HETEROCYCLES, Vol. 91, No. 2, 2015, pp. 265 - 274. © 2015 The Japan Institute of Heterocyclic Chemistry
Received, 19th November, 2014, Accepted, 19th January, 2015, Published online, 23rd January, 2015
DOI: 10.3987/COM-14-13132

IRIOMOTEOLIDE-2a, A CYTOTOXIC 23-MEMBERED MACROLIDE FROM MARINE BENTHIC DINOFLAGELLATE *AMPHIDINIUM* SPECIES

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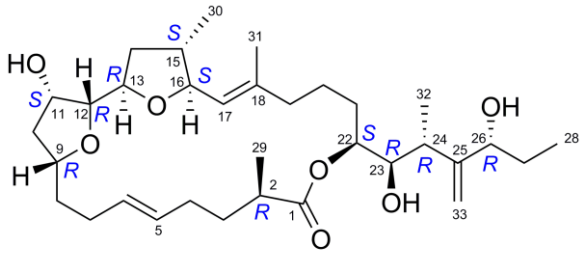
Abstract – The first 23-membered macrolide, iriomoteolide-2a (**1**), was isolated from cultivated algal cells of a marine benthic dinoflagellate *Amphidinium* species. The structure was determined based on detailed analysis of 2D NMR data. The relative stereochemistries were assigned on the basis of coupling constants and ROESY data, and the absolute configurations were elucidated by analyzing the NMR data of the MTPA esters for **1** and its reduced product. Iriomoteolide-2a (**1**) exhibited potent cytotoxic activities in vitro and in vivo.

INTRODUCTION

Marine phytoplankton such as dinoflagellates have been proven to produce a variety of chemically unique and biologically interesting secondary metabolites such as long-chain polyketides and polyethers.[1] It is well known that marine dinoflagellates of the genus *Amphidinium* produce unique metabolites such as macrolides and/or linear polyketides.[2] These macrolides possess various carbon chains forming 12–29-membered macrolactone rings, including odd-numbered lactone rings. Biosynthetically unique structural features such as vicinally located C_1 branches are found in a majority of these molecules, and most exhibit potent cytotoxicity.

Our continuing search for new cytotoxic metabolites from marine benthic dinoflagellate *Amphidinium* species[3] has resulted in the isolation of the first naturally occurring 23-membered macrolide, iriomoteolide-2a (**1**) (Scheme 1), consisting of a continuous carbon chain. Compound **1** exhibited potent

cytotoxic activity against tumor cells and in vivo activity against tumor. Here, we describe the isolation, structural elucidation, and biological activities of **1**.



1

Scheme 1. Structure of iriomoteolide-2a (**1**)

RESULTS AND DISCUSSION

The dinoflagellate *Amphidinium* species (HYA024 strain) was monoclonally separated from benthic sea sand collected off Iriomote Island, Japan. 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_2O. The toluene-soluble materials were subjected to chromatography with SiO_2 gel, C_{18}, and amino-SiO_2 gel columns, and then C_{18} HPLC, to afford iriomoteolide-2a[4] (**1**, 0.032% from dry weight).

Iriomoteolide-2a (**1**) was obtained as an optically active colorless oil {$[\alpha]_D^{20}$ +44 (*c* 0.23, $CHCl_3$)}. The molecular formula of **1** was established to be $C_{33}H_{54}O_7$ by HRESIMS data [*m/z* 585.3749 $(M + Na)^+$, Δ −1.8 mmu], indicating seven degrees of unsaturation. ^1H and ^{13}C NMR data ($CDCl_3$; Table 1) showed the presence of an ester carbonyl, two sp^2 quaternary carbons, three sp^2 methines, an sp^2 methylene, eleven sp^3 methines including eight oxygenated ones, ten sp^3 methylenes, and five methyls, accounting for four degrees of unsaturation. The remaining double bond equivalents were inferred that there are three rings in **1**. Proton resonances of the ten sp^3 methylenes were nonequivalent and close to each other, so their corresponding carbons were assigned using CH_2-selected E-HSQC correlations.

