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Absolute stereostructures of cytotoxic metabolites, chaetomugilins A–C, produced by a *Chaetomium* species separated from a marine fish

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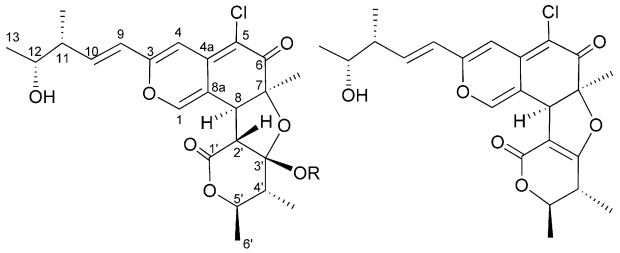
Abstract

Chaetomugilins A–C were isolated from a strain of *Chaetomium globosum* originally isolated from the marine fish *Mugil cephalus*, and their absolute stereostructures have been elucidated on the basis of spectroscopic analyses using 1D and 2D NMR techniques, some chemical transformations, and an X-ray analysis. This compound exhibited significant cytotoxicity against cultured P388 cells and HL-60 cells.

Based on the fact that some of the bioactive materials isolated from marine animals have been produced by bacteria, we have focused our attention on new antitumour materials from microorganisms separated from marine organisms.[1–4] As part of this study, we have made a search for antitumour compounds from a strain of *Chaetomium globosum* OUPS-T106B-6 originally obtained from the marine fish *Mugil cephalus* and found three new cytotoxic metabolites designated as chaetomugilins A–C (**1**–**3**), which belong to azaphilones, from the culture broth of this fungal strain. Azaphilones have various bioactivities,[5] and the metabolites, which were isolated in this investigation, exhibited significant cytotoxic activity against the murine P388 leukemia cell line and the human HL-60 leukemia cell line. We describe herein the absolute stereostructure and biological activities of these compounds.

The microorganism from *M. cephalus* fish was cultured at 27 °C 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. After incubation, the AcOEt extract of the culture filtrate was purified by bioassay-directed fractionation (cytotoxicities against P388 cell line) employing stepwise combination of Sephadex LH-20, silica gel column chromatography and reverse phased HPLC to afford chaetomugilins A (**1**, 72.2 mg), B (**2**, 7.7 mg), and C (**3**, 10.2 mg).



1 R = H
2 R = CH_3

3

Chaetomugilin A (**1**)[6] had the molecular formula $C_{23}H_{27}ClO_7$ established by the $[M+H]^+$ peak in high-resolution fast atom bombardment mass spectrometry (HRFABMS) and the ratio of intensity of isotope peaks ($MH^+/[MH+2]^+$). Its IR spectrum exhibited bands at 3437, 1719, and 1618 cm^{-1}, characteristic of hydroxyl, ester, and conjugated carbonyl groups. A close inspection

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of the ^{1}H and ^{13}C NMR spectra (Table 1) of **1** by DEPT and HMQC experiments revealed the presence of four secondary methyl (11-CH_3, C-13, 4′-CH_3 and C-6′), one tertiary methyl (7-CH_3), four sp^2-hybridized methines (C-1, C-4, C-9 and C-10) including oxygen-bearing carbon (C-1), six sp^3-methines (C-8, C-11, C-12, C-2′, C-4′ and C-5′) including two oxymethines (C-12 and C-5′), two quarternary oxygen-bearing sp^3-carbons (C-7 and C-3′) including a hemiacetal carbon (C-3′), four quarternary sp^2- carbons (C-3, C-4a, C-5 and C-8a) and two carbonyls (C-6 and C-1′). The ^{1}H– ^{1}H COSY analysis of **1** led to three partial structural units as shown by bold-faced lines in Figure 1. The geometrical configuration of the double bond moieties (C-9 – C-10) was deduced as *trans* from the coupling constants of the olefinic protons ($J_{9,10} = 15.0$ Hz). The connection of these units and the remaining functional groups was determined on the basis of the key HMBC correlations, summarized in Figure 1. The connection of a chlorine atom to C-5 was reasonable from its chemical shift (δ_C 110.43). Thus the planar structure of **1** was elucidated as shown in Fig. 1. The relative stereochemistry of **1** was deduced from NOESY experiments (Fig. 2). NOEs correlations (5′-H/8-H, H-5′/7-CH_3, H-2′/H-4′ and H-4′/H-6′) revealed the relative configuration expect C-11 and C-12 for **1** as shown in Figure 2. The absolute stereochemistry for compound **1** was established by derivation of chaetomugilin B (**2**) described later.

