

ORIGINAL ARTICLE

New isochaetochromin, an inhibitor of triacylglycerol synthesis in mammalian cells, produced by *Penicillium* sp. FKI-4942: II. structure elucidation

Narihiro Ugaki, Hiroyuki Yamazaki, Ryuji Uchida and Hiroshi Tomoda

The structure of a new congener of chaetochromin, an inhibitor of triacylglycerol synthesis in CHO-K1 cells produced by *Penicillium* sp. FKI-4942, was elucidated by spectroscopic methods, including various NMR experiments. Isochaetochromin A₁ has a bis-naphtho- γ -pyrone moiety.

The Journal of Antibiotics (2012) 65, 21–24; doi:10.1038/ja.2011.106; published online 30 November 2011

Keywords: fungal metabolites; isochaetochromin; naphtho- γ -pyrone; structure elucidation

INTRODUCTION

During the course of screening for inhibitors of triacylglycerol synthesis in CHO-K1 cells, a new compound named isochaetochromin A₁ was isolated along with structurally related known isochaetochromins B₁ and B₂¹ from the culture broth of *Penicillium* sp. FKI-4942. These compounds were found to inhibit triacylglycerol synthesis in CHO-K1 cells. The taxonomy of the producing fungus, fermentation, isolation and biological properties of isochaetochromins were described in a previous paper.² In this study, the physico-chemical properties and structure elucidation of isochaetochromin A₁ are described.

MATERIALS AND METHODS

General experimental procedures

UV spectra were recorded on a spectrophotometer (8453 UV-Visible spectrophotometer; Agilent Technologies, Santa Clara, CA, USA). IR spectra were recorded on a Fourier transform IR spectrometer (FT-710; Horiba, Kyoto, Japan). Optical rotations were measured with a digital polarimeter (DIP-1000; JASCO, Tokyo, Japan). CD spectra were measured with a spectrometer (J-720; JASCO). ESI-TOF-MS and HRESI-TOF-MS spectra were recorded on a mass spectrometer (JMS-T100LP; JEOL, Tokyo, Japan). Various NMR spectra were measured with a spectrometer (XL-400; Agilent Technologies, Santa Clara, CA, USA).

RESULTS

Physico-chemical properties of isochaetochromins

The physico-chemical properties of isochaetochromins A₁, B₁ and B₂ are summarized in Table 1 and have a similar pattern with absorption maxima at 234, 291–292 and 328 nm in the UV spectra. The IR absorption of isochaetochromins A₁, B₁ and B₂ at 1342–1348, 1390, 1433–1448, 1635 and 3398–3425 cm⁻¹ suggested the presence of carbonyl and hydroxy moieties in the molecule. These data indicated

that isochaetochromin A₁ is structurally related to isochaetochromins B₁ and B₂.

Structure of Isochaetochromin A₁

The molecular formula of isochaetochromin A₁ was determined to be C₃₀H₂₆O₁₀ on the basis of HRESI-TOF-MS measurement (Table 1). The ¹³C NMR spectrum (CDCl₃) showed 30 resolved signals, which were classified into four methyl carbons, four *sp*² methine carbons, two *sp*³ methine carbons, two oxygenated *sp*³ methine carbons, eight *sp*² quaternary carbons, eight oxygenated *sp*² quaternary carbons and two carbonyl carbons by analysis of the distortionless enhancement by polarization transfer (DEPT) and ¹³C-¹H HSQC spectra. The ¹H NMR spectrum (CDCl₃) displayed 24 proton signals, which were classified into 12 methyl protons, 8 methine protons and 4 hydroxy protons (δ 9.78, 9.94, 15.32 and 15.59). Taking the molecular formula into consideration, the presence of two more hydroxy protons was suggested. The connectivity of proton and carbon atoms was established by interpretation of the HSQC spectrum (Table 2). Analysis of ¹H-¹H COSY data revealed the presence of two partial structures, as shown in Figure 1. Furthermore, ¹³C-¹H long-range couplings of ²J and ³J observed in the ¹³C-¹H heteronuclear multiplet bond connectivity (HMBC) spectrum (Figure 2) provided further structural information: (1) the cross peaks from H-2 (δ 4.52) to C-4 (δ 202.6) and C-10a (δ 156.0), from H-3 (δ 2.64) to C-4 and C-4a (δ 101.5) and from 3-CH₃ (δ 1.22) to C-4 supported the connectivity from 2-CH₃ (δ 1.33) to C-4a. Considering the number of oxygen atoms present in isochaetochromin A₁ and the chemical shifts of C-2 (δ 75.6) and C-10a, one oxygen atom should be inserted between these carbons. (2) The cross peaks from 5-OH (δ 15.59) to C-4a, C-5 (δ 165.6) and C-5a (δ 105.7), from 6-OH (δ 9.94) to C-5a, C-6 (δ 158.6) and C-7 (δ 100.1), from H-7 (δ 6.56) to C-5a, C-6, C-8 (δ 161.2) and C-9