Interpretation of the ^1H–^1H COSY and TOCSY spectra revealed the following three proton–proton connectivities: from H-2 to H-17, H_3-29, and H_3-30; from H_2-19 to H-24 and H_3-32; and from H-26 to H_3-28 (Figure 1). These networks were supported by CH_2-selected E-HSQC-TOCSY correlations for H-5/C-3, H-6/C-8, H_2-19/C-20, H_2-19/C-21, H-22/C-20, and H_3-29/C-3. It was deduced from the *J*(H-5/H-6) value (15.5 Hz) that the disubstituted double bond at C-5–C-6 had *E* geometry. The presence of the *E*-trisubstituted double bond at C-17–C-18 was suggested by HMBC correlations for H_3-31/C-17, H_3-31/C-18, and H_3-31/C-19 and the intensive ROESY correlations for H-16/H_3-31 and H-17/H_2-19. Attachment of the C-25–C-33 exomethylene to C-24 and C-26 was confirmed from HMBC correlations for H-26/C-25, H_3-32/C-25, and H_2-33/C-25. HMBC correlations for H-22/C-1 and H_3-29/C-1 suggested that C-22 was involved in an ester linkage with C-1. The presence of two tetrahydrofuran rings (C-9–

Table 1. ^{1}H and ^{13}C NMR data for iriomoteolide-2a (**1**) in $CDCl_3$

Positn	^{13}C	^{1}H (mult, *J* = in Hz)	Positn	^{13}C	^{1}H (mult, *J* = in Hz)
1	176.6 C		17	124.1 CH	5.09 d, 8.5
2	37.6 CH	2.46 m	18	141.5 C	
3	33.2 CH_2	1.78 m	19	39.7 CH_2	2.03 m
		1.45 m			1.99 m
4	29.5 CH_2	2.09 m	20	24.7 CH_2	1.48 m
		1.96 m			1.35 m
5	129.1 CH	5.36 brdt, 15.5, 7.0	21	26.5 CH_2	1.82 m
6	131.2 CH	5.46 brdt, 15.5, 6.2			1.49 m
7	28.7 CH_2	2.21 m	22	75.1 CH	5.05 dt, 11.3, 2.3
		2.07 m	23	78.4 CH	3.62 dd, 10.3, 2.3
8	36.5 CH_2	1.81 m	24	35.6 CH	2.43 m
9	77.6 CH	3.97 tt, 8.5, 4.6	25	153.9 C	
10	41.4 CH_2	2.20 ddd, 13.1, 8.5, 5.4	26	77.2 CH	4.01 t, 7.0
		1.74 m	27	29.1 CH_2	1.64 m
11	72.8 CH	4.43 m	28	10.2 CH_3	0.88[a] t, 7.3
12	83.1 CH	3.73 dd, 3.9, 3.1	29	16.0 CH_3	1.13[a] d, 7.0
13	77.5 CH	4.44 m	30	16.7 CH_3	1.01[a] d, 6.6
14	36.3 CH_2	2.17 ddd, 11.6, 8.5, 6.2	31	17.2 CH_3	1.70[a] s
		1.73 m	32	17.8 CH_3	1.11[a] d, 6.9
15	39.8 CH	2.08 m	33	112.4 CH_2	5.10 s
16	82.8 CH	4.08 t, 8.5			5.03 s

[a]3H.

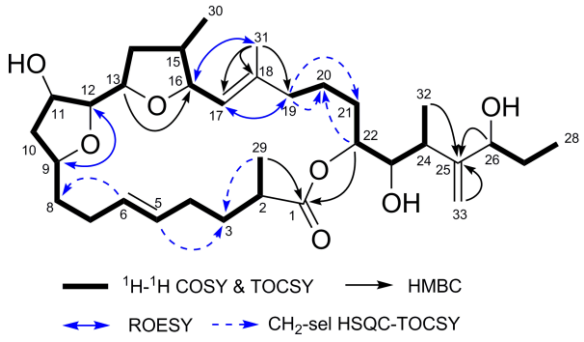


Figure 1. Selected 2D NMR correlations for iriomoteolide-2a (**1**)

C-12 and C-13–C-16) was implied by a ROESY correlation for H-9/H-12 and an HMBC correlation for H-13/C-16. The planar structure of iriomoteolide-2a was therefore concluded to be **1**.

