Biosynthesis of Chaetochromin A, a Bis(naphtho-γ-pyrone), in *Chaetomium* spp.

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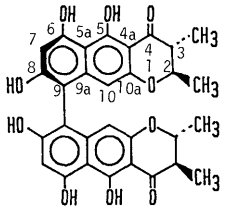
The biosynthesis of chaetochromin A, a metabolite of *Chaetomium gracile*, has been studied using [$^{13}CH_3$]methionine, sodium [1-^{13}C]acetate, sodium [1,2-$^{13}C_2$]acetate, sodium [1-^{13}C,2,2,2-$^{2}H_3$]acetate, and sodium [1-^{13}C,1,1-$^{18}O_2$]acetate as precursors. The folding pattern of the polyketide chain in chaetochromin A, biosynthesized from sodium [1,2-$^{13}C_2$]acetate as the precursor, was determined to be the same as that of rubrofusarin by carbon-13 nuclear magnetic resonance (^{13}C-NMR) analysis. By using [$^{13}CH_3$]methionine as a precursor, the source of 2-CH_3 was determined. When sodium [1-^{13}C,2,2,2-$^{2}H_3$]acetate was fed, a β-isotope-shifted peak was observed only for carbon 2. In the ^{13}C-NMR spectra of chaetochromin A and of its hexamethyl ether derived from sodium [1-^{13}C,1,1-$^{18}O_2$]acetate, isotope-shifted peaks were observed for carbons 4, 5, 6, 8 and 10a, but not for carbon 2. These results showed that oxygen 1 originated from the same unit of acetate as carbon 10a.

Keywords chaetochromin A; bis(naphtho-γ-pyrone); mycotoxin; biosynthesis; ^{13}C-NMR; 2D-INADEQUATE; isotope shift

Chaetochromin A, first isolated from *Chaetomium virescens* UDAGAWA (*C. thielavioideum* CHEN),[1] is a bis(naphtho-γ-pyrone) mycotoxin exhibiting toxicity to experimental animals[2] and antitumor activities.[3] The absolute configuration of the compound was established by X-ray analysis of the 6-*O*-*p*-bromobenzoate of the 5,5′,6′,8,8′-pentamethyl ether.[4] Three congeners from *C. gracile*[5] and seven related compounds from *Claviceps virens* (COOKE) TAKAHASHI (anamorph: *Ustilaginoidea virens*)[6,7] were isolated and their structures (including the atropisomerism of 9—9′) were established mainly by analysis of the proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) and circular dichroism (CD) spectra.[4] From the structures established, the biosynthetic origin of the dimeric naphtho-γ-pyrone mold metabolites as dimers of heptaketides is quite obvious. However, the folding pattern of the polyketide chain and the origin of the oxygen atom at the 1-position have not been determined.



chaetochromin A(**1**)

In this study, we examined these points, employing chaetochromin A produced by *C. gracile*.

In the previous papers,[1,5] some ambiguities remained in the ^{13}C-NMR assignments of chaetochromin A. ^{13}C–^{1}H correlation spectroscopy (COSY) (Fig. 1) and ^{13}C–^{1}H long range COSY (Fig. 2) of chaetochromin A were measured in deuterated chloroform ($CDCl_3$) and acetone (acetone-d_6) and the assignments shown in Table I were firmly estab-

TABLE I. ^{13}C-NMR Chemical Shifts (δ) of Chaetochromin A (**1**)

Carbon atom	Solvent	
	$CDCl_3$	Acetone-d_6
2	78.4	79.0
3	46.2	46.6
4	200.8	201.7
4a	102.0	102.3
5	164.4	165.4
5a	105.5	106.4
6	160.6	161.6
7	99.8	100.9
8	159.9	160.7
9	102.1	105.6
9a	142.0	143.3
10	99.4	100.1
10a	156.2	156.5
2-Me	19.6	19.8
3-Me	9.9	10.0

