Epigenetic stimulation of polyketide production in *Chaetomium cancroideum* by an NAD^+-dependent HDAC inhibitor

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Exposure of the fungus *Chaetomium cancroideum* to an NAD$^+$-dependent HDAC inhibitor, nicotinamide, enhanced the productions of aromatic and branched aliphatic polyketides, which allowed us to isolate new secondary metabolites, chaetophenol G and cancrolides A and B. Their structures were determined using spectroscopic analyses, and their absolute configuration was elucidated with electronic circular dichroism (ECD), vibrational circular dichroism (VCD), and chemical transformations. Biosynthesis of the branched aliphatic polyketide skeletons in cancrolides A and B was evidenced by conducting a feeding experiment using compounds labeled with ^{13}C stable isotope.

Introduction

Fungi produce a wide range of secondary metabolites and are thus attractive sources for novel drug leads. Recent analyses of fungal genome have shed light on the exsistence of a vast number of unidentified secondary metabolites, revealing the potential of fungi for the production of numerous novel compounds.[1,2] However, these cryptic metabolites are not readily accessible because the genes responsible for their production are generally silenced under normal culture conditions.[3] Recently, the chemical epigenetic method using histone deacetylase (HDAC) and DNA methyl transferase inhibitors has been recognized as a promising approach to obtaining compounds encoded by silent biosynthetic genes.[4-12] For example, we obtained various novel compounds by activating the production of secondary mtetabolites in several fungal species with nicotinamide, an NAD$^+$-dependent HDAC inhibitor.[13,14] In our present research, we found that the addition of nicotinamide to the medium in which the fungus *Chaetomium cancroideum* was cultured significantly enhanced its polyketide production. This enabled us to isolate an aromatic polyketide, chaetophenol G (**1**), and two branched aliphatic polyketides, cancrolides A (**2**) and B (**3**) together with known chaetophenols B (**4**) and C (**5**) (Fig. 1). In this paper, we discuss the isolation and structure elucidation of new compounds (**1**–**3**) and propose a plausible pathway that accounts for their biosynthetic relationship (Scheme 1).

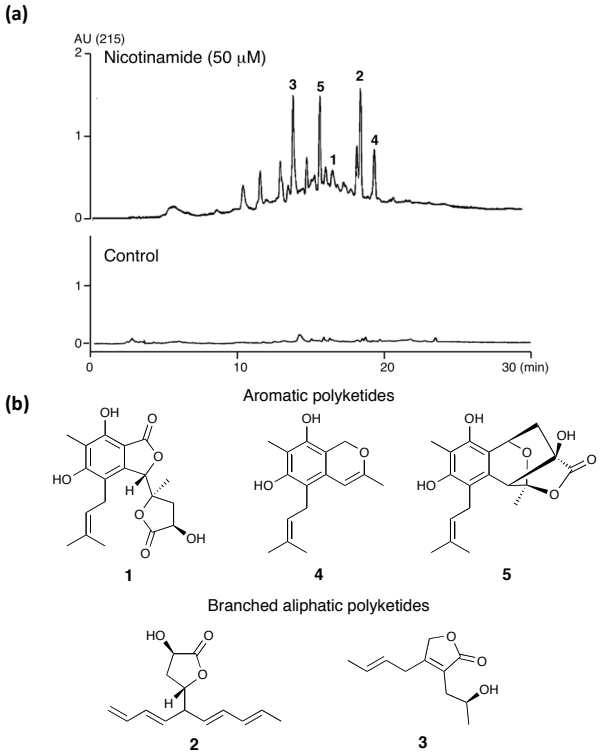

Fig. 1 (a) HPLC profiles of the EtOAc extracts of *C. cancroideum* cultivated in the presence of nicotinamide 50 μM (upper) and without the addition of nicotinamide (bottom), as detected by UV absorption at 215 nm. (b) Structures of 1–5.

Results and Discussion

C. cancroideum was cultivated in potato dextrose broth (PDB) medium containing 50 μM nicotinamide under shaking at 25 °C for 14 d. The culture medium (9.0 L) was extracted with ethyl acetate, and the extract (1.9 g) was separated by column chromatographies on Sephadex LH-20 and silica gel to afford three new polyketides,

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†Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra of compounds **1**–**3**. See DOI: 10.1039/x0xx00000x

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chaetophenol G (**1**, 1.8 mg), cancrolides A (**2**, 20.4 mg) and B (**3**, 33.7 mg) as well as chaetophenols B (**4**, 26.2 mg) and C (**5**, 21.1 mg).