The stereostructure of **1** was elucidated using the following procedure: 1) elucidation of the relative stereochemistries of C-9–C-16 and C-22–C-27, 2) determination of the absolute configurations of C-11, C-23, and C-26, and 3) elucidation of the absolute configuration of C-2. The ^{1}H–^{1}H coupling constants needed for stereochemical assignments were obtained from resolution-enhanced ^{1}H NMR spectra, and the long-range ^{13}C–^{1}H coupling constants for *J*-based configuration analysis[5] were estimated from the intensities of the correlations in the phase-sensitive HMBC spectrum.[6]

The relative stereochemistries of the bis-tetrahydrofuran moiety at C-9–C-16 were investigated using ROESY data, as shown in Figure 2a. For the C-9–C-12 moiety, *syn* relations for H-9–H-11 and H-9–H-12 were assigned by ROESY correlations for H-9/H-11 and H-9/H-12; *anti* and *syn* relations were deduced for H-13–H-15 and H-13–H-16, respectively, of the other tetrahydrofuran ring (C-13–C-16) from ROESY correlations for H-13/H-16 and H-13/H_3-30. The rotation of the C-12–C-13 bond between the two tetrahydrofuran rings was analyzed using *J*-based configuration analysis as shown in Figure 2b. The *J*(H-12/H-13) value (3.1 Hz) indicated a *gauche* relation for H-12–H-13, and the ^2J(C-12/H-13) and ^2J(C-13/H-12) values (almost 0 Hz) suggested *anti* relations for H-12–13-O and H-13–12-O. Considering the HSQC-ROESY correlation for H-11/C-13 and ROESY correlation for H-12/H-14a,[7] the relative stereochemistry for C-12–C-13 was concluded to be *threo*.

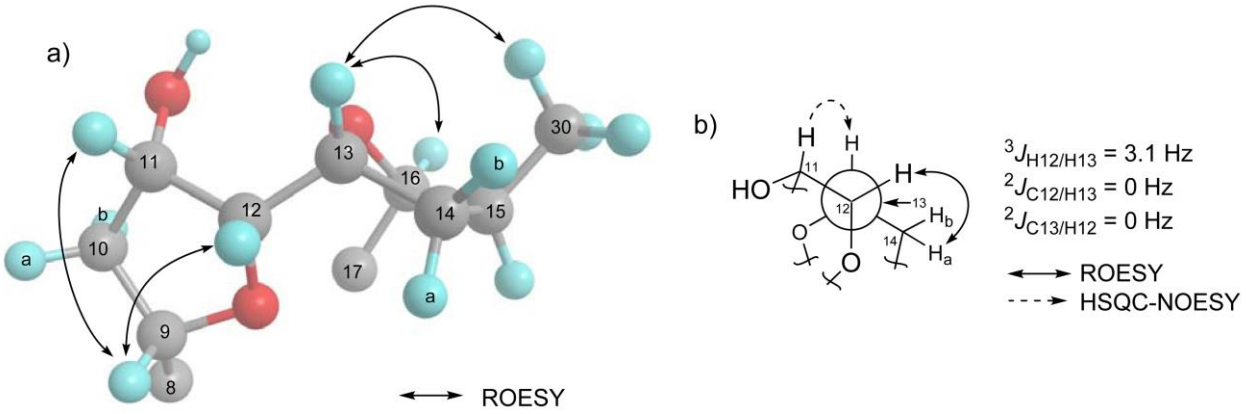


Figure 2. (a) Stereostructure of the bis-tetrahydrofuran portion at C-9–C-16 and (b) rotation model for C-12–C-13 bond in iriomoteolide-2a (**1**)

For the C-22–C-23 bond (Figure 3a), a *gauche* orientation for H-22–H-23 was implied by the relative small *J*(H-22/H-23) value (2.3 Hz), and the relatively large *J*(C-22/H-23) and small *J*(C-21/H-23) values (−6 and 7 Hz, respectively) indicated *gauche* and *anti* orientations for H-23–O-22 and H-23–C-21, respectively. ROESY correlations for H-21b/H-24 and H-22/H_3-32 suggested that the C-22–C-23 bond had the *erythro* configuration. Based on the relatively large *J*(H-23/H-24) (10.3 Hz) and *J*(C-23/H-24) (−8 Hz) values and the ROESY correlation for H-22/H_3-32, the relative configuration for C-23–C-24 was inferred to be *erythro* (Figure 3b). ROESY correlations for H-23/H-26, H-24/H-33b, and H-27b/H_3-32 indicated a 1,3-*syn* configuration for C-24–C-26 through the exomethylene unit. Therefore, the relative configurations of C-22, C-23, C-24, and C-26 were concluded as shown in Figure 3c.

