Four New Azaphilones from *Chaetomium globosum* var. *flavo-viridae*

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Four new azaphilones of angular type, named chaetoviridins A, B, C and D, were isolated from the culture of *Chaetomium globosum* var. *flavo-viridae*. The absolute configuration of chaetoviridin A was established on the basis of the spectral data and chemical reactions. The structures of chaetoviridins B, C and D were suggested by the spectral data. The result of a biosynthetic study of chaetoviridin B from sodium [1,2-$^{13}C_2$]acetate gave support to the angular structure.

Keywords *Chaetomium globosum* var. *flavo-viridae*; chaetoviridin A; chaetoviridin B; chaetoviridin C; chaetoviridin D; azaphilone; biosynthesis; ^{13}C-NMR

Fungal secondary metabolites having a pyrano-quinone structure are called azaphilones because of the affinity of these compounds for ammonia, yielding vinylogous γ-pyridones. More than twenty compounds such as sclerotiorin, monascorubrin and monascoflavin have so far been characterized from a variety of fungal sources. Some of these fungi have been used as coloring matters for foodstuffs and alcoholic beverages in certain Asian countries and some extractives are now used as food additives.

In the course of our studies on mycotoxin production by the fungi of *Chaetomium* genus,[1] one of the strains, *Chaetomium globosum* var. *flavo-viridae* (TRTC 66.631a) was found to produce red pigments assumed to be azaphilones. This paper deals with the isolation and structure elucidation of four new compounds from the fungus.

The mold was cultured on wheat and the dichloromethane extract was subjected to silica gel chromatography to obtain a mixture of red pigments. High performance liquid chromatography (HPLC) of the mixture afforded four red pigments named chaetoviridins; chaetoviridin A (**1**) as the major metabolite and B (**2**), C (**3**) and D (**4**) as the minor congeners. Chaetoviridin A (**1**) showed deep red coloration with ammonia characteristic to azaphilones and, from the ultraviolet (UV) spectra and the positive Beilstein reactions, all these pigments were suspected to be chlorine-containing azaphilones resembling sclerotiorin (**5**).[2]

Chaetoviridin A (**1**) has the molecular formula, $C_{23}H_{25}ClO_6$ (by mass spectrometry (MS)). The infrared (IR) absorptions (3450, 1780, 1620 cm^{-1}) indicated the presence of hydroxyl, γ-lactone and conjugated carbonyl groups. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** (Table I) indicated the presence of five aliphatic methyl groups (one triplet, three doublets and one singlet), one methylene and three methine protons, one *trans*-olefinic group (J=15.7 Hz), and two uncoupled olefinic protons. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table II) showed the presence of three carbonyl carbons, ten olefinic carbons and ten sp^3 carbons. These spectral data were consistent with a chlorine-containing azaphilone having a conjugated γ-lactone. To confirm these assignments and to identify the structures of the substituents on the *O*-heterocyclic rings, ^{1}H–^{1}H correlation spectroscopy (H–H COSY) and ^{13}C–^{1}H COSY (C–H COSY) were performed and the presence of 3-methyl-1-pentenyl and 2-butanol-3-yl groups on the ring was established. The C–

TABLE I. ^{1}H-NMR Data[a] for Chaetoviridins (400 MHz in $CDCl_3$)