The HREIMS spectrum for **1** showed a molecular ion peak at *m/z* 362.1347 $[M]^+$, which corresponds to the molecular formula $C_{19}H_{22}O_7$, indicating nine degrees of unsaturation. Its ^{1}H NMR spectrum showed distinct signals for a 3,3-dimethylallyl moiety attached to an aromatic ring [δ_H 5.21 (m, H-13), 3.49 (m, H_2-12), 1.86 (brs, H_3-16), 1.81 (brs, H_3-15)] (Table 1). The HMBC correlations of H_2-12/C-4, C-5, C-6, H_3-11/C-6, C-7, C-8, H-3/C-4, C-5, C-9 implied a fully substituted benzene ring with the dimethylallyl group at C-5, two phenols at C-6 and C-8, and a methyl group at C-7 (Fig. 2). In addition, the H-3/C-10 HMBC correlation demonstrated C-3–C-10 connectivity through an ester bond in a phthalide skeleton (Fig. 2). The long-range C-H correlations of H_3-1/C-2, C-3, H_2-3′/C-1, H-2′/C-1′ and ^{1}H-^{1}H COSY correlation of H_2-3′/H-2′ revealed the presence of an α-hydroxy-γ-lactone structure linked to the phthalide moiety through the C-2/C-3 bond (Fig. 2). Based on these findings, we determined the planar structure of **1** (Fig. 2). Relative configurations of C-2 and C-2′ in **1** were elucidated from the 1D NOE correlations of H_3-1/H-2′ and H_3-1/Ha-3′ (Fig. 2). To determine the absolute stereochemistry at C-2′, we introduced an (*S*)-MTPA ester to OH-2′; however, the scarcity of hydrogen atoms around the C-2′ chiral center limited the reliability of the advanced Mosher's method. We therefore applied the VCD exciton chirality method[15,16] to the mono (*S*)-MTPA ester of **1** (**1a**). As shown in Fig. 3a, **1a** displayed a positive–negative couplet (from lower to higher frequencies) that suggested a clockwise orientation between the two adjacent carbonyl groups at C-1′ and the MTPA ester, where C=O in the MTPA ester is in a *syn* relationship to the methine hydrogen at C-2′ (see Fig. 3a, inset). Therefore, we concluded that the absolute configuration of C-2′ as *R*, and consequently C-2 as *R* considering its relative configuration. Meanwhile, the absolute configuration of the remaining C-3 chiral center was elucidated by comparison of the experimental and theoretical ECD spectra. Fig. 3c shows the observed ECD spectrum of **1** and calculated ones for (2*R*,2′*R*,3*S*)-**1** and (2*R*,2′*R*,3*R*)-**1**. The calculated ECD curve for (2*R*,2′*R*,3*S*)-**1** showed a good agreement with the observed one: a broad negative Cotton effect at around 260 nm and a negative signal at around 230 nm. In contrast, the one for (2*R*,2′*R*,3*R*)-**1** showed a broad positive first Cotton effect in 250-300 nm, and a weak positive band at around 230 nm. These comparisons clearly suggested that the absolute configuration of C-3 to be *S*. The structure of **1** was thus determined as shown in Fig. 1.

Table 1. ^{13}C (150 MHz) and ^{1}H NMR (600 MHz) data for 1.[a,b]

Position	^{13}C	^{1}H	Position	^{13}C	^{1}H
1	20.0	1.03 (3H, s)	12	26.9	3.49 (2H, m)
2	83.0	-----	13	120.7	5.21 (1H, m)
3	85.2	5.49 (1H, s)	14	137.5	-----
4	141.3	-----	15	25.9	1.81 (3H, brs)
5	114.6	-----	16	18.4	1.86 (3H, brs)
6	162.0	-----	1'	175.6	-----
7	113.4	-----	2'	67.4	4.64 (1H, t, 8.8)
8	154.6	-----	3'	41.4	2.67 (1H, dd, 13.6, 8.8, Ha)
9	103.6	-----			2.58 (1H, dd, 13.6, 8.8, Hb)
10	171.6	-----	6-OH		6.37 (1H, s)
11	7.7	2.13 (3H, s)	8-OH		7.86 (1H, s)