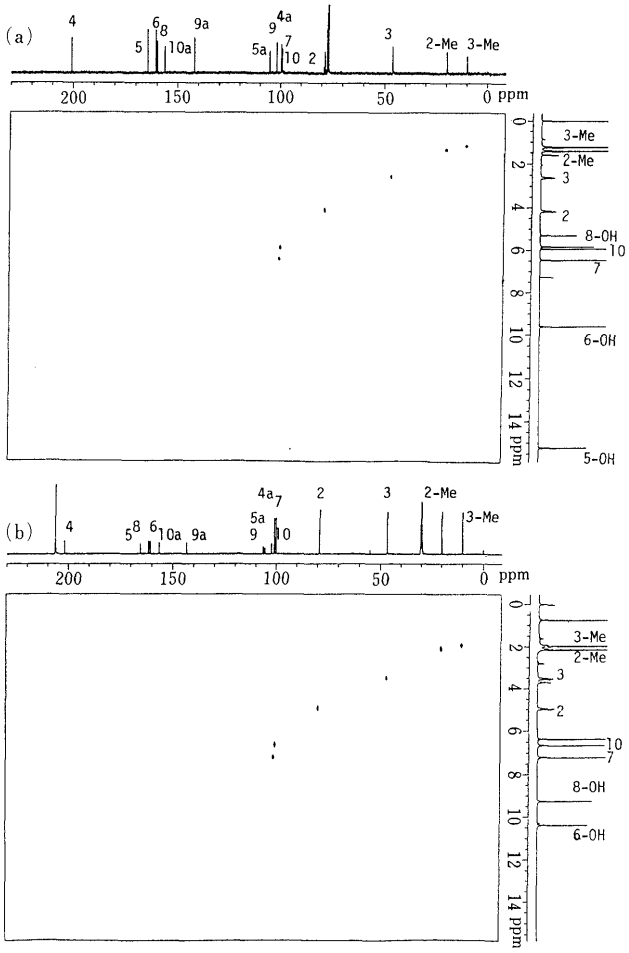


Fig. 1. ^{1}H–^{13}H Correlation Spectra of Chaetochromin A (**1**)
(a) In $CDCl_3$. (b) In acetone-d_6.

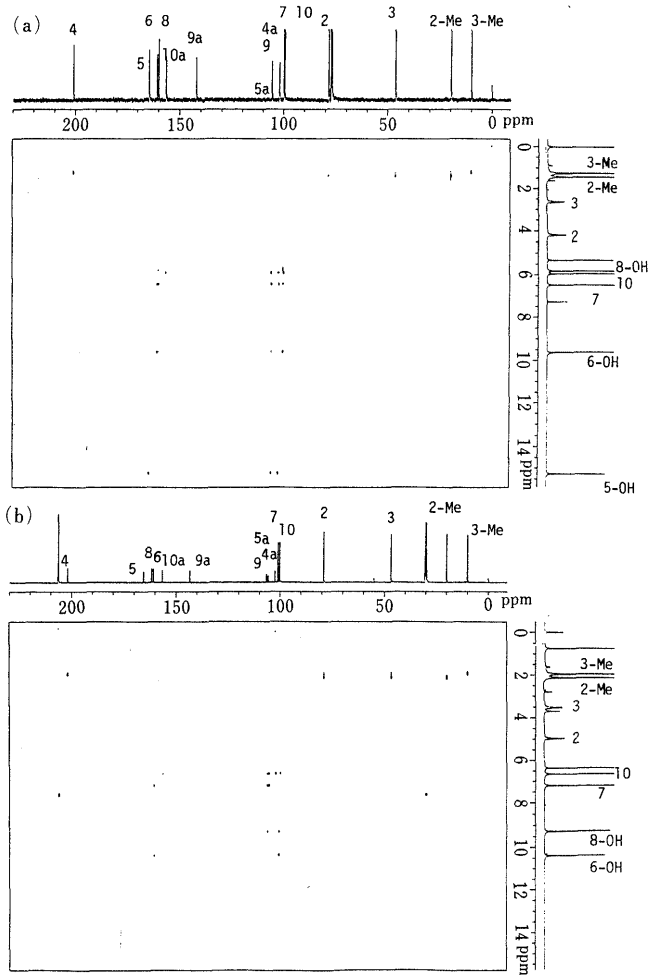


Fig. 2. ^{1}H–^{13}C Long-Range Correlation Spectra of Chaetochromin A (**1**) ($J = 10$ Hz)

