11- and 4′-Epimers of Chaetomugilin A, Novel Cytostatic Metabolites from Marine Fish-Derived Fungus *Chaetomium globosum*

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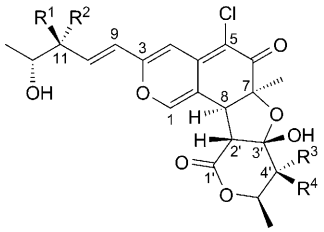
11- and 4′-epichaetomugilin A were isolated from a strain of *Chaetomium globosum* that was originally isolated from the marine fish *Mugil cephalus*. 11-Epichaetomugilin A is the first compound to have an opposite absolute configuration at C(11) to that of other azaphilones isolated to date. Their absolute configurations were elucidated on the basis of spectroscopic analyses, including 1D- and 2D-NMR techniques, and some chemical transformations. 11-Epichaetomugilin A weakly inhibited the growth of cultured P388 cells and HL-60 cells (IC_{50} 88.9 and 66.7 μM, resp.).

Introduction. – Marine microorganisms are potentially prolific sources of highly bioactive secondary metabolites that might serve as useful leads in the development of new pharmaceutical agents. Based on the fact that some bioactive compounds isolated from marine animals are produced by bacteria, we have focused our attention on new antitumor compounds from microorganisms separated from marine animals [1–5]. As part of this study, we conducted a search for antitumor compounds from a strain of *Chaetomium globosum* OUPS-T106B-6 originally obtained from the marine fish *Mugil cephalus* and isolated six new cytotoxic metabolites designated as chaetomugilins A–F from the culture broth of this fungal strain [6][7]. These metabolites are classified as azaphilones which are compounds that exhibit antimicrobial activity, nitric oxide inhibition [8], gp120-CD4 binding inhibition [9], monoamine oxidase inhibition [10][11], and platelet-derived growth-factor binding inhibition [12]. An examination using a disease-oriented panel of 39 human cancer cell lines (HCC panel) [13][14] suggested that the mode of action of chaetomugilins A (**1**), C, and F might be different from that shown by any other anticancer drugs developed to date [7]. Our continuing search for cytotoxic metabolites from this fungal strain led to the isolation of two new azaphilones designated as 11-epichaetomugilin A (**2**) and 4′-epichaetomugilin A (**3**). The cytotoxicity of these compounds was examined using the murine P388 leukemia cell line, the human HL-60 leukemia cell line, the murine L1210 leukemia cell line, and the human KB epidermoid carcinoma cell line. Compound **2** exhibits moderate activity against P388 and HL-60 cell lines. We describe herein their absolute configurations and biological activities.

Results and Discussion. – *Chaetomium globosum*, OUPS-T106B-6, was cultured at 27° for 6 weeks in a medium (50 l) containing 1% soluble starch and 0.1% casein in 50% artificial seawater adjusted to pH 7.4, as reported in [6][7]. After incubation, the

AcOEt extract of the culture filtrate was purified by bioassay-directed fractionation (cytotoxicity to P388 cell line) employing a stepwise combination of *Sephadex LH-20* chromatography, silica-gel column chromatography, and reversed-phase (RP) HPLC to afford 11-epichaetomugilin A (**2**) and 4′-epichaetomugilin A (**3**).