[a]Assignment for **1** was based on COSY, HMQC and HMBC experiments.
[b]*J* in Hz.
[c]Recorded in $CDCl_3$

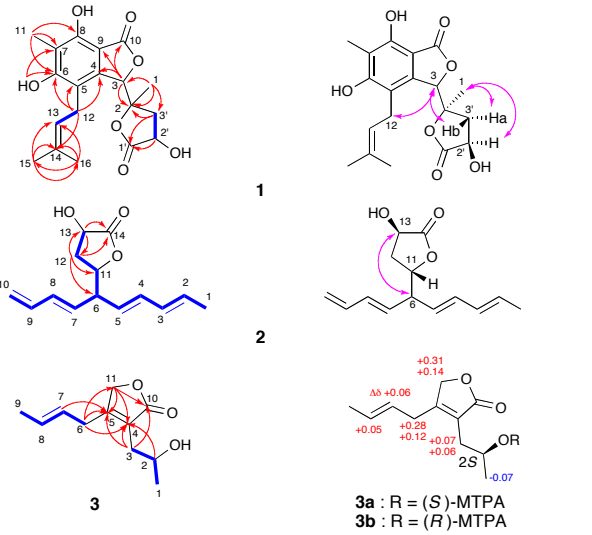

Fig.2. Key HMBC (red allows) and ^{1}H-^{1}H COSY (blue bold lines) correlations of 1–3, relative stereochemistries of 1 and 2 based on 1D NOE experiments (purple arrows), and absolute configuration of 3 based on Δδ(3a–3b) values.

The molecular formula of cancrolide A (**2**) was determined to be $C_{14}H_{18}O_3$ by HREIMS at *m/z* 234.1262 $[M]^+$. The IR and ^{13}C NMR spectra indicated the presence of a γ-lactone (1773 cm^{-1} (KBr), δ_C 177.4), while COSY correlations through H-11 to H-13 , long-range correlation of H-12/C-14 and ^{1}H chemical shift of H-11 (δ_H 4.64) implied an α-hydroxy-γ-lactone structure (C-11-C-14) similar to **1**. In addition, the ^{13}C NMR spectrum displayed seven tertiary sp^2 carbons, one secondary sp^2 carbon, one methine and one methyl (Table 2). In the ^{1}H NMR spectrum, nine olefinic proton signals at δ_H 6.31 (dt, *J* = 17.0, 10.4 Hz, H-9), 6.14 (dd, *J* = 15.4, 10.4 Hz, H-8), 6.11 (dd, *J* = 15.4, 10.3 Hz, H-4), 6.02 (brdd, *J* = 14.7, 10.3 Hz, H-3), 5.69 (dq, *J* =14.7, 7.0 Hz, H-2), 5.61 (dd, *J* = 15.4, 7.4 Hz, H-7), 5.41 (dd, *J* = 15.4, 7.9 Hz, H-5), 5.19 (d, *J* = 17.0 Hz, Ha-10), 5.09 (d, *J* = 10.4 Hz, Hb-10) suggested the existence of three *trans*-olefins and an end olefin. In the ^{1}H-^{1}H COSY spectrum, sequential correlations from H_3-1 to H_2-10 and the cross peak based on H-6/H-11 coupling, which were attributed to the CH_3-CH=CH-CH=CH-CH-CH=CH-CH=CH_2 (C-1–C-10) chain structure and C-6/C-11 connectivity respectively, indicated the branched planer structure of **2** (Fig. 2). The NOE correlation of H-13/H-6 revealed the spatial relationship between the 13-hydroxy group and H-11 (Fig. 2). The absolute stereochemistry at C-13 was also solved using the VCD exciton chirality method. The acetyl derivative **2a** displayed a positive–negative VCD couplet similar to **1a**, indicating the 13*R* configuration (Fig. 3b). The configuration at C-6 remains to be determined because of the virtually symmetrical nature of the C-6 chiral center.