(a) In $CDCl_3$. (b) In acetone-d_6.

lished before the biosynthetic studies.

To obtain labelled chaetochromin A, labelled precursors were added to a culture of *C. gracile* in the medium including potato extract, glucose, peptone, KH_2PO_4, and $MgSO_4 \cdot 7H_2O$. After incubation for 7 d, the culture was freeze-dried and extracted with ethyl acetate. Labelled chaetochromin A was isolated by silica gel chromatography and high-performance liquid chromatography (HPLC) on Nucleosil 50-5 as reported in the previous paper.[5]

By addition of sodium [1-^{13}C]acetate to growing culture of *C. gracile*, chaetochromin A enriched at carbons 2, 4, 5, 6, 8, 9a, and 10a was obtained, as deduced from the ^{13}C-NMR spectrum (Fig. 3). When [$^{13}CH_3$]methionine was added as a precursor, the intensity of the signal due to 3-CH_3 was enhanced relative to the natural abundance. Thus, the formation of a half-molecule of chaetochromin A from one C_1-unit and seven units of acetate/malonate was verified.

Two folding patterns of the heptaketide chain in naphtho-γ-pyrone derivatives have been reported (Fig. 4). The folding of fonsecin from the culture of *Aspergillus carbonarius* was shown to occur in pattern A from J(^{13}C–^{13}C) data.[8] In the case of rubrofusarin from the culture of *Fusarium culmorum*, the result shown as pattern B was obtained by the same method.[9]

In order to determine the folding pattern of seven units of

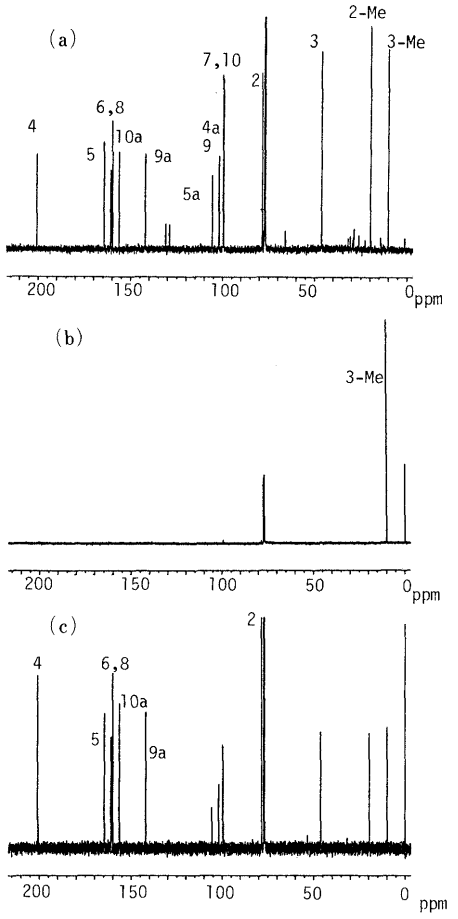
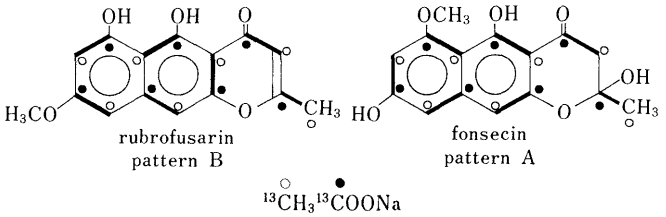
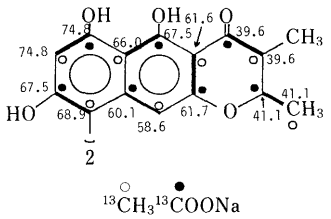