	R^1	R^2	R^3	R^4
1	H	Me	Me	H
2	Me	H	Me	H
3	H	Me	H	Me

11-Epichaetomugilin A (**2**) had the molecular formula $C_{23}H_{27}ClO_7$, which was deduced from the $[M+H]^+$ peak in high-resolution fast atom-bombardment mass spectrometry (HR-FAB-MS) and the ratio of the intensities of isotope peaks ($[M+H]^+/[M+H+2]^+$). Its IR spectrum exhibited bands at 3440, 1720, and 1617 cm^{-1}, characteristic of OH, ester, and conjugated C=O groups. Close inspection of the ^{1}H- and ^{13}C-NMR spectra (*Table 1*) of **2** by DEPT and HMQC experiments revealed the presence of four secondary Me groups (Me–C(11), Me(13), Me–C(4′), and Me–C(5′)), one tertiary Me group (Me–C(7)); four sp^2-hybridized CH groups (H–C(1), H–C(4), H–C(9), and H–C(10)), including one O-bearing C-atom (H–C(1)); six sp^3-CH groups (H–C(8), H–C(11), H–C(12), H–C(2′), H–C(4′), and H–C(5′)), including two CH–O groups (H–C(12) and H–C(5′)); two quaternary O-bearing sp^3-C-atoms (C(7) and C(3′)), including a hemiacetal C-atom (C(3′)); four quaternary sp^2-C-atoms (C(3), C(4a), C(5), and C(8a)); and two C=O groups (C(6) and C(1′)). ^{1}H,^{1}H-COSY Analysis of **2** led to three partial structural units as shown by bold lines in *Fig. 1*. The configuration of the double bond C(9)=C(10) was deduced to be (*E*) from the coupling constants of the olefinic H-atoms ($J(9,10) = 15.5$ Hz). The connection of these units with the remaining functional groups was determined on the basis of key HMBC correlations outlined in *Fig. 1*. The connection of the Cl-atom to C(5) was reasonable from its chemical shift (δ(C) 110.33). The above data revealed that the planar structure of **2**, elucidated as shown in *Fig. 1*, is the same as that of chaetomugilin A (**1**). The general features of the ^{1}H- and ^{13}C-NMR spectra of **2** closely resembled those of **1** except for the chemical-shift difference of the H-atom signals for H–C(11) (δ(H) 2.39) and H–C(12) (δ(H) 3.77), and the C-atom signal for C(11)

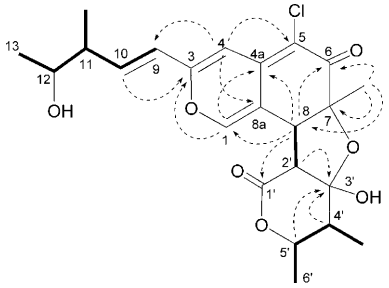


Fig. 1. *Selected ^{1}H,^{1}H-COSY* (—) *and HMBC correlations* (H → C) *in 11-epichaetomugilin A* (**2**)

(δ(C) 16.05) of **2**, relative to those of **1**. In NOESY experiments of **2**, NOE correlations (H–C(5′)/H–C(8), H–C(5′)/Me–C(7), H–C(2′)/H–C(4′), and H–C(4′)/Me(6′)) revealed that the relative configuration of **2** corresponded to that of **1** except for the side chain. *Chen et al.* have shown that the sign of the specific rotation of azaphilones is apparently controlled by the absolute configuration at C(7) [15]. *Steyn* and *Vleggaar* established the absolute configuration at C(7) of (+)-sclerotiorin and consequently of azaphilones on the basis of the CD spectra. In addition, they concluded that the *Cotton* curve remained unaffected by the functional groups at C(8) [16]. In agreement with these data, a negative *Cotton* effect ($\Delta\varepsilon_{337}$ −1.3) was observed for compound **1**, the absolute configuration of which at C(7) had been already established as (*S*) [6]. Based on these evidences, the absolute configuration at C(7) of **2** was determined to be (*S*), because its CD spectrum showed a negative *Cotton* effect ($\Delta\varepsilon_{336}$ −1.7; *Fig. 2*). This fact allowed the assignment of the absolute configuration of the stereogenic centers except C(11) and C(12) (7*S*, 8*S*, 2′*R*, 3′*R*, 4′*R*, and 5′*R*). These findings suggested that **2** was the stereoisomer of **1** at C(11) or C(12) in the side chain. The modified *Mosher*'s method [17] was applied to **2** to determine the absolute configuration at C(12). The ^{1}H chemical-shift differences between (*R*)- and (*S*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) esters **2a** and **2b** of compound **2** are shown in *Fig. 3*. The results revealed (*R*)-configuration at C(12) in **2**, which is the same as that in **1**. Hence, we deduced that **2** was the stereoisomer of **1** at C(11) with the (11*S*)-configuration. The above evidence led to the elucidation of the absolute configuration of 11-epichaetomugilin A (**2**).