Proton	Chaetoviridins			
	A (**1**)	B (**2**)	C (**3**)	D (**4**)
1	8.80 (s)	7.30 (s)	7.40 (s)	7.30 (s)
4	6.56 (s)	6.55 (s)	6.48 (s)	6.61 (s)
8	—	3.01 (d, J=10.1)	3.91 (d, J=12.1)	2.98 (d, J=10.1)
9	6.10 (d, J=15.7)	6.06 (d, J=15.7)	6.03 (d, J=15.7)	6.15 (d, J=15.7)
10	6.62 (dd, J=15.7, 8.3)	6.53 (dd, J=15.7, 8.0)	6.51 (dd, J=15.7, 8.0)	6.61 (dd, J=15.7, 8.0)
11	2.30 (m)	2.26 (m)	2.27 (m)	2.45 (m)
12	1.45 (m)	1.43 (m)	1.43 (m)	3.81 (m)
13	0.92 (t, J=7.4)	0.91 (t, J=7.4)	0.90 (t, J=7.4)	1.20 (d, J=6.6)
7-CH_3	1.70 (s)	1.40 (s)	1.60 (s)	1.40 (s)
11-CH_3	1.10 (d, J=6.6)	1.08 (d, J=6.6)	1.25 (d, J=6.3)	1.13 (d, J=6.9)
12-OH	—	—	—	1.52 (br)[b]
2′	—	3.07 (d, J=10.1)	4.22 (d, J=12.1)	3.06 (d, J=10.1)
4′	3.64 (m)	1.91 (m)	3.21 (m)	1.90 (m)
5′	3.86 (m)	4.32 (m)	3.81 (m)	4.30 (m)
6′	1.17 (d, J=6.6)	1.41 (d, J=5.2)	1.06 (J=6.1)	1.41 (d, J=5.5)
3′-OH	—	3.19 (br)	—	3.21 (br)
4′-CH_3	1.17 (d, J=6.6)	1.14 (d, J=7.2)	1.08 (d, J=7.0)	1.13 (d, J=6.9)
5′-OH	2.28 (br)	1.86 (br)	1.31 (br)	1.66 (br)[b]

a) Chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard and coupling constants are given in Hz (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). *b*) Assignments may be interchanged.

TABLE II. ^{13}C-NMR Data for Chaetoviridins (100 MHz in $CDCl_3$)

Carbon atom	Chaetoviridins			
	A (1)	B (2)	C (3)	D (4)
1	151.5	145.7	147.2	146.9
3	157.1	157.8	157.8	158.6
4	105.3	105.0	105.9	106.9
4a	139.7	140.6	140.9	141.6
5	108.9	110.1	110.3	111.8
6	183.4	189.3	184.4	190.7
7	87.5	83.9	84.5	85.4
8	162.6	50.5	53.8	52.0
8a	110.4	114.4	114.1	115.8
9	119.7	120.2	120.9	123.5
10	148.0	146.9	146.4	144.0
11	38.9	38.9	39.8	45.7
12	30.1	29.2	30.2	72.3
13	11.6	11.7	12.8	20.5
7-CH_3	26.2	23.4	24.6	23.3
11-CH_3	19.2	19.4	20.3	14.8
1′	167.9	170.8	168.9	172.0
2′	125.1	58.3	59.8	59.7
3′	201.1	104.1	207.1	105.6
4′	51.0	45.0	44.1	46.3
5′	70.8	77.3	74.3	78.6
6′	13.4	18.7	14.1	18.7
4′-CH_3	21.4	8.8	23.4	8.8

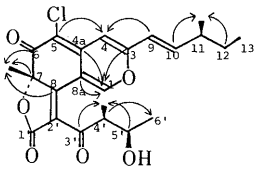

Fig. 1. ^1H–^{13}C Long-Range Correlation Spectrum of Chaetoviridin A (1) (J=10 Hz) (C→H)

H long-range COSY indicated the connections shown in Fig. 1 in the molecule and the attachment of the 3-methyl-1-pentenyl group at the 3-position of the ring and of the 2-butanol-3-yl group at the conjugated carbonyl group.

Among azaphilones bearing a five-membered lactone, two types of junction are known: the linear type as in rotiorin[3,4] (**6**), monascorubrin[5] (**7**), and monascoflavin[6] (**8**) and the angular type as in rubrorotiorin[7] (**9**) and ocrephilone[8] (**10**). The UV spectrum of A (**1**) (λ_{max} 305, 365, 450 nm) favored the latter structure (this was confirmed by a biosynthetic experiment (*vide infra*)). Thus the structure **1** (without the stereochemistry), an analog of rubrorotiorin (**9**), was suggested.