The absolute configuration of **1** was examined by application of modified Mosher's method.[8] Treatment of **1** with (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-trifluoro-2-phenylacetyl chloride (MTPACl) gave 11,23,26-tris-(*S*)- and 11,23,26-tris-(*R*)-MTPA esters (**2a** and **2b**, respectively) of **1**. The ^{1}H NMR data

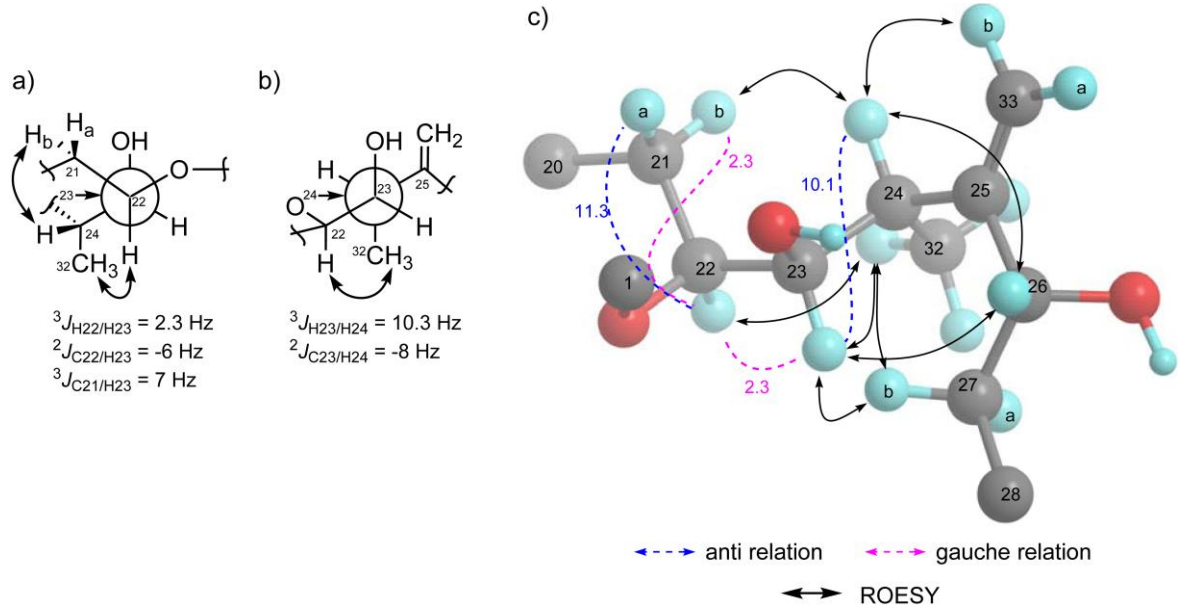


Figure 3. Rotation models for (a) C-22–C-23 and (b) C-23–C-24 bonds and (c) stereostructure of the C-22–C-26 portion in iriomoteolide-2a (**1**)

for **2a** and **2b** were assigned by analyzing the ^{1}H–^{1}H COSY and TOCSY spectra, and $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$) were calculated as shown in Figure 4. Around C-11 of the MTPA ester, the $\Delta\delta$ values of H-12, H-13, H_2-14, and H_3-30 were positive and those of H-9 and H_2-10 were negative, suggesting that C-11 had the *S* configuration. In contrast, positive $\Delta\delta$ values for H-26, H_2-27, and H_3-28 and negative values for H-22 and H-23 corresponded to $\Delta\delta$ distribution patterns typical of the *anti*-1,4-diols with chiral anisotropic reagents described by Riguera *et al.*,[9] suggesting the 23*R*,26*R*-configuration for **1**. Considering the relative stereochemistries, the absolute configurations at C-9, C-12, C-13, C-15, C-16, C-22, and C-24 were elucidated as *R*, *R*, *R*, *S*, *S*, *S*, and *R*, respectively.

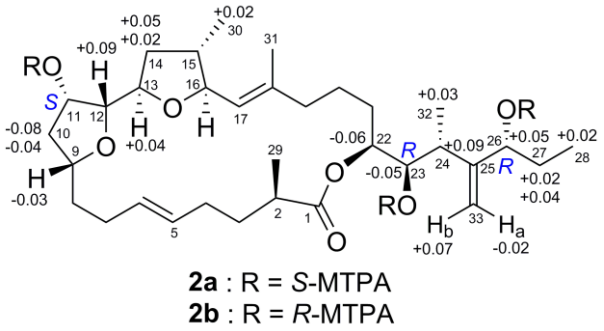


Figure 4. Δδ Values [Δδ (in ppm)] $\delta_S - \delta_R$] obtained for the 11,23,26-tris-(*S*)- and (*R*)-MTPA esters (**2a** and **2b**, respectively) of iriomoteolide-2a (**1**)

The absolute configuration at C-2 with a methyl group was elucidated on the basis of signal patterns of the two geminal protons at C-1[10] for the MTPA esters of the seco-alcohol of **1**. Reduction of **1** with LAH followed by treatment with (*R*)- and (*S*)-MTPACl gave mainly **3a** and **3b** (Figure 5a). The methylene

protons of C-1 in **3a** were observed as an overlapping 2H signal at δ_H 4.15 (Figure 5b), whereas those in **3b** appeared as separate signals at δ_H 4.09 and 4.23 (Figure 5c), indicating that the absolute configuration at C-2 was *R*.