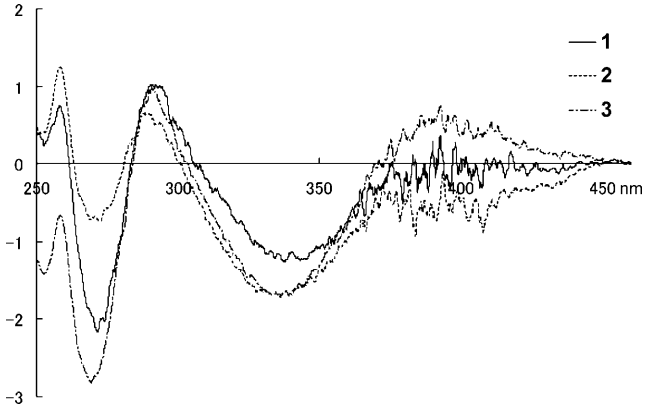


Fig. 2. *CD Spectra of* **1**–**3**

4′-Epichaetomugilin A (**3**) had the same molecular formula as **1** and **2** as deduced from HR-FAB-MS. The general features of its UV, IR, and NMR spectra (*Table 1*) closely resembled those of **1** except for the chemical-shift differences of the H-atom signals for H–C(1) (δ(H) 7.39), H–C(8) (δ(H) 3.08), H–C(2′) (δ(H) 2.90), H–C(4′) (δ(H) 2.19), H–C(5′) (δ(H) 4.68), and Me–C(4′) (δ(H) 1.02), and the C-atom signals for C(2′) (δ(C) 56.52), C(4′) (δ(C) 41.83), C(6′) (δ(C) 17.32), and Me–C(4′) (δ(C) 7.47) in **3**, relative to those of **1**. The planar structure of **3**, which was confirmed by

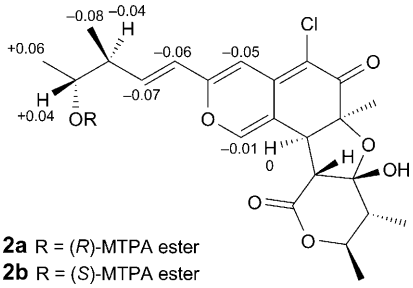


Fig. 3. *^1H Chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the* (R)*- and* (S)*-MTPA esters* **2a** *and* **2b**, *respectively, of 11-epichaetomugilin A* (**2**)

Table 1. *NMR-Spectral Data for* **1**–**3** *in $CDCl_3$* (500 MHz)[a])

	1		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
H−C(1)	7.27 (*s*)	145.67 (*d*)	7.29 (*s*)	145.54 (*d*)	7.39 (*s*)	145.73 (*d*)
C(3)		157.11 (*s*)		157.14 (*s*)		157.02 (*s*)
H−C(4)	6.57 (*s*)	105.47 (*d*)	6.57 (*s*)	105.40 (*d*)	6.57 (*s*)	105.45 (*d*)
C(4a)		140.07 (*s*)		140.23 (*s*)		140.14 (*s*)
C(5)		110.43 (*s*)		110.33 (*s*)		110.57 (*s*)
C(6)		189.25 (*s*)		189.20 (*s*)		188.96 (*s*)
C(7)		83.98 (*s*)		83.78 (*s*)		83.51 (*s*)
H−C(8)	2.98 (*d*, *J* = 10.0)	50.55 (*d*)	3.00 (*d*, *J* = 10.0)	50.41 (*d*)	3.08 (*d*, *J* = 9.1)	49.88 (*d*)
C(8a)		114.29 (*s*)		114.37 (*s*)		114.94 (*s*)
H−C(9)	6.15 (*d*, *J* = 15.0)	122.10 (*d*)	6.16 (*d*, *J* = 15.5)	122.45 (*d*)	6.15 (*d*, *J* = 15.5)	122.20 (*d*)
H−C(10)	6.61 (*dd*, *J* = 15.0, 6.2)	142.52 (*d*)	6.62 (*dd*, *J* = 15.5, 6.2)	142.54 (*d*)	6.61 (*dd*, *J* = 15.5 , 6.2)	142.24 (*d*)
H−C(11)	2.45 (*sext.*, *J* = 6.2)	44.32 (*d*)	2.39 (*sext.*, *J* = 6.2)	44.75 (*d*)	2.46 (*sext.*, *J* = 6.2)	44.28 (*d*)
H−C(12)	3.81 (br. *s*)	70.90 (*d*)	3.77 (*quint.*, *J* = 6.2)	71.02 (*d*)	3.79–3.85 (*m*)	70.91 (*d*)
Me(13)	1.20 (*d*, *J* = 6.2)	20.52 (*q*)	1.22 (*d*, *J* = 6.2)	20.88 (*q*)	1.20 (*d*, *J* = 6.2)	20.37 (*q*)
Me−C(7)	1.40 (*s*)	23.23 (*q*)	1.40 (*s*)	23.33 (*q*)	1.41 (*s*)	23.75 (*q*)
Me−C(11)	1.13 (*d*, *J* = 6.2)	14.85 (*q*)	1.12 (*d*, *J* = 6.2)	16.05 (*q*)	1.13 (*d*, *J* = 6.2)	14.89 (*q*)
C(1′)		170.50 (*s*)		170.65 (*s*)		171.50 (*s*)
H−C(2′)	3.06 (*d*, *J* = 10.0)	58.24 (*d*)	3.05 (*d*, *J* = 10.0)	58.22 (*d*)	2.90 (*d*, *J* = 9.1)	56.52 (*d*)
C(3′)		104.17 (*s*)		104.04 (*s*)		104.87 (*s*)
H−C(4′)	1.90 (*dq*, *J* = 10.3, 6.2)	44.89 (*d*)	1.90 (*dq*, *J* = 10.3, 6.5)	44.91 (*d*)	2.19 (*qd*, *J* = 7.0, 2.1)	41.83 (*d*)
H−C(5′)	4.30 (*dq*, *J* = 10.3, 6.5)	76.89 (*d*)	4.31 (*dq*, *J* = 10.3, 6.5)	77.20 (*d*)	4.65–4.70 (*m*)	76.48 (*d*)
Me−C(5′)	1.41 (*d*, *J* = 6.5)	18.70 (*q*)	1.41 (*d*, *J* = 6.5)	18.66 (*q*)	1.38 (*d*, *J* = 6.3)	17.32 (*q*)
Me−C(4′)	1.13 (*d*, *J* = 6.2)	8.79 (*q*)	1.13 (*d*, *J* = 6.5)	8.75 (*q*)	1.02 (*d*, *J* = 7.0)	7.47 (*q*)