Fig. 3. ^{13}C-NMR Spectra of Chaetochromin A (**1**) (a) Natural (b) Derived from [$^{13}CH_3$]Methionine (c) Derived from Sodium [1-^{13}C]Acetate



$^{13}CH_3{}^{13}COONa$

Fig. 4. The Folding Patterns of Naphtho-γ-pyrone Derivatives



$^{13}CH_3{}^{13}COONa$

Fig. 5. ^{13}C–^{13}C Coupling Constants (Hz) of Chaetochromin A (**1**) Derived from Sodium [1,2-$^{13}C_2$]Acetate

acetate/malonate in the half-molecule of chaetochromin A, sodium [1,2-$^{13}C_2$]acetate was added as the precursor to a growing culture of *C. gracile*. The folding of labelled chaetochromin A was determined to occur in pattern B, the same as that of rubrofusarin, from J(^{13}C–^{13}C) data (Fig. 5) and the 2D-INADEQUATE spectrum (Fig. 6).

Next, to determine the origin of hydrogen and oxygen atoms of chaetochromin A, sodium [1-^{13}C,2,2,2-$^{2}H_3$]-acetate and sodium [1-^{13}C,1,1-$^{18}O_2$]acetate were used as precursors respectively. In chaetochromin A derived from

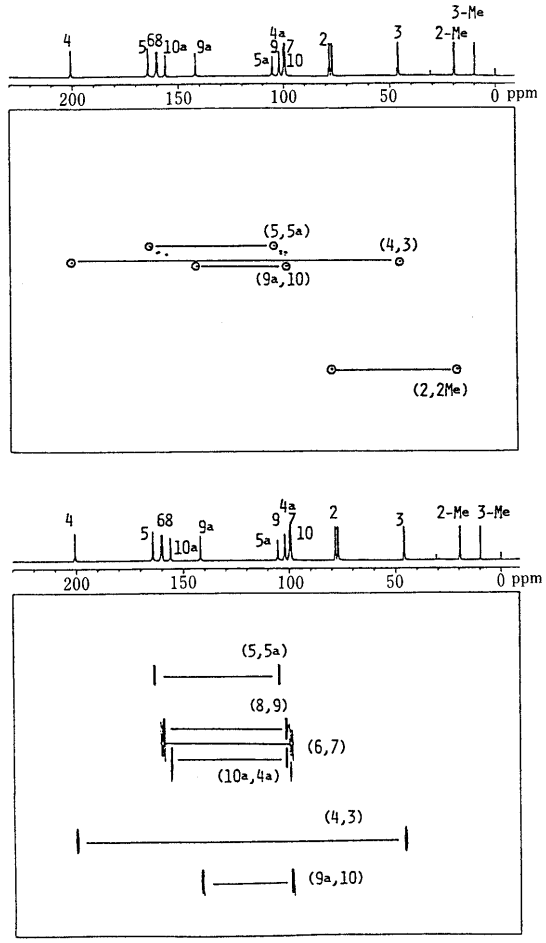


Fig. 6. 2-D INADEQUATE Spectrum of Chaetochromin A (**1**) Derived from Sodium [1,2-$^{13}C_2$]Acetate

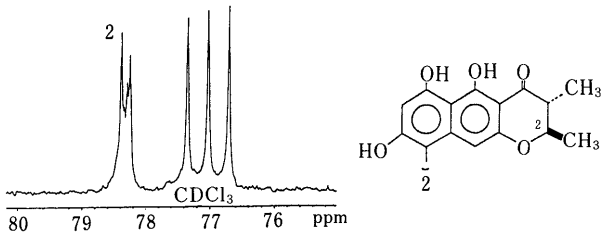


Fig. 7. ^{13}C-NMR Spectrum of Chaetochromin A (**1**) Derived from Sodium [1-^{13}C,2,2,2-$^{2}H_3$]Acetate