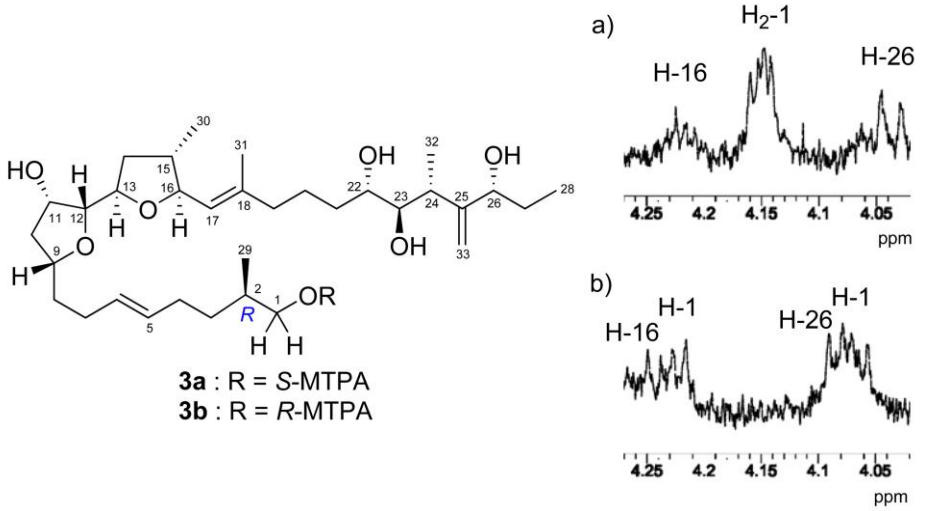


Figure 5. Structures and proton signal patterns of H_2-1 of (a) 1-(*S*)- and (b) (*R*)-MTPA esters (**3a** and **3b**, respectively) of the seco-alcohol of iriomoteolide-2a (**1**)

Iriomoteolide-2a (**1**) exhibited potent cytotoxicity against human B lymphocyte DG-75 cells (IC_{50}: 0.006 μg/mL) and human cervix adenocarcinoma HeLa cells (IC_{50}: 0.03 μg/mL). Although the compound showed *in vivo* activity against murine tumor P388 (T/C 132%) at a dose of 0.2 mg/kg, therapeutic effects did not appear at higher doses. Investigations for molecular targets of **1** using surface plasmon resonance revealed to have an affinity for actin. Amphidinolide H,[11] a potent cytotoxic macrolide from the *Amphidinium* dinoflagellate, is actin inhibitor that covalently binds to actin,[12] however, **1** seems to bind non-covalently with actin because the resonance disappeared through washing with a running solution.

CONCLUSION

Iriomoteolide-2a (**1**) is the first reported natural product possessing a 23-membered macrolactone ring consisting of a continuous polyketide chain.[13] More than half of the macrolides obtained from *Amphidinium* species have odd-numbered lactone rings, e.g., 13-, 15-, 17-, 19-, 25-, 27-, and 29-membered macrolides.[2] Although *Amphidinium* macrolides such as amphidinolides C,[14] F,[15] and M[16] have two tetrahydrofuran rings in their molecules, macrolide **1**, possessing a bis-tetrahydrofuran moiety, is unique.[3] Vicinally-locating C_1 branches (C-32 and C-33) on a C-23–C-25 portion are also characteristic of *Amphidinium* macrolides. Compound **1** showed the potent cytotoxic activity against tumor cells and in vivo activity against tumor.

EXPERIMENTAL

General. Optical rotation and IR were measured on a JASCO DIP-370 polarimeter and a JASCO FT/IR-5300 spectrophotometer, respectively. NMR data were recorded using 2.5 mm microcells for $CDCl_3$ (Shigemi Co., Ltd.). NMR spectra were measured on a Bruker AMX-500 spectrometer equipped with 2.5 mm C/H dual probe. Chemical shifts in $CDCl_3$ are reported in ppm with reference to the solvent residual proton and carbon signals (δ_H 7.26 and δ_C 77.0). ESIMS spectra were obtained on a JEOL JMS 700-TZ spectrometer.