The stereochemistry of the C_7-position of azaphilones so far known has firmly been established by optical rotations, circular dichroism (CD) and X-ray analysis.[9,10] The CD of chaetoviridin A (**1**) ($\Delta\varepsilon_{367}$ −1.1) (Fig. 2) clearly showed (*S*)-configuration of C_7. By the application of Horeau's method, the secondary alcohol group at $C_{5'}$ was shown to be (*R*). The stereochemistry of $C_{4'}$ was established as (*S*) by the following facts: i) The presence of a hydrogen bond between the $C_{5'}$-hydroxyl group and $C_{3'}$-carbonyl group was shown by the IR spectra in carbon tetrachloride solutions. ii) The coupling constant (8.0 Hz) between the $C_{5'}$ and $C_{4'}$ protons suggested the eclipsed conformation. iii) Nuclear Overhauser effect (NOE) between the two

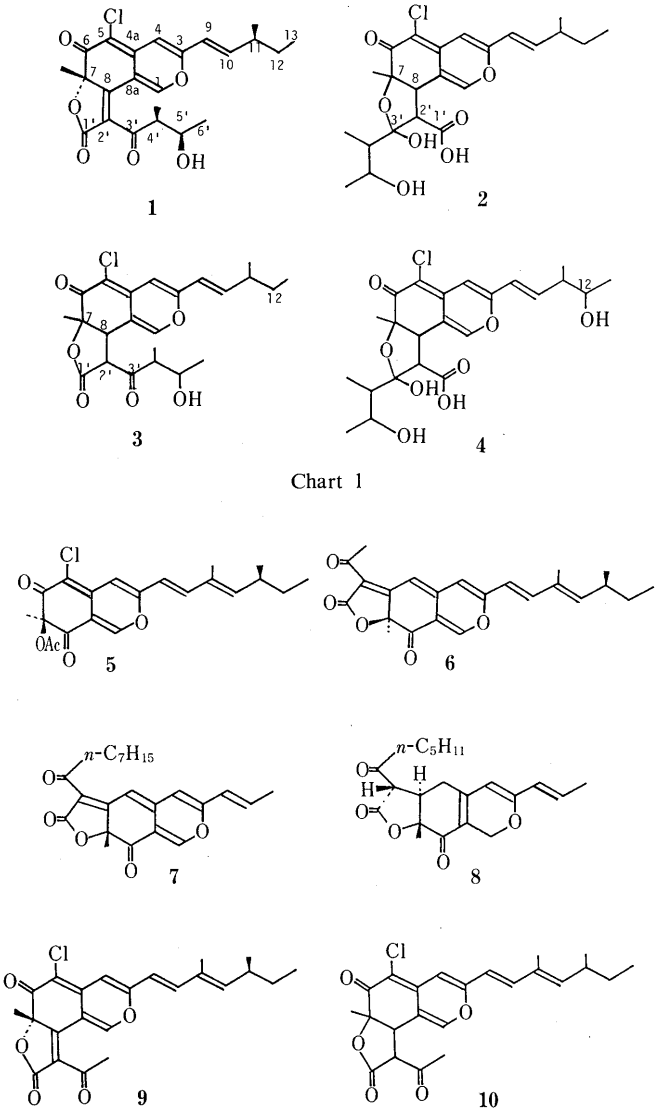

Chart 1

Chart 2

protons was observed in an NOE difference spectrum. Since $C_{5'}$ was (*R*), $C_{4'}$ was established as (*S*). Chromium trioxide oxidation[11,12] of A (**1**) gave 2-methylbutanoic acid showing (+)-rotation.[13] Thus the stereochemistry of C_{11} in the side chain at C_3 was established as (*S*).

From these results, the absolute configuration of chaetoviridin A (**1**) was established as 5-chloro-9-[(2*S*, 3*R*)-3-hydroxy-3-methyl-1-oxobutyl]-6a-(*S*)-methyl-3-[(*S*)-3-methyl-1-pentenyl)]-6*H*-furo[2,3-*h*]-2-benzopyran-6,8-(6a*H*)-dione.