Table 1 Physico-chemical properties of isochaetochromins

	<i>Isochaetochromin A₁</i>	<i>Isochaetochromin B₁</i>	<i>Isochaetochromin B₂</i>
Appearance	Yellow powder	Yellow powder	Yellow powder
Molecular weight	546	546	546
Molecular formula	C ₃₀ H ₂₆ O ₁₀	C ₃₀ H ₂₆ O ₁₀	C ₃₀ H ₂₆ O ₁₀
<i>HRESI-TOF-MS (m/z)</i>			
Calcd:	569.1423 (M+Na) ⁺	569.1423 (M+Na) ⁺	569.1423 (M+Na) ⁺
Found:	569.1462 (M+Na) ⁺	569.1459 (M+Na) ⁺	569.1464 (M+Na) ⁺
UV (MeOH) λ _{max} nm (ε)	234 (31 000), 291 (39 700), 328 (11 100)	234 (33 600), 291 (44 300), 328 (12 300)	234 (48 600), 292 (63 100), 328 (16 800)
[α] _D ²⁰	−229° (c=0.1, CHCl ₃)	+142° (c=0.1, CHCl ₃)	−548° (c=0.10, CHCl ₃)
IR (KBr) ν _{max} (cm ^{−1})	3406, 1635, 1448, 1390, 1342	3398, 1635, 1441, 1390, 1344	3425, 1635, 1433, 1390, 1348

Table 2 ¹H (400 MHz) and ¹³C NMR (100 MHz) chemical shifts of isochaetochromins in CDCl₃

No.	<i>Isochaetochromin A₁</i>		<i>Isochaetochromin B₁</i>		<i>Isochaetochromin B₂</i>	
	δ _C	δ _H (J in Hz)	δ _C	δ _H (J in Hz)	δ _C	δ _H (J in Hz)
2	75.6	4.52 dq (3.0, 6.5)	78.4	4.17 dq (10.8, 6.5)	78.4	4.11 dq (11.0, 6.5)
2'	75.5	4.60 dq (3.0, 6.5)	75.6	4.64 dq (3.0, 6.5)	75.5	4.55 dq (3.0, 6.5)
3	44.5	2.64 dq (3.0, 7.0)	46.1	2.66 dq (10.8, 7.0)	46.2	2.64 dq (11.0, 7.0)
3'	44.4	2.72 dq (3.0, 7.0)	44.4	2.75 dq (3.0, 7.0)	44.3	2.61 dq (3.0, 7.0)
4	202.6	—	202.2	—	202.3	—
4'	202.2	—	200.7	—	200.7	—
4a	101.5	—	101.5	—	101.3	—
4a'	101.4	—	101.4	—	101.3	—
5	165.6	—	165.6	—	165.4	—
5'	165.3	—	164.6	—	164.6	—
5a	105.7	—	105.5	—	105.5	—
5a'	105.0	—	105.0	—	104.9	—
6	158.6	—	158.5	—	158.8	—
6'	158.2	—	158.3	—	158.3	—
7	100.1	6.56 s	100.2	6.52 s	100.1	6.49 s
7'	99.9	6.56 s	99.8	6.56 s	100.0	6.53 s
8	161.2	—	161.0	—	161.0	—
8'	159.8	—	159.5	—	159.8	—
9	102.3	—	102.2	—	102.6	—
9'	102.0	—	102.0	—	102.0	—
9a	142.7	—	142.6	—	142.0	—
9a'	141.3	—	141.3	—	141.3	—
10	99.8	6.14 s	99.1	6.15 s	99.1	6.09 s
10'	99.8	6.73 s	99.1	6.74 s	99.1	6.66 s
10a	156.0	—	156.1	—	156.1	—
10a'	155.5	—	155.4	—	155.4	—
2-CH ₃	16.6	1.33 d (6.5)	19.6	1.44 d (6.5)	19.6	1.40 d (6.5)
2'-CH ₃	16.5	1.43 d (6.5)	16.5	1.44 d (6.5)	16.4	1.39 d (6.5)
3-CH ₃	9.6	1.22 d (7.0)	10.0	1.26 d (7.0)	9.9	1.22 d (7.0)
3'-CH ₃	9.5	1.25 d (7.0)	9.6	1.25 d (7.0)	9.7	1.20 d (7.0)
5-OH		15.59 s		15.48 s		15.19 s
5'-OH		15.32 s		15.32 s		15.16 s
6-OH		9.94 s		9.90 s		9.81 s
6'-OH		9.78 s		9.70 s		9.64 s