Isolation. Material, cultivation and extraction of the dinoflagellate were described previously.[17] The toluene-soluble fraction (2 g) of the extract was subjected to SiO_2 gel column chromatography (40 X 200 mm) using a stepwise elution of $CHCl_3$ (200 mL) and $CHCl_3$–MeOH (98:2, 200 mL and then 95:5, 200 mL). The fraction eluted with 95% $CHCl_3$–MeOH was chromatographed successively by using C_{18} (MeCN–H_2O, 7:3) and then NH_2–SiO_2 gel columns (*n*-hexane–EtOAc, 2:1). A macrolide-containing fraction was separated by C_{18} HPLC [YMC-Pack Pro C18, 5 **μ**m, YMC Co., Ltd., 10 X 250 mm; eluent, MeCN–H_2O (60:40); flow rate, 2 mL/min; UV detection at 210 nm] to afford iriomoteolide-2a (**1**, 4.3 mg, 0.032% from dry weight).

Iriomoteolide-2a (1). Colorless oil; $[\alpha]_D^{20}$ +44 (*c* 0.23, $CHCl_3$); IR (neat) ν_{max} 3430 (broad), 2920 1712, 1219, and 1005 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESIMS (pos.) *m/z* 585 $(M+Na)^+$; ESIMS (neg.) *m/z* 561 $(M-H)^-$, 597 $(M+^{35}Cl)^-$, and 599 $(M+^{37}Cl)^-$; HRESIMS *m/z* 585.3749 calcd for $C_{33}H_{54}O_7Na$, $(M+Na)^+$, 585.3767].

11,23,26-Tris-(*S*)-MTPA ester (2a) of iriomoteolide-2a (1).

To a solution of iriomoteolide-2a (**1**, 0.2 mg) in 1% DMAP solution in CH_2Cl_2 (20 μL) were added triethylamine (2 μL) and (*R*)-(-)-MTPACl (1 μL), and the mixture was stirred at 4 °C for 15 h. After addition of *N,N*-dimethyl-1,3-propanediamine (2 μL), the solvent was evaporated in vacuo. The residue was passed through a SiO_2 gel column (hexane/acetone, 4:1) to afford 5-(*S*)-MTPA ester (**2a**) of **1** (0.02 mg): ^1H NMR ($CDCl_3$); δ 0.80 (3H, t, *J* = 6.8 Hz, H_3-28), 0.98 (3H, d, *J* = 6.8 Hz, H_3-30), 1.12 (3H, d, *J* = 6.8 Hz, H_3-29), 1.18 (3H, d, *J* = 6.8 Hz, H_3-32), 1.32 (1H, m, H-20b), 1.40 ~ 1.53 (4H, m, H-3b, H-27b, H-20a, and H-21b), 1.63 (1H, m, H-8b), 1.64 (1H, m, H-27a), 1.64 (3H, s, H_3-31), 1.72 (1H, H-14b), 1.80~1.85 (4H, m, H-3a, H-8a, H-10b, and H-21a), 1.90~2.04 (3H, m, H-4b, and H_2-19), 2.07 (1H, m, H-7b), 2.09 (1H, m, H-4a), 2.20 (1H, m, H-15), 2.21 (1H, m, H-7a), 2.34 (1H, m, H-14a), 2.37 (1H, m, H-10a), 2.49 (1H, m, H-2), 2.53 (1H, m, H-24), 3.51 (3H, s, OCH_3), 3.53 (6H, s, 2 X OCH_3), 3.68 (1H, m, H-12), 3.90 (1H, m, H-9), 4.20 (1H, m, H-16), 4.47 (1H, m, H-13), 4.97 (1H, s, H-33b), 4.98 (1H, m, H-22), 5.00 (1H, s, H-33a), 5.01 (1H, m, H-17), 5.14 (1H, m, H-23), 5.32 (1H, m, H-26), 5.35 (1H, m, H-11), 5.37 (1H, m, H-5), 5.46 (1H, m, H-6), 7.38 (9H, m, Ph), and 7.50 (6H, m, Ph); HRESIMS *m/z* 1233.4958 [calcd for $C_{63}H_{75}O_{13}F_9Na$, $(M+Na)^+$: 1233.4916].