Chaetoviridin C (**3**) has the molecular formula, $C_{23}H_{27}ClO_6$, corresponding to a dihydro derivative of A (**1**). The ^1H- and ^{13}C-NMR (Tables I and II) of C (**3**) showed nearly the same signals except for those corresponding to $C_{2'}$ and C_8. The UV absorption (λ_{max} 386, 406, 430) showed a hypsochromic shift as compared with A (**1**). In the ^{13}C-NMR of C (**3**), two sp^2 carbon signals at $C_{2'}$ and C_8 in A (**1**) disappeared and, instead, two sp^3 signals (59.8, 50.5 ppm) were observed. In ^1H-NMR, new proton signals (3.91, 4.22 ppm), showing a vicinal coupling (J= 12.1 Hz), appeared. Monascorubrin[5] (**7**) and rubrorotiorin[7] (**9**) have an α,β-unsaturated γ-lactone ring, while monascoflavin[6] (**8**) and ochrephilone[8] (**10**) have a satu-

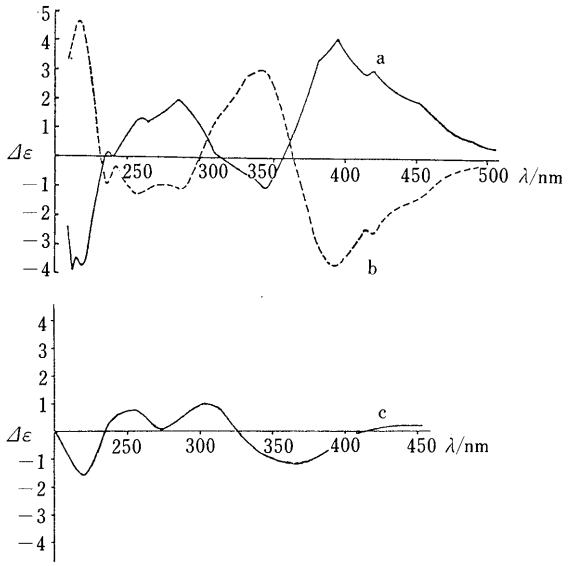

Fig. 2. CD Spectra of (a) (+)-Sclerotiorin[9] (**5**), (b) (−)-7-Episclerotiorin[9] and (c) Chaetoviridin A (**1**)

rated γ-lactone ring. Comparison of the physical properties suggested that chaetoviridin C (**3**) is the *cis*-8,2′-dihydro derivative of chaetoviridin A (**1**).

Chaetoviridin B (**2**) showed higher polarity than A (**1**) and has the molecular formula, $C_{23}H_{29}ClO_7$ (by chemical ionization (CI) MS), corresponding to a hydrate of chaetoviridin C (**3**). The ^{1}H- and ^{13}C-NMR (Tables I and II) and also H–H and C–H COSY data clearly showed the presence of the same chlorine-containing furobenzopyran ring, the 3-methyl-1-pentenyl group at the 3-position and the 2-butanol-3-yl group as a side chain as in chaetoviridin C (**3**). The UV absorptions (λ_{max} 385, 405, 430 nm) were nearly superimposable on those of C (**3**). The angular type structure as in the case of chaetoviridin A (**1**) and C (**3**) was supported by the observed NOE between the C_8–C_1 protons. However, the IR absorptions corresponding to the γ-lactone carbonyls observed in A (**1**) and C (**3**) were replaced by a carboxyl carbonyl absorption (1720 cm^{-1}). In the ^{13}C-NMR of chaetoviridin B (**2**), the carbonyl carbon signals corresponding to those of $C_{3'}$ in A (**1**) and C (**3**) were replaced by an oxygen-bearing quaternary sp^3 carbon at 104.1 ppm. These observations, along with the molecular formula, are accommodated by the structure (**2**), in which the lactone ring connecting C_7–$C_{1'}$ in chaetoviridin C (**3**) has been cleaved and recyclized between C_7 and the $C_{3'}$ carbonyl in a hemiketal form. The relative stereochemistry of C_8–$C_{2'}$ was suggested to be *cis* as in C (**3**) by the coupling constant (J = 10.1 Hz).