[a]) ^{1}H Chemical-shift values (δ in ppm rel. to Me_4Si), followed by multiplicity and then the coupling constants (*J* in Hz) in parentheses.

analysis of ^{1}H,^{1}H-COSY correlations and HMBC correlations, was the same as those of **1** and **2**. In NOESY experiments of **3**, differences in NOE correlations (H−C(2′)/Me−C(4′) and H−C(4′)/H−C(5′)) from those of **1** and **2** (H−C(2′)/H−C(4′) and H−C(4′)/Me(6′)) were observed in the lactone ring (*Fig. 4*). This suggested that **3** was

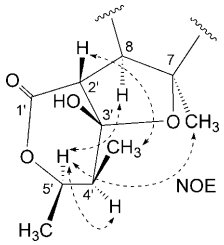
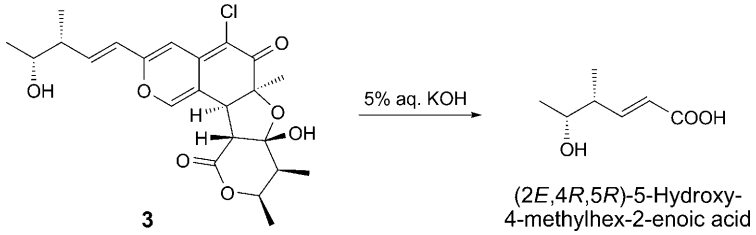


Fig. 4. *Observed NOE for the ring system of 4′-epichaetomugilin A* (**3**)

the stereoisomer of **1** at C(4′) in the lactone ring. The observation that the CD spectrum of **3** showed the same curve as those of **1** and **2** (*Fig. 2*) led us to deduce that the absolute configuration at C(7) was (*S*), and allowed us to assign the absolute configuration at C(4′) as (*S*). In addition, ^{1}H- and ^{13}C-NMR signals for 11- and 12-positions of **3** were similar to those of **1** and other chaetomugilins reported previously [6][7], suggesting the absolute configurations at C(11) and C(12) ((11*R*) and (12*R*)). To confirm the absolute configuration at C(11) and C(12), alkaline degradation of **3** was carried out. The degradation of **3** with 5% KOH afforded a carboxylic acid that was identified as (2*E*,4*R*,5*R*)-5-hydroxy-4-methylhex-2-enoic acid by comparison with the compound obtained from chaetomugilin A (**1**) in a similar manner (*Scheme*). Based on the above evidence, the absolute configuration for **3** was established as 4′-epichaetomugilin A.