11,23,26-Tris-(*R*)-MTPA ester (2b) of iriomoteolide-2a (1). A solution of iriomoteolide-2a (**1**, 0.2 mg) in CH_2Cl_2 (20 μL) containing 1% DMAP was treated with triethylamine (5 μL) and (*S*)-(+)-MTPACl (1.5 μL) by the same procedure as described above to afford 5-(*R*)-MTPA ester (**2b**) of **1** (0.12 mg): ^{1}H NMR ($CDCl_3$); δ 0.78 (3H, t, *J* = 6.8 Hz, H_3-28), 0.96 (3H, d, *J* = 6.8 Hz, H_3-30), 1.12 (3H, d, *J* = 6.8 Hz, H_3-29), 1.15 (3H, d, *J* = 6.8 Hz, H_3-32), 1.33 (1H, m, H-20b), 1.40 ~ 1.53 (4H, m, H-3b, H-27b, H-20a, and H-21b), 1.60 (1H, m, H-27a), 1.63 (1H, m, H-8b), 1.64 (3H, s, H_3-31), 1.70 (1H, m, H-14b), 1.80~1.85 (4H, m, H-3a, H-8a, H-10b, and H-21a), 1.90~2.04 (3H, m, H-4b, and H_2-19), 2.08 (1H, m, H-7b), 2.10 (1H, m, H-4a), 2.17 (1H, m, H-15), 2.21 (1H, m, H-7a), 2.29 (1H, m, H-14a), 2.41 (1H, m, H-10a), 2.49 (1H, m, H-2), 2.51 (1H, m, H-24), 3.51 (3H, s, OCH_3), 3.52 (3H, s, OCH_3), 3.53 (3H, s, OCH_3), 3.59 (1H, m, H-12), 3.93 (1H, m, H-9), 4.19 (1H, m, H-16), 4.43 (1H, m, H-13), 4.90 (1H, s, H-33b), 5.00 (H-17), 5.02 (1H, s, H-33a), 5.04 (1H, m, H-22), 5.06 (1H, m, H-23), 5.26 (1H, m, H-26), 5.33 (1H, m, H-11), 5.37 (1H, m, H-5), 5.46 (1H, m, H-6), 7.38 (9H, m, Ph), and 7.50 (6H, m, Ph); HRESIMS *m/z* 1233.4937 [calcd for $C_{63}H_{75}O_{13}F_9Na$, $(M+Na)^+$: 1233.4916].

1-(*S*)-MTPA ester (3a) of seco-alcohol of iriomoteolide-2a (1). To a solution of iriomoteolide-2a (**1**, 0.5 mg) in THF (20 μL) was added $LiAlH_4$ (2 mg) and the mixture was stirred at 4 °C for 1 h. After addition of MeOH (10 μL), the mixture was partitioned between EtOAc and brine. After evaporation of the organic solvent, the residue was treated with triethylamine (2 μL) and (*R*)-(-)-MTPACl (1 μL) in 1% DMAP solution in CH_2Cl_2 (20 μL), and the mixture was stirred at 4 °C for 15 h. After addition of *N,N*-dimethyl-1,3-propanediamine (2 μL), the solvent was evaporated in vacuo. The residue was passed through a SiO_2 gel column (hexane/acetone, 2:1) to afford 1-(*S*)-MTPA ester (**3a**) of seco-alcohol of **1** (0.05 mg): ^{1}H NMR ($CDCl_3$); δ 0.87 (3H, t, *J* = 6.8 Hz, H_3-28), 1.00 (3H, d, *J* = 6.8 Hz, H_3-30), 1.08 (3H, d, *J* = 6.8 Hz, H_3-29), 1.12 (3H, d, *J* = 6.8 Hz, H_3-32), 1.30~1.50 (7H, m), 1.64 (3H, s, H_3-31), 1.60~1.90 (5H, m), 1.90~2.27 (9H, m), 2.36 ~2.48 (2H, m, H-2 and H-24), 3.51 (3H, s, OCH_3), 3.65 (1H, m, H-12), 3.62 (1H, m, H-13), 3.78 (1H, m, H-22), 3.98 ~ 4.06 (2H, m, H-9 and H-26), 4.15 (2H, m, H_2-1), 4.23 (1H, m, H-16), 4.40 ~ 4.45 (2H, m, H-11 and H-13), 5.01 (1H, s, H-33a), 5.05 (1H, s, H-33b), 5.12 (1H, m, H-17), 5.40 ~ 5.50 (2H, m, H-5 and H-6), 7.38 (3H, m, Ph), and 7.50 (2H, m, Ph); HRESIMS *m/z* 805.4488 [calcd for $C_{43}H_{65}O_9F_3Na$, $(M+Na)^+$: 805.44784].