The molecular formula of chaetoviridin D (**4**), $C_{23}H_{29}ClO_8$, has one more oxygen atom than chaetoviridin B (**2**). All the spectral data of D (**4**) such as UV, IR and ^{1}H- and ^{13}C-NMR (Tables I and II), indicated a close similarity of the structure to that of chaetoviridin B (**2**). The only structural difference between these compounds existed in the C_{11}–C_{13} region of the side chain, as was suggested by the chemical shifts and the coupling patterns in the NMR. Decoupling experiments revealed the presence of a secondary hydroxyl group at the 4-position in the 3-methyl-1-pentenyl group (C_{12} in the formula **4**). Thus the structure of chaetoviridin D (**4**) was shown to correspond to 12-hydroxychaetoviridin B. The relative stereochemistry of the C_8—$C_{2'}$ positions was shown to be *cis* by the coupling constant (J = 10.1 Hz).

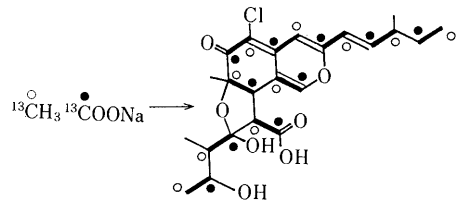

Fig. 3. Biosynthesis of Chaetoviridin B (**2**)

Due to the small amounts of the samples, the absolute configurations of chaetoviridins B (**2**), C (**3**) and D (**4**) have not been studied, but the negative optical rotations of the three compounds, opposite to chaetoviridin A (**1**), may suggest (*R*) configurations at C_7 of these compounds.[9,10]

Biosynthetic studies on sclerotiorin[14] (**5**), monascorubrin[15] (**7**), monascoflavin[15] (**8**) and ochrephilone[8] (**10**) clarified the polyketide origins of azaphilones, which are composed of a main chain starting from the side chain at C_3 and ending at C_1 with a subsidiary chain attached at the C_7-hydroxyl. As in the case of ochrephilone[8] (**10**), the labelling pattern from [1,2-$^{13}C_2$]acetate would be useful for the distinction between the linear type compounds (**6**—**8**) and angular type compounds (**9**, **10**): in the former, the C_5 atom bearing chlorine in the case of chaetoviridins (**1**—**4**) and the sp^3 or sp^2 carbon at C_6 are derived from the same acetate unit, while, in the latter, the C_5 atom and the carbonyl carbon atom at C_6 are derived from the same acetate unit.

In the shaking culture for the incorporation study of *C. flavo-vitidae*, the main metabolite was not chaetoviridin A (**1**) but B (**2**). The ^{13}C-NMR of chaetoviridin B (**2**) obtained by the feeding of [1,2-$^{13}C_2$]acetate showed the satellite doublet signals due to ^{13}C–^{13}C couplings at all carbon signals except those for the C_7-, C_{11}- and $C_{4'}$-methyl carbons originated from C_1 units. The ^{13}C–^{13}C coupling constants (see Experimental) indicated a labelling pattern originating from two polyketide chains as shown in Fig. 3; the observed coupling of C_5 with the carbonyl carbon and not with sp^3 carbon gave decisive evidence for the angular structure of chaetoviridin B (**2**).

Little is known about the biological activities of azaphilones. Quite recently inhibition of monoamine oxidase,[16] induction of chlamydospore-like cells in *Cochliobolus lunatus*,[17] and inhibition of growth and induction of curling of *Pyricuralia oryzae*[18] by some azaphilones have been reported. Chaetoviridin A (**1**) showed a weak inhibitory activity on monoamine oxidase (IC_{50} 1.2 × 10^{-2} g/ml), an induction of chlamydospore-like cells (40—50% at 100 μg/disc), and an inhibition of growth of *P. oryzae* (2.5 μg/ml).[19]

Experimental

All melting points were determined on a Yanagimoto MP micromelting point apparatus and uncorrected. 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 in $CDCl_3$ with tetramethylsilane as an internal standard. Chemical shifts are recorded in ppm (δ). MS were taken on JEOL JMS-D300 and Hitachi M-80B spectrometers. UV and IR spectra were measured with a Shimadzu UV-240 spectrophotometer and a JASCO A-102 infrared spectrophotometer. The $[\alpha]_D$ values were measured with a JASCO

DIP-140 digital polarimeter. CD spectra were recorded on a JASCO J-20 spectropolarimeter.