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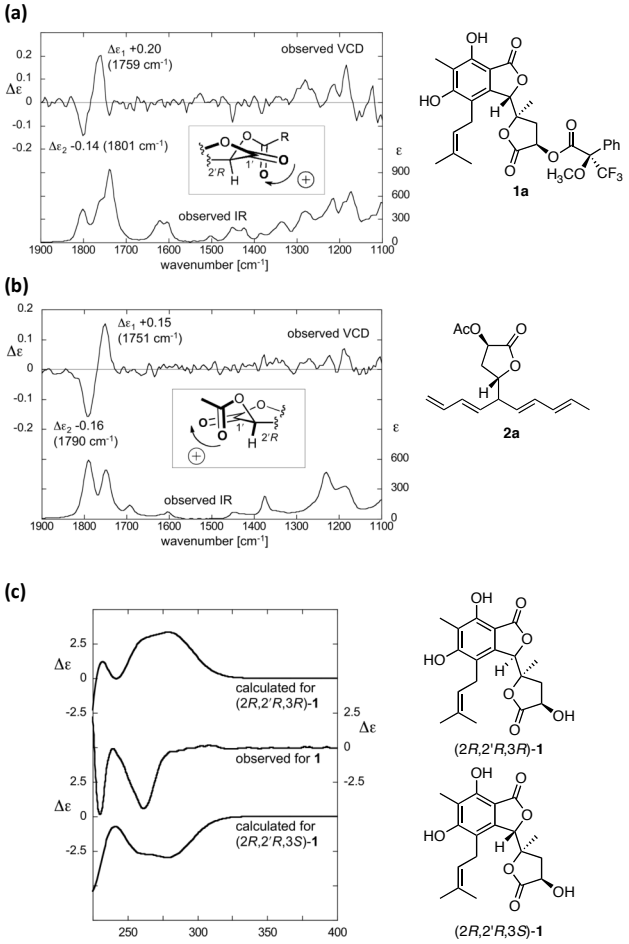


Fig3. (a) VCD spectra of 1a, (b) VCD spectra of 2a, and (c) ECD spectra of 1. VCD spectra were measured in $CDCl_3$ for 90 mins (*l* = 100 μm, *c* = 0.015 M for 1a and 0.025 M for 2a). ECD spectrum of 1 was measured in (CH_3CN) at the concentration of 74 μM.

The ^{13}C NMR and DEPT spectra of cancrolide B (**3**) [HRFABMS at *m/z* 197.1177 $[M+H]^+$ (197.1177 calcd. for $C_{11}H_{17}O_3$)] implied the presence of one ester carbonyl, two quaternary sp^2 carbons, two tertiary sp^2 carbons, one oxymethine, one oxymethylene, two

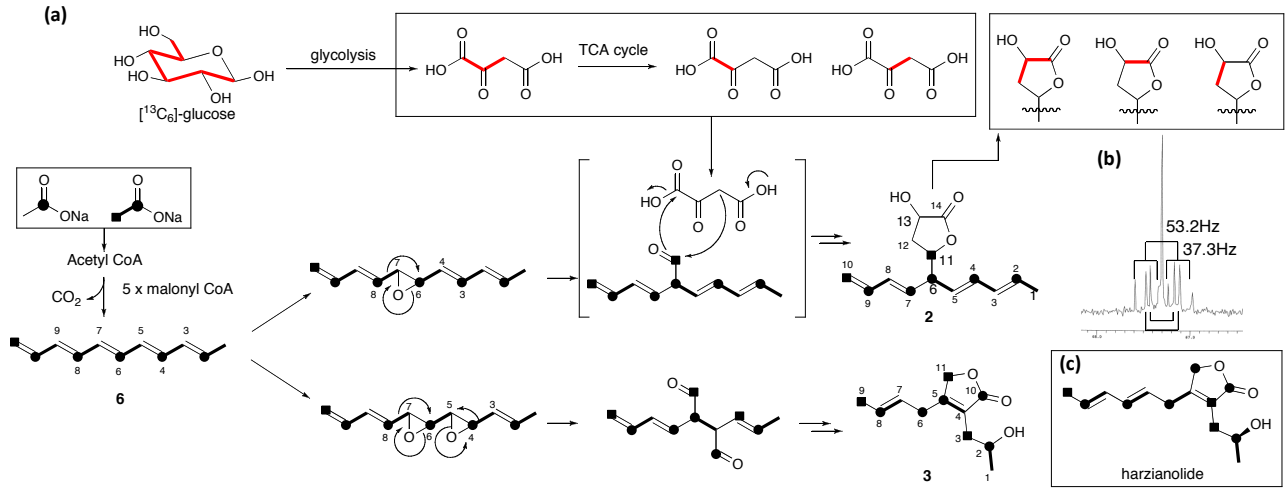
Table 2. 13C (150 MHz) and 1H NMR (600 MHz) data for 2 and 3.[a,b,c]