(δ 102.3) and from H-10 (δ 6.14) to C-4a, C-5a, C-9, C-9a (δ 142.7) and C-10a supported the existence of the naphthalene ring (C-4a to C-10a). Considering the chemical shift of C-8 and molecular formula, one hydroxy group should be connected at C-8; thus, the existence of

the naphtho-γ-pyrone (naphtho-γ-dihydropyrone) unit in isochaetochromin A₁ was confirmed as shown in Figure 2. The NMR data suggested the presence of one more naphtho-γ-pyrone in isochaetochromin A₁. Fortunately, the naphtho-γ-pyrone unit (C-2' to C-10'a)

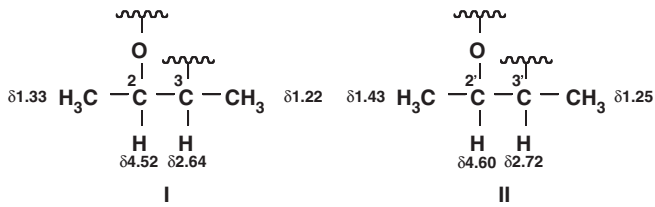


Figure 1 Partial structures of isochaetochromin A₁.

was assigned in the same way as the first naphtho- γ -pyrone unit (C-2 to C-10a). Taking all these findings together, the planar structure of isochaetochromin A₁ was elucidated to be a symmetrical dimer of the naphtho- γ -pyrone, as shown in Figure 3.

The relative stereochemistry at C-2, C-3, C-2' and C-3' of the γ -pyrone skeleton was investigated by the J values and NOE experiments. The J values ($J_{2-3}=3.0$ Hz and $J_{2'-3'}=3.0$ Hz) of isochaetochromin A₁ indicated that both the vicinal methyl groups are *cis*.¹

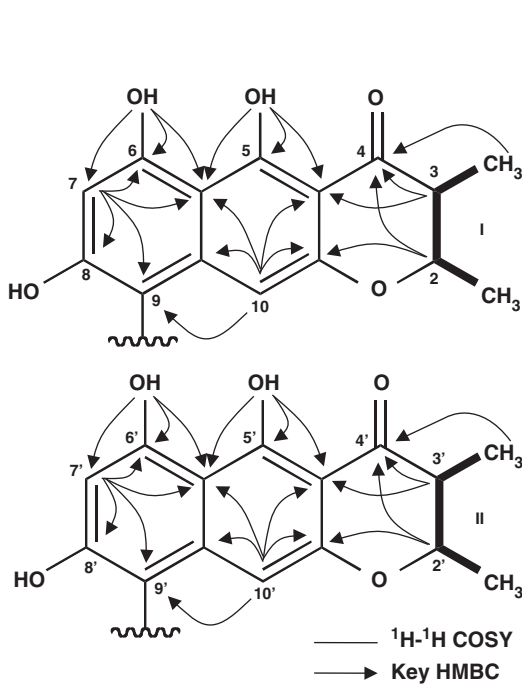


Figure 2 ¹H-¹H COSY and ¹³C-¹H HMBC experiments of isochaetochromin A₁.