Kieselgel $60F_{254}$ (Merck) precoated plates were used for thin-layer chromatography (TLC). 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-7 UV detector.

Isolation of Chaetoviridins A—D (1—4) from *Chaetomium globosum* var. *flavo-viridae* The strain (TRTC 66.631a) was grown in stationary culture on sterilized wheat (150 g × 40) at 26 °C for 10 d. The moldy wheat was extracted three times with CH_2Cl_2 (250 ml × 40) for 24 h at room temperature. The extract was concentrated to give the residue (48 g), which was chromatographed over silica gel using a gradient mixture of hexane–EtOAc to give three colored bands. The bands were eluted with hexane–EtOAc (2 : 1), (1 : 1) and (1 : 5), respectively, collected and subjected to HPLC on a Develosil 50-5 column (for bands 2 and 3, a column pretreated with oxalic acid solution) employing hexane–EtOAc (2 : 1), (3 : 2) and (1 : 3), respectively, as the developers to give chaetoviridin A (**1**) (1.1 g) from band 1, B (**2**) (31 mg) and C (**3**) (5.2 mg) from band 2, and D (**4**) (2.1 mg) from band 3.

Chaetoviridin A (1) Red needles (MeOH), mp 121—124 °C, $[\alpha]_D^{30}$ +98° (c = 0.05, $CHCl_3$). CD (MeOH) $\Delta\varepsilon$ (nm): 0 (200, −1.6 (220), 0 (234), +0.8 (253), +0.1 (272), +1.0 (303), 0 (326), −1.1 (365), 0 (412). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 305 (4.42), 365 (4.27), 450sh (3.67). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2950, 1780 (γ-lactone), 1680 (C=O), 1620 (C=O). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1780, 1680, 1620. MS m/z: 432.1214 (M^+, Calcd for $C_{23}H_{25}{}^{35}ClO_6$: 432.1341), 434. ^{1}H-NMR (Table I); ^{13}C-NMR (Table II).

Chaetoviridin B (2) Yellow powder ($CHCl_3$), mp 111—113 °C, $[\alpha]_D^{30}$ −104° (c = 0.13, $CHCl_3$). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 290 (4.14), 385 (4.43), 405 (4.38), 430sh (4.05). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2950, 1720 (C=O), 1620 (C=O), 1560 (C=C), 1520 (C=C). CI-MS (isobutane) m/z: 452.1684 (M^+, Calcd for $C_{23}H_{29}{}^{35}ClO_7$: 452.1604). ^{1}H-NMR (Table I); ^{13}C-NMR (Table II).

Chaetoviridin C (3) Yellow powder ($CHCl_3$), mp 117—120 °C, $[\alpha]_D^{30}$ −138° (c = 0.03, $CHCl_3$). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 294 (3.88), 386 (4.15), 406 (4.10), 430sh (3.74). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2950, 1780 (γ-lactone), 1600 (C=O). CI-MS (isobutane) m/z: 434.1458 (M^+, Calcd for $C_{23}H_{27}{}^{35}ClO_6$: 434.1498). ^{1}H-NMR (Table I); ^{13}C-NMR (Table II).

Chaetoviridin D (4) Yellow powder ($CHCl_3$), mp 113—115 °C, $[\alpha]_D^{24}$ −91° (c = 0.06, $CHCl_3$). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 290 (3.95), 395 (4.21), 410 (4.14), 430sh (3.79). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2950, 1720 (C=O), 1620 (C=O). CI-MS (isobutane) m/z: 467.1400 ($M^+ - 1$, Calcd for $C_{23}H_{28}{}^{35}ClO_8$: 467.1475). ^{1}H-NMR (Table I); ^{13}C-NMR (Table II).