Scheme



In a primary screen for antitumor activity, cancer cell-growth inhibitory properties of **2** and **3** were examined using the murine P388 leukemia cell line, the human HL-60 leukemia cell line, the murine L1210 leukemia cell line, and the human KB epidermoid carcinoma cell line. Compound **2** exhibited moderate activity against P388 and HL-60 cell lines, but marginal activity against L1210 and KB cell lines ($IC_{50} > 100$ μM) (*Table 2*). On the other hand, compound **3** was only marginally active against all cell lines. Henceforth, the sensitivities of various cancer cell lines to these compounds will be studied by examining cytotoxicities using a disease-oriented panel of 39 human cancer cell lines (HCC panel) [13][14].

Experimental Part

General. Liq. chromatography over silica gel (mesh 230–400) was performed at medium pressure. HPLC was run on a *Waters ALC-200* instrument equipped with a differential refractometer (*R 401*) and

Table 2. *Cytotoxity* (IC_{50} [μM])[a]) *of the Metabolites against P388, HL-60, L1210, and KB Cell Lines*

	P388	HL-60	L1210	KB
11-Epichaetomugilin A (**2**)	88.9 ± 2.8	66.7 ± 2.7	> 100	> 100
4′-Epichaetomugilin A (**3**)	> 100	> 100	> 100	> 100
5-Fluorouracil[b])	1.7 ± 0.2	2.7 ± 0.2	1.1 ± 0.2	7.7 ± 0.2

[a]) DMSO was used for vehicle. [b]) Positive control.

Shim-pack PREP-ODS column (particle size: 2.5 μm, column size: 25 cm × 20 mm i.d.). M.p.: *Yanagimoto* micro-melting point apparatus; uncorrected. Anal. TLC was performed on precoated *Merck* aluminium sheets (DC-Alufolien *Kieselgel 60* F_{254}, 0.2 mm) with the solvent system CH_2Cl_2/MeOH (9:1), and compounds were viewed under a UV lamp and sprayed with 10% H_2SO_4, followed by heating. Optical rotations were recorded on a *Jasco J-820* polarimeter. UV Spectra: *Hitachi U-2000* spectrophotometer; λ_{max} (log ε) in nm. CD: *Jasco J-820* polarimeter; λ ($\Delta\varepsilon$) in nm. IR Spectra: *Jasco FT/IR-680 plus*; ν_{max} in cm^{-1}. NMR Spectra: *Varian Unity Inova-500* and *Mercury 300* spectrometers; at 27° with Me_4Si as internal reference; δ in ppm, *J* in Hz. FAB-MS and HR-FAB-MS: *Jeol JMS-700* (Ver. 2) mass spectrometer; matrix: *p*-nitrobenzyl alcohol; *m/z* (rel. int. %).

Fermentation. Chaetomium globosum OUPS-T106B-6 was initially isolated from the marine fish *Mugil cephalus* captured in Katsuura Bay, Japan, in October 2000. The marine fish was disinfected with EtOH, and its gastrointestinal tract was applied to the surface of nutrient agar layered in a *Petri* dish. Serial transfers of one of the resulting colonies provided a pure strain of *C. globosum*. The fungal strain was cultured at 27° for 6 weeks in a liquid medium (50 l) containing 1% soluble starch and 0.1% casein in 50% artificial seawater adjusted to pH 7.4. The culture was filtered under suction, and the culture filtrate was extracted three times with AcOEt (150 l). The combined extracts were evaporated *in vacuo* to give a crude extract (10.2 g) that exhibited cytotoxicity (IC_{50} 35.8 μg/ml).

Isolation of Metabolites. The above AcOEt extract was passed through a *Sephadex LH-20* column using $CHCl_3$/MeOH 1:1 as the eluent. The second fraction (3.4 g), exhibiting strong activity, was chromatographed on a silica-gel column with $CHCl_3$/MeOH gradient as the eluent. The MeOH/$CHCl_3$ 2:98 eluate (1.1 g) was purified by HPLC with MeOH/H_2O 50:50 to afford *Fr. 1* (323.8 mg) that exhibited cytotoxicity. *Fr. 1* was further purified by HPLC with MeCN/H_2O 35:65 to afford **3** (5.4 mg) and **2** (6.2 mg) (t_R 93 and 100 min, resp.).

