu$ L) and (*R*)-(-)-MTPACl (1  $\mu$ L), and the mixture was stirred at 4 °C for 15 h. After addition of *N,N*-dimethyl-1,3-propanediamine (2  $\mu$ L), the solvent was evaporated in vacuo. The residue was passed through a silica gel column (hexane/acetone, 4:1) to afford 5,22-bis-(*S*)-MTPA ester (**2a**) of **1** (0.12 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>);  $\delta$  0.92 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-28), 0.97 (3H, d, *J* = 6.5 Hz, H<sub>3</sub>-29), 1.09 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-23), 1.13 (1H, m), 1.18 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-25), 1.25 (3H, s, H<sub>3</sub>-27), 1.60 to ~1.68 (2H, m), 1.70 (1H, m, H-21), 1.76 (1H, m), 1.87 (1H, m), 1.92 to ~2.07 (2H, m), 2.07 (3H, s, H<sub>3</sub>-24), 2.08 to ~2.27 (3H, m), 2.38 (1H, d, *J* = 13.7 Hz, H-12), 2.49 (1H, m, H-4), 3.48 (1H, m, H-9), 3.51 (3H, s), 3.60 (3H, s), 5.82 (2H, s, H<sub>2</sub>-26), 5.02 (1H, m, H-22), 5.09 (1H, m, H-19), 5.25 (1H, m, H-5), 5.47 (1H, m, H-6), 5.50 (2H, m, H-15 and H-16), 5.68 (1H, m, H-7), 5.78 (1H, s, H-2), 7.34 (6H, m), and 7.52 (4H, m); ESIMS (pos) *m/z* 961.5 (M + Na)<sup>+</sup>; HRESIMS *m/z* 961.3915 [calcd for C<sub>49</sub>H<sub>60</sub>O<sub>11</sub>F<sub>6</sub>Na, (M + Na)<sup>+</sup>: 961.3927].

**5,22-Bis-(R)-MTPA Ester (2b) of Iriomoteolide-1a (1).** Iriomoteolide-1a (**1**, 0.2 mg) was treated with 4-dimethylaminopyridine

(50  $\mu$ g), triethylamine (5  $\mu$ L), and (*S*)-(+)-MTPACl (1.5  $\mu$ L) by the same procedure as described previously to afford 5,22-bis-(*R*)-MTPA ester (**2b**) of **1** (0.08 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>);  $\delta$  0.88 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-28), 0.94 (3H, d, *J* = 6.5 Hz, H<sub>3</sub>-29), 1.07 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-25), 1.11 (1H, m), 1.24 (3H, s, H<sub>3</sub>-27), 1.25 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-23), 1.62 (1H, m, H-21), 1.63 to ~1.70 (3H, m), 1.81 (1H, m), 2.02 (1H, m), 2.05 (3H, s, H<sub>3</sub>-24), 2.06 (1H, m), 2.08 to ~2.30 (4H, m), 2.48 (1H, m, H-4), 3.49 (1H, m, H-9), 3.51 (3H, s), 3.53 (3H, s), 4.85 (2H, s, H<sub>2</sub>-26), 5.09 (1H, m, H-22), 5.10 (1H, m, H-19), 5.55 (1H, m, H-5), 5.61 (1H, m, H-6), 5.70 (2H, m, H-15 and H-16), 5.72 (1H, s, H-2), 5.74 (1H, m, H-7), 7.39 (6H, m), and 7.51 (4H, m); ESIMS (pos) *m/z* 961.5 (M + Na)<sup>+</sup>; HRESIMS *m/z* 961.3944 [calcd for C<sub>49</sub>H<sub>60</sub>O<sub>11</sub>F<sub>6</sub>Na, (M + Na)<sup>+</sup>: 961.3927].

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**Supporting Information Available:** Spectral data for **1**. This material is available free charge via the Internet at <http://pub.acs.org>.

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