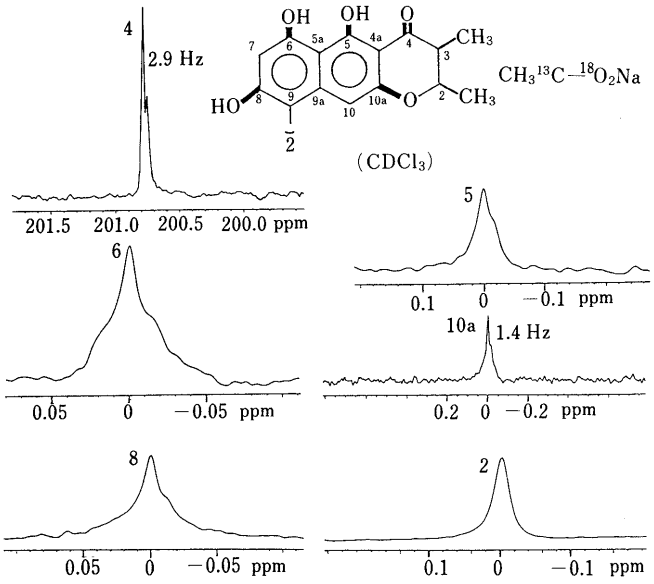


Fig. 8. ^{13}C-NMR Spectrum and ^{13}C–^{18}O Coupling Constants of Chaetochromin A (**1**) Derived from Sodium [1-^{13}C,1,1-$^{18}O_2$]Acetate

The unlabeled carbons at 2,5,6,8 and 10a are arbitarily assigned the value 0.0 ppm. Spectral measurement: 50—100 scans, 160-Hz sweep width, and 32 K data points were used to record the spectra at 2-C, 5-C, 6-C, 8-C and 10a-C.

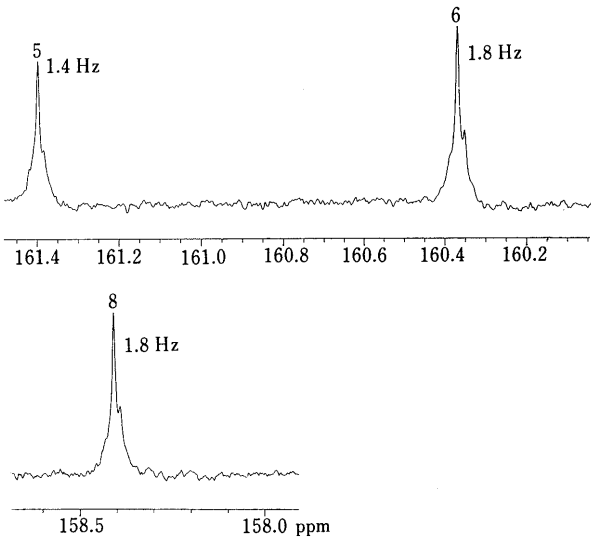


Fig. 9. ^{13}C-NMR Spectrum and ^{13}C–^{18}O Coupling Constants of Chaetochromin A Hexamethyl Ether

Spectral measurement: 2780 scans, 1000-Hz sweep width, and 32 K data points were used to record the spectrum.

sodium [1-^{13}C,2,2,2-$^{2}H_3$]acetate, β-isotope shift[10] was induced only at C-2 in the ^{13}C-NMR spectrum. The C-2-methyl unit was considered as a starter unit of the polyketide chain (Fig. 7). In the case of sodium [1-^{13}C,1,1-$^{18}O_2$]acetate as the precursor, α-isotope shifts were observed at C-4, C-5, C-6, C-8 and C-10a (Fig. 7). Since no isotope shift was observed at C-2, the incorporation pattern of precursors was concluded to be as shown in Fig. 8. The isotope shift values were measured for C-4 and C-10a but not for C-5, C-6 and C-8, as shown in Fig. 8. In order to clarify the values of isotope shifts, the hexamethyl ether[5] of chaetochromin A derived from sodium [1-^{13}C,1,1-$^{18}O_2$]acetate was prepared. As shown in Fig. 9, the isotope shift values became obvious.