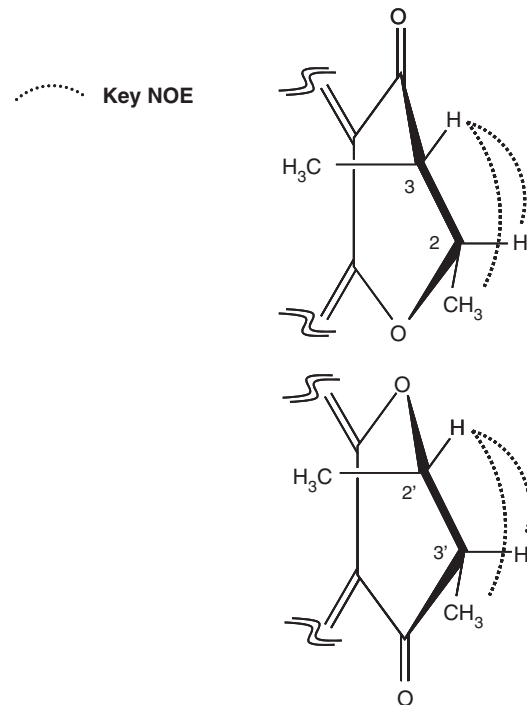


Figure 4 NOE experiments of isochaetochromin A₁.

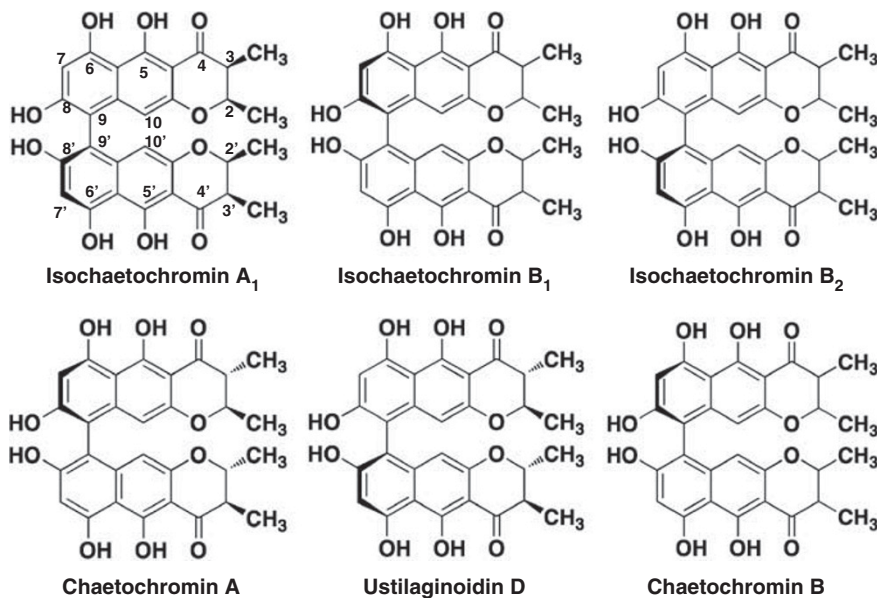


Figure 3 Structures of isochaetochromins A₁, B₁ and B₂, chaetochromin A, ustilaginoidin D and chaetochromin B.

Furthermore, NOEs were observed between H-2 and H-3, between 2-CH₃ and H-3, between H-3 and H-2/2-CH₃, between H-2' (δ 4.60) and H-3' (δ 2.72)/3'-CH₃ (δ 1.25), between H-3' and H-2' and between 3'-CH₃ and H-2' (Figure 4). From these data, the relative stereochemistry of iso chaetochromin A₁ was elucidated as shown in Figure 3.

To elucidate the absolute axial stereochemistry, the specific rotation and the CD data of iso chaetochromin A₁ was compared with those reported for iso chaetochromins B₁ and B₂.¹ The specific rotation of iso chaetochromin A₁ (-229°) showed a negative value, as did iso chaetochromin B₂ (-543.9° , aR), but iso chaetochromin B₁ ($+142.6^\circ$, aS) showed a positive value. Furthermore, the CD spectrum of iso chaetochromin A₁ showed the first negative cotton effect at 292 nm and the second positive cotton effect at 268 nm. These spectra conformed to those of iso chaetochromin B₂. Accordingly, the respective absolute axis stereochemistry of iso chaetochromin A₁ was elucidated to be aR.