Reaction of (+)-2-Phenylbutanoic Anhydride to Chaetoviridin A (1) Chaetoviridin A (**1**) (218 mg) and (±)-2-phenylbutanoic anhydride (467 mg) in pyridine (4 ml) were kept at room temperature for 14 h under stirring. H_2O (0.5 ml) was added to the reaction mixture, then benzene was added after 30 min, and the aqueous layer was neutralized with 0.1 N NaOH. The benzene layer was evaporated and the residue was subjected to HPLC on a Develosil 50-5 column using hexane–EtOAc (11 : 1) as the developer to give two peaks of diastereomeric esters, showing m/z: 578.2058 and 578.2026, respectively, by MS (M^+, Calcd for $C_{33}H_{35}{}^{35}ClO_7$, 578.2073). The aqueous layer was washed with $CHCl_3$ and, after acidification, extracted with benzene. 2-Phenylbutanoic acid, obtained after evaporation (yield, 61%), showed $[\alpha]_D^{25}$ +5.3° (c = 0.61, benzene) and optical yield, 6.1% ee.

Chromium Trioxide Oxidation of Chaetoviridin A (1) Chaetoviridin A (**1**) (320 mg) was oxidized with a reagent prepared from CrO_3 (0.6 g), water (0.5 ml), H_2SO_4 (0.5 ml) and HOAc (18 ml) for 20 h at room temperature under stirring. The reaction mixture was extracted with EtOAc and the extract was washed with water and evaporated. The residue was applied to HPLC on a column of Develosil 60-5 treated with oxalic acid using hexane–acetone (2 : 1) as the developer. Each fraction was checked by TLC for an acidic substance (with bromocresol green as the reagent) and an acidic substance was obtained in a yield of 26% as a colorless oil, $[\alpha]_D^{24}$ +12.7° (c = 0.20, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.95 (3H, t, J = 7.42, $-CH_2-\underline{CH}_3$), 1.18 (3H, d, J = 7.14, $-CH-CH_3$), 1.51, 1.70 (each 1H m, $-C\underline{H}_2-$), 2.41 (1H, m, HOOC-C$\underline{H}$<), 11.44 (1H, br, $\underline{H}$OOC–). The acid was identical with a commercial sample of (*S*)-2-methylbutanoic acid (Aldrich Chem. Co.), $[\alpha]_D^{20}$ +19°.

Biosynthesis of Chaetoviridin B (2) *Chaetomium globosum* var. *flavo-viridae* (TRTC 66.631a) was cultured in a Sakaguchi flask containing 400 ml of fluid prepared from boiled wheat (10 g) at 26 °C for 14 d on a rotary shaker. Sodium [1-^{13}C]acetate (99% ^{13}C) or sodium [1,2-$^{13}C_2$]acetate (99% ^{13}C) (Cambridge Isotope Laboratories) (400 mg) was dissolved in water (8 ml) and divided into four portions. One portion was given to the culture each day on days 3—6. The whole culture was lyophilized and extracted with CH_2Cl_2 three times at room temperature. The extract was purified by the same procedure as in the case of isolation of chaetoviridins A—D (**1**—**4**) described above. The yield of chaetoviridin B (**2**) was 2.7 mg and the incorporation ratio[20] in the case of sodium [1-^{13}C]acetate was 0.034%. ^{13}C–^{13}C coupling constants (Hz) observed in chaetoviridin B (**2**) from [1,2-$^{13}C_2$]acetate were as follows: $J_{1'-2'}$ = 55.0, $J_{3'-4'}$ = 44.4, J_{4-4a} = 57.2, J_{5-6} = 61.6, J_{7-8} = 33.8, J_{8a-1} = 77.8, J_{3-9} = 66.8, J_{10-11} = 41.1, J_{12-13} = 35.2.

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