	2		3	
	^{13}C	^1H	^{13}C	^1H
1	18.0	1.75 (3H, brd, 7.0)	23.3	1.23 (3H, d, 6.2)
2	130.3	5.69 (1H, dq, 14.7, 7.0)	66.3	4.04 (1H, m)
3	130.7	6.02	33.2	2.48 (1H, dd, 14.3, 3.7)
		(1H, ddq, 14.7, 10.3, 1.4)		2.41 (1H, dd, 14.3, 7.7)
4	134.3	6.11 (1H, dd, 15.4, 10.3)	124.2	-----
5	126.2	5.41 (1H, dd, 15.4, 7.9)	161.9	-----
6	50.5	3.03 (1H, dt, 7.9, 7.4)	30.4	3.14 (2H, d, 6.6)
7	130.3	5.61 (1H, dd, 15.4, 7.4)	124.4	5.39 (1H, m)
8	134.0	6.14 (1H, dd, 15.4, 10.4)	129.5	5.60 (1H, dq, 15.1, 6.6)
9	136.3	6.31 (1H, dt, 17.0, 10.4)	17.7	1.69 (3H, dd, 6.6, 1.1)
10	117.5	5.19 (1H, d, 17.0)	176.0	-----
		5.09 (1H, d, 10.4)		
11	80.3	4.64 (1H, m)	71.8	4.71 (2H, s)
12	33.0	2.38 (1H, ddd, 13.6, 8.3, 4.0)		
		2.26 (1H, dt, 13.6, 8.3)		
13	67.3	4.43 (1H, t, 8.3)		
14	177.4	-----		

[a]Assignment for **2** and **3** were based on COSY, HMQC and HMBC experiments.
[b]*J* in Hz.
[c]Recorded in $CDCl_3$.

methylenes and two methyls (Table 2). The ^1H NMR signals at δ 5.60 (dq, *J* = 15.1, 6.6 Hz, H-8) and 5.39 (m, H-7) showed the presence of a *trans*-olefin. The ^1H-^1H COSY correlations (Fig. 2) indicated the connectivity from C-1 to C-3 and C-6 to C-9. The HMBC correlations of H_2-6/C-4, C-5, C-11 and H_2-3/C-4, C-5, C-10 and H_2-11/C-10 revealed a butenolide structure (C-4–C-5–C-11–O–C-10), which was corroborated by the IR spectrum (1742 cm^{-1} (KBr)) (Fig. 2). In addition, the long-range correlations of H-7/C-5 and H-2/C-4 suggested bonding of C-5 to C-6 and C-3 to C-4, respectively. The 2*S* configuration was determined based on the Δδ values (**3a–3b**) (Fig. 2). Therefore, the structure of **3** was identified as shown Fig. 1.

To determine the origin of the branched skeletons in **2** and **3**, we conducted feeding experiments using compounds labeled with stable isotope (results were summarized in Scheme 1).



Scheme 1. (a) Proposed biosynthetic pathway for 2 and 3, based on feeding experiments using compounds labeled with ^{13}C stable isotope, (b) ^{13}C NMR signal for C-13 in labeled 2 by [$^{13}C_6$]-glucose, (c) ^{13}C labeling pattern of harzianolide.

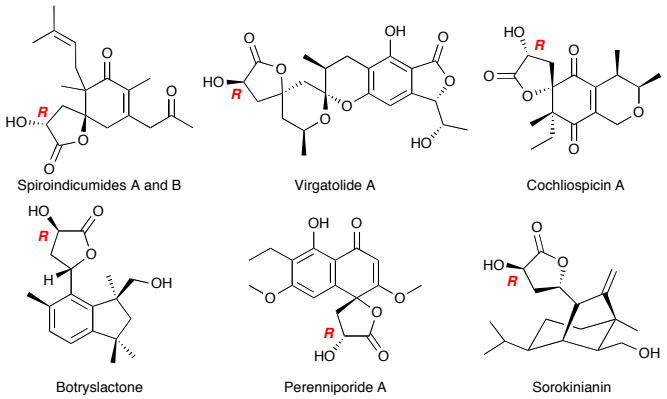


Fig. 4. Examples of fungal secondary metabolites with α-hydroxy-γ-lactone moiety.