Chaetomugilin B (**2**)[7] was assigned the molecular formula $C_{24}H_{29}ClO_7$ deduced from HRFABMS. The general

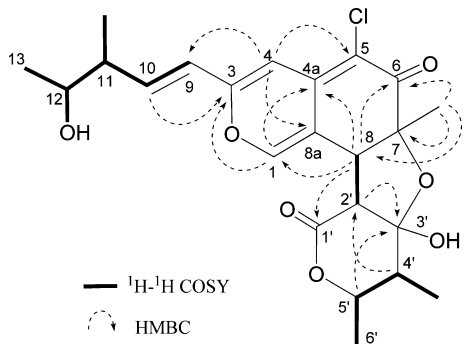

Fig. 1. Selected ^{1}H–^{1}H COSY and HMBC correlations in chaetomugilin A (**1**).

features of the ^{1}H and ^{13}C NMR spectra (Table 1) closely resembled those of **1** except that the proton signal for H-4′ (δ_H 2.32), the carbon signal for C-4′ (δ_C 37.74), C-3′ (δ_C 106.65), and 4′-CH_3 (δ_C 13.54) in **2** revealed a chemical shift difference relative to those of **1**. In addition, the signal for a methoxyl group (δ_H 3.25, δ_C 49.66) was observed newly. The ^{1}H– ^{1}H COSY and HMBC correlations (from 10-H to C-3, from 4-H to C-4a and C-5, from 1-H to C-3, C-4a, 8 and 8a, from 8-H to C-6, C-7, 7-CH_3, C-3′ and C-1′, from 5′-H to C-3′ and C-1′, and from the methoxyl group to C-3′ led to the planar structure for **2**. In order to determine the absolute stereostructure of **2**, an X-ray crystal-structure analysis was carried out for a single crystal of **2** (obtained by recrystallization from $CHCl_3$–MeOH).[8] The result (Fig. 3) allowed assignment of the