11-Epichaetomugilin A (=*(6a*S*,7a*R*,8*R*,9*R*,11a*R*,11b*S*)-5-Chloro-7a-hydroxy-3-[(1*E*,3*S*,4*R*)-4-hydroxy-3-methylpent-1-en-1-yl]-6a,8,9-trimethyl-6a,7a,8,9,11a,11b-hexahydro-6*H*,11*H*-pyrano[3′,4′:4,5]furo[2,3-*h*]-2-benzopyran-6,11-dione*; **2**). Yellow powder. M.p. 127–129°. $[\alpha]_D^{22} = -185.1$ ($c = 0.18$, EtOH). UV (EtOH): 285 (3.78), 373 (3.75), 414 (3.82). CD ($c = 3.51 \times 10^{-4}$ M, EtOH): 449 (0), 336 (−1.72), 301 (0), 288 (0.64), 281 (0), 272 (−0.73), 263 (0), 258 (1.24). IR (KBr): 3440, 1720, 1617, 1560, 1519. ^{1}H- and ^{13}C-NMR: see *Table 1*. FAB-MS: 451 ($[M+H]^+$, 100), 453 ($[M+H+2]^+$, 37.7). HR-FAB-MS: 451.1518 ($[M+H]^+$, $C_{23}H_{28}{}^{35}ClO_7^+$; calc. 451.1524).

4′-Epichaetomugilin A (=*(6a*S*,7a*R*,8*S*,9*R*,11a*R*,11b*S*)-5-Chloro-7a-hydroxy-3-[(1*E*,3*R*,4*R*)-4-hydroxy-3-methylpent-1-en-1-yl]-6a,8,9-trimethyl-6a,7a,8,9,11a,11b-hexahydro-6*H*,11*H*-pyrano[3′,4′:4,5]furo[2,3-*h*]-2-benzopyran-6,11-dione*; **3**). Yellow powder. M.p. 135–137°. $[\alpha]_D^{22} = -262.5$ ($c = 0.12$, EtOH). UV (EtOH): 288 (3.72), 373 (3.75), 405 (3.81). CD ($c = 2.60 \times 10^{-4}$ M, EtOH): 373 (0), 336 (−1.69), 303 (0), 290 (1.00), 283 (0), 270 (−2.77). IR (KBr): 3432, 1720, 1617, 1560, 1519 (C=C). ^{1}H- and ^{13}C-NMR: see *Table 1*. FAB-MS: 451 ($[M+H]^+$, 100), 453 ($[M+H+2]^+$, 43.3). HR-FAB-MS: 451.1523 ($[M+H]^+$, $C_{23}H_{28}{}^{35}ClO_7^+$; calc. 451.1524).

*Formation of (*R*)- and (*S*)-MTPA Esters* **2a** *and* **2b** *from 11-Epichaetomugilin A* (**2**). (*R*)-MTPA (2.0 mg), dicyclohexylcarbodiimide (DCC; 2.0 mg), and 4-(dimethylamino)pyridine (DMAP; 1.0 mg) were added to a CH_2Cl_2 soln. (0.5 ml) of **2** (2.4 mg), and the mixture was left at r.t. for 6 h. The solvent was evaporated under reduced pressure, and the residue was purified by HPLC MeCN/H_2O 65:35 to afford (*R*)-MTPA ester **2a** (0.24 mg) as an amorphous powder. The same reaction with **2** (2.3 mg) using (*S*)-MTPA (2.1 mg) gave ester **2b** (0.30 mg).