From these results, the formation of the γ-pyrone moiety was considered to occur as shown in Chart 1. Since a

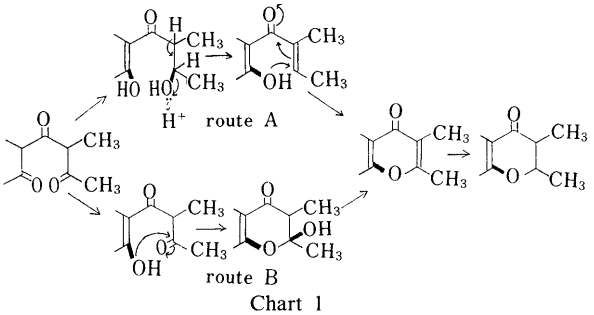


Chart 1

compound such as fonsecin, bearing a hydroxy group at the 2-position, has not been isolated from *Chaetomium gracile*, there is no evidence regarding the biosynthetic route A or B

(Chart 1) of the naphtho-γ-pyrone moiety of chaetochromin A.

Experimental

The ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GSX-400 (^{1}H 400 MHz and ^{13}C 100 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are recorded in ppm (δ). Mass spectra (MS) were taken on a JEOL JMS-D300.

Kieselgel 60 F_{254} (Merck) precoated plates were used for thin-layer chromatography (TLC) and the spots were detected by UV illumination. Column chromatography was carried out on 70—230 mesh silica gel (Merck). HPLC were carried out by using a Waters M45J pump with an Oyo-Bunko Uvilog 5IIIA UV detector.

[$^{13}CH_3$]methionine (97%, ^{13}C), sodium [1-^{13}C]acetate (99%, ^{13}C), sodium [1,2-$^{13}C_2$]acetate (99%, ^{13}C), sodium [1-^{13}C,2,2,2-$^{2}H_3$]acetate (99%, ^{13}C; 98%, D_3), and sodium [1-^{13}C,1,1-$^{18}O_2$]acetate (99%, ^{13}C; 96%, $^{18}O_2$) were purchased from Cambridge Isotope Laboratories.

Medium Potato (100 g) was extracted with distilled water (1000 ml). After filtration, the extract was supplemented with glucose (20 g), peptone (5 g), KH_2PO_4 (5 g), $MgSO_4 \cdot 7H_2O$ (2.5 g), and distilled water to 1000 ml.

Culture Conditions Medium (300 ml) was placed in a Sakaguchi flask, autoclaved, and inoculated with a small amount of spore suspension of the strain (73-S-T-Y-3). The flask was incubated for 7 d at 26 °C in the dark on a reciprocatory shaker (90 rev./min). For precursor feedings, the labelled acetate was added (100 mg each on days 0, 1, 2 and 3) in a sterile manner through a Millipore filter. In the case of labelled methionine 50 mg was added on each day.

Isolation The cultures were freeze-dried and extracted 3 times with EtOAc for 24 h at room temperature. The details of the isolation of chaetochromin A were given in the preceding paper.[5] Chaetochromin A was obtained in yields of 3 mg for [$^{13}CH_3$]methionine incorporation and 46 mg for sodium [1-^{13}C]acetate incorporation.

Incorporation Rate The incorporation rates were determined by comparison of the MS spectra of labelled and unlabelled chaetochromin A according to the method of Nakanishi *et al.*[11] The incorporation rates were determined to be 0.58% for [$^{13}CH_3$]methionine and 0.61% for sodium [1-^{13}C]acetate.

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