Table 1
NMR spectral data of chaetomugilins A–C (**1**–**3**) in $CDCl_3$

Position	**1**				**2**				**3**			
	δ_H[a]	*J*/Hz	δ_C		δ_H[a]	*J*/Hz	δ_C		δ_H[a]	*J*/Hz	δ_C	
1	7.27 s		145.67	(d)	7.50 s		145.89	(d)	7.89 s		147.60	(d)
3			157.11	(s)			156.47	(s)			157.24	(s)
4	6.57 s		105.47	(d)	6.52 s		105.37	(d)	6.56 s		105.54	(d)
4a			140.07	(s)			139.34	(s)			142.07	(s)
5			110.43	(s)			110.84	(s)			110.00	(s)
6			189.25	(s)			186.89	(s)			182.07	(s)
7			83.98	(s)			83.29	(s)			91.23	(s)
8	2.98 d	10.0 (2′)	50.55	(d)	3.14 d	10.0 (2′)	48.71	(d)	4.22 s		44.75	(d)
8a			114.29	(s)			114.94	(s)			113.93	(s)
9	6.15 d	15.0 (10)	122.10	(d)	6.14 d	15.0 (10)	122.27	(d)	6.14 d	15.0 (10)	122.15	(d)
10	6.61 dd	15.0 (9), 6.2 (11)	142.52	(d)	6.57 dd	15.0 (9), 6.2 (11)	141.62	(d)	6.61 dd	15.0 (9), 6.2 (11)	142.38	(d)
11	2.45 sex	6.2 (10, 12, 11-Me)	44.32	(d)	2.44 sex	6.2 (10, 12, 11-Me)	44.26	(d)	2.45 sex	6.2 (10, 12, 11-Me)	44.29	(d)
12	3.81 br s		70.90	(d)	3.80 quint	6.2 (11, 13)	70.91	(d)	3.80 quint	6.2 (11, 13)	70.88	(d)
13	1.20 d	6.2 (12)	20.52	(q)	1.19 d	6.2 (12)	20.50	(q)	1.19 d	6.2 (12)	20.50	(q)
7 –Me	1.40 s		23.23	(q)	1.40 s		23.78	(q)	1.72 s		24.89	(q)
11 –Me	1.13 d	6.2 (11)	14.85	(q)	1.13 d	6.2 (11)	14.88	(q)	1.13 d	6.2 (11)	14.84	(q)
1′			170.50	(s)			171.36	(s)			164.07	(s)
2′	3.06 d	10.0 (8)	58.24	(d)	3.01 d	10.0 (8)	56.90	(d)			101.10	(s)
3′			104.17	(s)			106.65	(s)			174.91	(s)
4′	1.90 dq	10.3 (5′), 6.2 (4′-Me)	44.89	(d)	2.32 qd	6.8 (4′-Me), 5.2 (5′)	37.74	(d)	2.58 quint	6.2 (5′, 4′-Me)	35.06	(d)
5′	4.30 dq	10.3 (4′), 6.5 (6′)	76.89	(d)	4.40 qd	7.0 (6′), 5.2 (4′)	79.29	(d)	4.29 quint	6.2 (4′, 6′)	79.42	(d)
6′	1.41 d	6.5 (5′)	18.70	(q)	1.44 d	7.0 (5′)	19.22	(q)	1.41 d	6.2 (5′)	19.48	(q)
4′ –Me	1.13 d	6.2 (4′)	8.79	(q)	1.11 d	6.8 (4′)	13.54	(q)	1.24 d	6.2 (4′)	14.05	(q)
3′ –OMe					3.25 s		49.66	(q)				

[a] ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (*J*/Hz). Figures in parentheses indicate the proton coupling with that position.

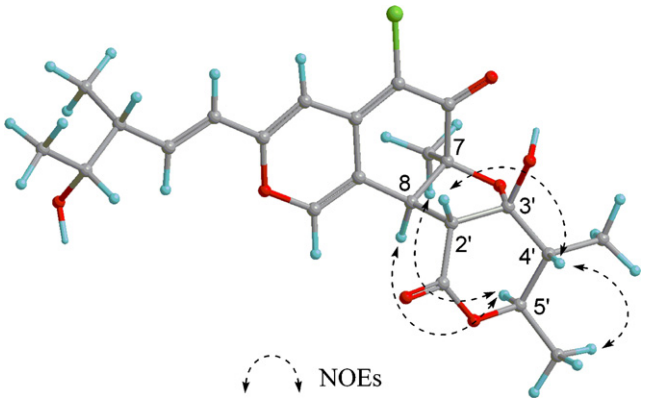

Fig. 2. Key NOEs correlations in **1** (graphical representation using the program CHEM 3D).

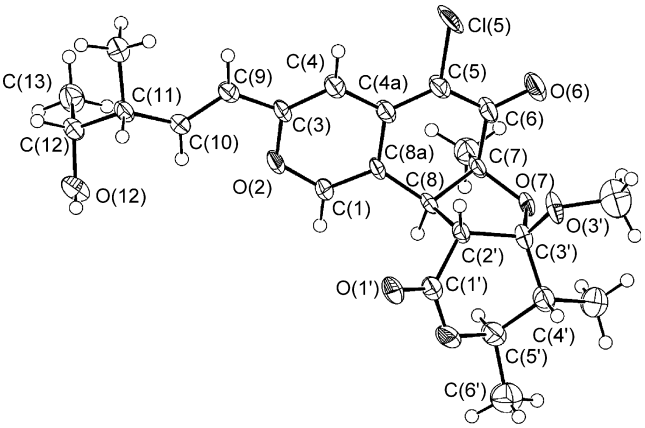

Fig. 3. X-ray structure for **2**.

absolute configuration of all the asymmetric centers (7*S*, 8*S*, 11*R*, 12*R*, 2′*R*, 3′*R*, 4′*R* and 5′*R*) and the conformation for **2**.