Ester **2a**. ^{1}H-NMR ($CDCl_3$): 1.13 (*d*, *J* = 6.2, *Me*−C(11)); 1.13 (*d*, *J* = 6.5, *Me*−C(4′)); 1.29 (*d*, *J* = 6.2, Me(13)); 1.41 (*s*, *Me*−C(7)); 1.41 (*d*, *J* = 6.5, Me(6′)); 1.90 (*dq*, *J* = 10.3, 6.2, H−C(4′)); 2.62–2.68 (*m*, H−C(11)); 2.98 (*d*, *J* = 10.0, H−C(8′)); 3.07 (*d*, *J* = 10.0, H−C(2′)); 3.50 (*s*, MeO(MTPA)); 4.30 (*dq*, *J* = 10.3, 6.5, H−C(5′)); 5.11 (*quint.*, *J* = 6.2, H−C(12)); 6.08 (*d*, *J* = 15.5, H−C(9)); 6.46 (*dd*, *J* = 15.5, 6.2, H−C(10)); 6.51 (*s*, H−C(4)); 7.26 (*s*, H−C(1)); 7.40–7.44 (*m*, 3 arom. H); 7.50 – 7.59 (*m*, 2 arom. H). FAB-MS: 667 ($[M+H]^+$, 54.0), 669 ($[M+H+2]^+$, 21.5). HR-FAB-MS: 667.1917 ($[M+H]^+$, $C_{33}H_{35}F_3{}^{35}ClO_9^+$; calc. 667.1922).

Ester **2b**. ^{1}H-NMR ($CDCl_3$): 1.05 (*d*, *J* = 6.2, *Me*−C(11)); 1.13 (*d*, *J* = 6.5, *Me*−C(4′)); 1.35 (*d*, *J* = 6.2, Me(13)); 1.40 (*s*, *Me*−C(7)); 1.41 (*d*, *J* = 6.5, Me(6′)); 1.90 (*dq*, *J* = 10.3, 6.2, H−C(4′)); 2.58–2.64 (*m*, H−C(11)); 2.98 (*d*, *J* = 10.0, H−C(8′)); 3.07 (*d*, *J* = 10.0, H−C(2′)); 3.55 (*s*, MeO(MTPA)); 4.30 (*dq*, *J* = 10.3, 6.5, H−C(5′)); 5.15 (*quint.*, *J* = 6.2, H−C(12)); 6.02 (*d*, *J* = 15.5, H−C(9)); 6.39 (*dd*, *J* = 15.5, 6.2, H−C(10)); 6.46 (*s*, H−C(4)); 7.25 (*s*, H−C(1)); 7.39–7.45 (*m*, 3 arom. H); 7.51–7.60 (*m*, 2 arom. H). FAB-MS: 667 ($[M+H]^+$, 27.6), 669 ($[M+H+2]^+$, 11.4). HR-FAB-MS: 667.1923 ($[M+H]^+$, $C_{33}H_{35}{}^{35}ClF_3O_9^+$; calc. 667.1922).

Degradation of **3** *by KOH.* 4′-Epichaetomugilin A (**3**; 11.3 mg) was dissolved in 10 ml of 5% aq. KOH, and the mixture was stirred for 3 h at 100°. Then, the mixture was extracted with 10 ml of $CHCl_3$. The H_2O layer was adjusted to pH 3.0 with 9% H_2SO_4 and re-extracted with 10 ml of AcOEt. The org. extract was concentrated to dryness *in vacuo*. The residue was purified by HPLC with MeCN/H_2O gradient (0:100 → 60:40) to afford (2*E*,4*R*,5*R*)-5-hydroxyl-4-methylhex-2-enoic acid (0.5 mg).

Assay for Cytotoxicity to Cancer Cell Lines. Cytotoxic activities of **2** and **3** were examined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method. P388, HL-60, L1210, and KB cells were cultured in RPMI 1640 medium (10% fetal calf serum) suspension at 37° in 5% CO_2. The test material was dissolved in DMSO to reach a concentration of 10 mM, and the soln. was further diluted with RPMI 1640 medium to achieve concentrations of 200, 20, and 2 μM. Each soln. was combined with each cell suspension (1×10^5 cells/ml) in the medium, resp. After incubating at 37° for 72 h in 5% CO_2, grown cells were labeled with 5 mg/ml MTT in phosphate-buffered saline (PBS), and the absorbance of formazan dissolved in 20% sodium dodecyl sulfate (SDS) in 0.1N HCl was measured at 540 nm using a microplate reader (*Model 450*, *BioRad*). Each absorbance value was expressed as percentage relative to the control cell suspension that was prepared without the test substance using the same procedure as that described above. All assays were performed three times. Semilogarithmic plots were constructed from the averaged data, and the effective dose of the substance required to inhibit cell growth by 50% (IC_{50}) was determined.

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