1-(*R*)-MTPA ester (3b) of seco-alcohol of iriomoteolide-2a (1). Iriomoteolide-2a (0.5 mg) was reduced using $LiAlH_4$ and then esterified with (*S*)-(+)-MTPACl by the same procedure as described above to afford 1-(*R*)-MTPA ester (**3b**) of seco-alcohol of **1** (0.08 mg): ^{1}H NMR ($CDCl_3$); δ 0.87 (3H, t, *J* = 6.8 Hz, H_3-28), 0.97 (3H, d, *J* = 6.8 Hz, H_3-30), 1.07 (3H, d, *J* = 6.8 Hz, H_3-29), 1.10 (3H, d, *J* = 6.8 Hz, H_3-32), 1.30~1.50 (7H, m), 1.64 (3H, s, H_3-31), 1.60~1.90 (5H, m), 1.86~2.27 (9H, m), 2.34 ~2.50 (2H, m, H-2 and H-24), 3.58 (1H, m, H-12), 3.60 (1H, m, H-13), 3.87 (1H, m, H-22), 3.97 (1H, m, H-9), 4.07 (1H, m,

H-26), 4.09 (1H, m, H-1a), 4.23 (1H, m, H-1b), 4.26 (1H, m, H-16), 4.30 (1H, m, H-11), 4.34 (1H, m, H-13), 4.97 (1H, s, H-33a), 5.07 (1H, s, H-33b), 5.08 (1H, m, H-17), 5.40 ~ 5.50 (2H, m, H-5 and H-6) 7.38 (3H, m, Ph), and 7.50 (2H, m, Ph); HRESIMS *m/z* 805.4488 [calcd for $C_{43}H_{65}O_9F_3Na$, $(M+Na)^+$: 805.44784].

Cytotoxic assay. Cytotoxic assay using DG-75 and Hela cells was carried out at a density of 5,000 cells per well into 96-well plates in DMEM medium containing 10% fetal calf serum at 37 ºC in 5% CO_2. After 72 h, the number of viable cells was counted using Cell Counting Kit 8 (Dojindo Co., Kumamoto, Japan) according to the manufacturer's instructions. The assay reagent is a tetrazolium compound (WST-8) that is reduced by live cells into a colored formazan product measured at 450 nm using a microplate reader (Bio-Rad, USA). The experiments were repeated in triplicate wells. The viability of the treated groups was estimated as a percentage of control groups. The cytotoxicity was shown as the concentration causing a 50% reduction of cell growth (IC_{50}). Doxorubicin (IC_{50} against DG-75 and Hela cells: 0.03 and 0.04 μg/mL) was used as a positive control.

In vivo antitumor activity. The ascites fluid containing 10^6 cells of murine leukemia P388 cell were transplanted i.p. into CDF1 mice. Iriomoteolide-2a (**1**) was dissolved in EtOH. The 0.1 mL solution of **1** was administered at day 1 and day 5 after the tumor inoculation (day 0). Dose of **1** was in the range of 1 ~ 20 μg per mice. Antitumor activity was expressed by T/C (the mean survival time of the treated group divided by that of the untreated group) at the each dosage of **1**. Five mice were used for each experimental group.

Surface plasmon resonance assay. The binding affinities of objective samples to the actin were determined using SPR biosensor technology (BIACORE J, GE Healthcare, Wisconsin, USA). The purified tubulin and actin were immobilized onto the CM5 chip surface by amino coupling method using HBS-N (10 mM Hepes/0.15 M NaCl, pH7.4) buffer as a running buffer. 1μM DMSO solution of iriomoteolide-2a (**1**) was injected for 2 min over the immobilized surface .The specific binding profiles of **1** to immobilized tubulin and actin were obtained after subtracting the response signal from the control flow cells. All the data were collected at 25 °C with running buffer HBS-N at a constant flow of 30 μl/min. Binding affinities of the compounds to actin were evaluated with Biacore Evaluation Version 3.0 software (GE Healthcare, Wisconsin, USA).

ACKNOWLEDGEMENTS

We thank R. Iwamoto, Y. Okammoto, Y. Endo, Y. Nagakita, and Y. Fukuda, for dinoflagellate cultivation and measurement of bioactivity and S. Oka and A. Tokumitsu of the Equipment Management Center, Hokkaido University, for ESI-MS measurements. This study was partially supported by a Grant-in-aids for Scientific Research (Nos. 25860079 to K. K. and 26670045 to M. T.) from the Ministry

of Education, Culture, Sports, Science, and Technology (MEXT) of Japan.

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