Since the absolute configuration for **1** has remained undecided, the hemiacetal hydroxyl group at C-3′ in **1** had been replaced to methoxyl group. The treatment with *p*-TsOH of **1** in MeOH gave two products **2** and **3** (yields 34% and 23%, respectively). These reaction products were confirmed to be identified with natural substances **2** and **3** in IR, UV, NMR spectra and optical rotations. These evidences revealed the absolute stereostructures for chaetomugilins A (**1**) and C (**3**) described later. Compound **1** had been stable in MeOH for a few days. Therefore, this evidence support that both **2** and **3** were not artefacts of **1**.

Chaetomugilin C (**3**)[9] was assigned the molecular formula $C_{23}H_{25}ClO_6$ which contained two hydrogen and one oxygen atoms less than that of **1**. The general features of its UV, IR, and NMR spectra (Table 1) closely resembled those of **1** except that the proton signal for H-8 (δ_H 4.22), 7-CH_3 (δ_H 1.72), H-2′ (disappearance), and H-4′ (δ_H 2.58), and the carbon signal for C-7 (δ_C 91.23), C-8 (δ_C 44.75), C-2′ (δ_C 101.10), C-3′ (δ_C 174.91), C-4′ (δ_C 35.06), C-1′ (δ_C 164.07), and 4′-CH_3 (δ_C 14.05) in **3** revealed a chemical shift difference relative to those of **1**. The ^1H–^1H COSY correlations and the key HMBC correlations (from 8-H to C-1′ and C-3′, from 4′-H to C-2′ and C-3′, and from 5′-H to C-1′ and C-3′) showed that a double bond was present at C-2′–C-3′. The above evidences, together with the molecular formula of **3**, suggested the presence of the ether linkage between C-7 and C-3′. Thus, the planar structure of **3** was elucidated. Observed NOEs (4′-CH_3/8-H and 4′-CH_3/7-CH_3) implied that 4′-CH_3 is oriented *cis* to 8-H, 7-CH_3 in axial arrangements. In addition, NOE correlation between 4′-CH_3 and 6′-H was observed very weakly. Based on the evidence summarized above, the relative configuration of C-7, C-8, C-4′ and C-5′ in **3** is the same as those of **1** and **2**. Consequently, the absolute stereostructure for **3** was established by the derivation from **1**.

Table 2
Cytotoxity of the metabolites against P388 and HL-60 cells

Compounds		Cell line P388 IC_{50} (μM)[a]	Cell line HL-60 IC_{50} (μM)[a]
Chaetomugilin	A (**1**)	8.7	7.3
	B (**2**)	18.7	16.5
	C (**3**)	3.6	2.7
5-FU[b]		1.7	2.7

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

The cancer cell growth inhibitory properties of chaetomugilins A–C (**1**–**3**) were examined using the murine P388 leukemia cell line and the human HL-60 leukemia cell line. Compounds **1** and **3** exhibited significant cytotoxic activity against P388 and HL-60 cell lines equal to 5-FU (Table 2). In future, the cancer cell growth inhibitory properties of these compounds will be examined using a disease-oriented panel of 39 human cell lines and molecular target inhibitory activities of the substance will also be tested using protein kinase, histone deacetylase, farnesyl transferase, telomerase, and proteasome.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.04.060.

References and notes

1. Iwamoto, C.; Yamada, T.; Ito, Y.; Minoura, K.; Numata, A. *Tetrahedron* **2001**, *57*, 2997–3004.
2. Yamada, T.; Iritani, M.; Doi, M.; Minoura, K.; Ito, Y.; Numata, A. *J. Chem. Soc., Perkin Trans. 1* **2001**, 3046–3053.