DISCUSSION

A new isomer named iso chaetochromin A₁ was isolated along with known isomers iso chaetochromins B₁ and B₂¹ from the culture broth of *Penicillium* sp. FKI-4942 as inhibitors of TG synthesis in CHO-K1 cells.² Iso chaetochromins B₁ and B₂ were reported to be produced by *Fusarium* sp.,¹ whereas all the iso chaetochromins described in this study were produced by *Penicillium* sp.²

From the structure elucidation of iso chaetochromins A₁, B₁ and B₂, these compounds have the same planar structures, although the structural difference among the isomers comes from the combination of the stereochemistry at 2/2' and 3/3' methyl residues and from the 9/9' axis (aS or aR) of the two monomers. Theoretically, there are 16 isomers for the same structure of this dimer. Among them, six isomers, chaetochromins A and B,^{3,4} ustilaginoidin D⁶ and iso chaetochromins A₁, B₁ and B₂^{1,2} (Figure 3), have been reported so far. Chaetochromin A is a symmetrical dimer having two *trans*-2,3-dimethyl groups on the 5,6,8-trihydroxy-naphtho- γ -pyrone ring, and the absolute stereochemistry was established by CD and X-ray crystallography of *p*-bromobenzoate derivative.⁵ Ustilaginoidin D is also a symmetrical dimer having two *trans*-2,3-dimethyl groups, and the 9/9' axial stereochemistry was elucidated to be aR by comparative CD

spectroscopy. However, the absolute stereochemistry was not determined.⁶ On the other hand, chaetochromin B and iso chaetochromins B₁/B₂ were unsymmetrical dimers having one *trans*-2,3-dimethyl group and one *cis*-2,3-dimethyl group, and these axial stereochemistries were elucidated to be aS, aS and aR by CD spectroscopy, respectively. However, it appeared difficult to elucidate the complete stereochemistry of these compounds. It would be done by X-ray crystallographic Analysis. In fact, the complete stereochemistry of chaetochromin B³ and iso chaetochromins B₁/B₂¹ has not been reported to date.

This series of isomers was produced by fungal genera *Chaetomium*,^{3,4,7} *Claviceps*,⁶ *Fusarium*¹ and *Penicillium*.² As they were completely different in taxonomical characteristics, these fungi share the similar biosynthetic genes to produce naphtho- γ -pyrones. Distinct isomers will be further isolated from the culture broth of these fungi.

ACKNOWLEDGEMENTS

We express our thanks to Ms N Sato for performing NMR experiments, and Dr K Nagai and Ms A Nakagawa for measuring mass spectra. This work was supported by a grant-in-aid for Scientific Research (B) 18390008 (to HT) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

- 1 Singh, S. B. *et al.* Four novel bis-(naphtho- γ -pyrones) isolated from *Fusarium* species as inhibitors of HIV-1 integrase. *Bioorg. Med. Chem. Lett.* **13**, 713–717 (2003).
- 2 Ugaki, N. *et al.* New iso chaetochromin, an inhibitor of triacylglycerol synthesis in mammalian cells, produced by *Penicillium* sp. FKI-4942: I. Taxonomy, fermentation, isolation and biological properties. *J. Antibiot.* **65**, 15–19 (2012).
- 3 Koyama, K. & Natori, S. Chaetochromins B, C and D, bis(naphtho- γ -pyrone) derivatives from *Chaetomium gracile*. *Chem. Pharm. Bull.* **35**, 578–584 (1987).
- 4 Sekita, S., Yoshihira, K. & Natori, S. Chaetochromin, a bis(naphthodihydropyran-4-one) mycotoxin from *Chaetomium thielavioideum*: application of ¹³C-¹H long-range coupling to the structure elucidation. *Chem. Pharm. Bull.* **28**, 2428–2435 (1980).
- 5 Koyama, K., Natori, S. & Iitaka, Y. Absolute configurations of chaetochromin A and related bis(naphtho- δ -pyrone) mold metabolites. *Chem. Pharm. Bull.* **35**, 4049–4055 (1987).
- 6 Koyama, K. & Natori, S. Further characterization of seven bis(naphtho- γ -pyrone) congeners of ustilaginoidins, coloring matters of *Claviceps virens* (*Ustilagoidea virens*). *Chem. Pharm. Bull.* **36**, 146–152 (1988).
- 7 Sekita, S. *et al.* Mycotoxin production by *Chaetomium* spp. and related fungi. *Can. J. Microbiol.* **27**, 766–772 (1981).