3. Yamada, T.; Iritani, M.; Minoura, K.; Numata, A. *J. Antibiot.* **2002**, *55*, 147–154.
4. Yamada, T.; Iritani, M.; Minoura, K.; Kawai, K.; Numata, A. *Org. Biomol. Chem.* **2004**, 2131–2135.
5. (a) Quang, D. N.; Stadler, M.; Fournier, J.; Tomita, A.; Hashimoto, T. *Tetrahedron* **2006**, *62*, 6349–6354; (b) Matsuzaki, K.; Tahara, H.; Inokoshi, J.; Tanaka, H. *J. Antibiot.* **1998**, *51*, 1004–1011; (c) Yoshida, E.; Fujimoto, H.; Yamazaki, M. *Chem. Pharm. Bull.* **1996**, *44*, 284–287; (d) Takahashi, M.; Koyama, K.; Natori, S. *Chem. Pharm. Bull.* **1990**, *38*, 625–628.
6. Chaetomugilin A (**1**): Yellow powder, mp 149–151 °C ($CHCl_3$–MeOH), $[\alpha]_D$ −50.1 (*c* 0.16, EtOH); UV λ_{max} (EtOH)/nm: 282 ($\log\varepsilon$ 3.92), 385 (4.03), 402 (3.99), 428 (3.76); IR ν_{max} (KBr)/cm^{-1}: 3437, 1719, 1618, 1561, 1520; HRFABMS *m*/*z* 451.1515 $[M+H]^+$ (calcd for $C_{23}H_{28}{}^{35}ClO_7$: 451.1506).
7. Chaetomugilin B (**2**): Yellow prisms, mp 215–217 °C ($CHCl_3$–MeOH), $[\alpha]_D$ −40.2 (*c* 0.03, EtOH); UV λ_{max} (EtOH)/nm: 285 ($\log\varepsilon$ 3.91), 384 (4.03), 405 (3.91), 429 (3.77); IR ν_{max} (KBr)/cm^{-1}: 3437, 1719, 1624, 1561, 1521; HRFABMS *m*/*z* 465.1679 $[M+H]^+$ (calcd for $C_{24}H_{30}{}^{35}ClO_7$: 465.1680).
8. *Crystal data* for **2**: $C_{24}H_{39}ClO_7$, $M = 464.92$, orthorhombic, space group $P2_12_12_1$, $a = 10.559(1)$, $b = 12.008(1)$, $c = 18.315(2)$ Å, $V = 2322.1(4)$ Å^3, $Z = 4$, $T = 240(2)$ K, $D_x = 1.330$ g cm^{-3}, $F(000) = 984$, m(Mo Kα) = 0.207 mm, Data collection was performed by Rigaku AFC5R using graphite-monochromated radiation ($\lambda = 0.7107$ Å); 21,847 reflections were collected until $\theta_{max} = 26.37$ Å, in which independent unique 3428 reflections ($R_{int} = 0.1847$) were observed ($I > 2\sigma(I)$). The crystal structure was solved by the direct method using SHELXS-97.[10] The structure was refined by the full matrix least-squares method on F^2 using SHELXL-97. [11] For the structure refinements, non-hydrogen atoms were refined with anisotropic temperature factors. Hydrogen atoms were calculated on the geometrically ideal positions and fitted the electron density map by the 'ride on' method, and were included in the calculation of structure factors with isotropic temperature factors. At the final stage, $R1 = 0.1076$, $Rw = 0.2033$ and $S = 1.295$ were obtained. CCDC 675994.
9. Chaetomugilin C (**3**): Yellow oil, $[\alpha]_D$ 103.3 (*c* 0.04, EtOH); UV λ_{max} (EtOH)/nm: 294 ($\log\varepsilon$ 3.93), 391 (4.06), 408 (4.02), 427 (3.68); IRν_{max} (liquid)/cm^{-1}: 3446, 1710, 1700, 1608, 1559, 1521; HRFABMS *m*/*z* 433.1413 $[M+H]^+$ (calcd for $C_{23}H_{26}{}^{35}ClO_6$: 433.1418).
10. Sheldrick, G. M. SHELXS-*97, Program for the Solution of Crystal Structures*; University of Göttingen: Germany, 1997.
11. Sheldrick, G. M. SHELXL-*97,* Program for the Solution of Crystal Structures from Diffraction Data; University of Göttingen: Germany, 1997.