Incorporation of ^{13}C at 11 carbon sites (C-1–C-11) in **2** was clearly observed but not at the remaining three sites in the α-hydroxy-γ-lactone (C-12–C-14). This indicates that **2** is composed of a C_{11} polyketide with a C_3 unit. The labeling pattern of **2** suggested that the branched skeleton derived from C_{11} polyene precursor (**6**) *via* rearrangement with the epoxide ring opening observed in aureonitol biosynthesis.[17,18] The ^{13}C labeling pattern for **3** indicated that a common C_{11} polyene (**6**) was used as precusor, and that rearrangement occurred twice during the formation of its double branched skeleton. The planer structure of **3** is similar to that of harzianolide. Nevertheless, the incorporation pattern around the butenolide ring indicates that the rearrangement manner in **3** is different from that in harzianolide (Scheme 1).[19] To determine the biosynthetic origin of the C_3 unit in **2**, we conducted a feeding experiment using fully labeled [$^{13}C_6$]-glucose. The signal at C-13 in the ^{13}C NMR spectrum for labeled **2** showed three types of ^{13}C–^{13}C coupling (dd, $^1J_{12\text{-}13}$ = 37.3 Hz, $^1J_{13\text{-}14}$ = 53.2 Hz; d, $^1J_{13\text{-}14}$ = 53.2 Hz; d, $^1J_{12\text{-}13}$ = 37.3 Hz) (Scheme 1b). These splitting patterns were consistent with evidence for oxaloacetic acid as the source of the C_3 unit in the TCA cycle.[20] Based on these findings, we propose a biosynthetic pathway for **2** and **3** (Scheme 1).

Conclusions

In conclusion, we have shown that nicotinamide, an NAD^+-dependent HDAC inhibitor, induced the production of cryptic secondary metabolites in *C. cancroideum*, leading to the isolation of three new polyketides. Our study also demonstrates the utility of the chemical epigenetic method in accessing new and diverse fungal secondary metabolites. Both **1** and **2** possess one α-hydroxy-γ-lactone moiety and the same configuration. Several other fungal secondary metabolites similarly containing an α-hydroxy-γ-lactone moiety have been reported (Fig. 4).[16,21-25] Interestingly, the stereocenters in the lactone ring have an *R* configuration, similar to **1** and **2**, indicating the existence of a common biosynthetic root for constructing α-hydroxy-γ-lactone in fungi. We examined the new compounds with several biological tests. However, we did not observe any significant biological activity; **1**-**3** showed no cytotoxicity against HCT-116 cell line (IC_{50} > 50 μM), or antiviral activity against HIV or adenovirus (EC_{50} > 50 μM). Compound **2** also displayed no potent activity against *Staphylococcus aureus*, *Enterococcus faecium* and *Pseudomonas aeruginosa* (MIC > 20 μg/ml, respectively).

Experimental

General procedure

Analytical were performed on silica gel 60 F254 (Merck) and RP-18 F254 (Merck). Column chromatography was carried out on silica gel 60 (70–230 and 40-50 mesh). NMR spectra were recorded on JEOL ECA-600. Chemical shifts for ^1H and ^{13}C NMR are given in parts per million (δ) relative to tetramethylsilane (δ_H 0.00) and residual solvent signals (δ_C 77.0) for $CDCl_3$ as internal standards. Mass spectra were measured on JEOL JMS-700 (EI-MS), JMS-DX303 (FAB-MS). VCD spectra were measured on a Bomem Chiral*ir* spectrometer equipped with a second photoelastic modulator. CD spectra were measured on a JASCO J-720 spectropolarimeter. UV spectra were recorded on a JASCO-V-550 spectrophotometer. IR spectra were recorded on a JASCO-FT/IR-4200 spectrometer. HPLC analysis was performed on HITACHI LaChrom Series HPLC equipped with L-7100 pump, L-7455 Diode Array Detector and D-7000 system manager.

Fungal material

C. canroideum was purchased from the Biological Resource Center (NBRC) as NBRC 9106, Chiba, Japan.

Fermentation, extraction and isolation

C. cancroideum maintained in potato dextrose agar at 25 °C was directly inoculated into 500 mL Erlenmeyer flasks containing 150 mL PDB fermentation medium (7.2 g dissolved in 1 L of distilled water) treated with 50 μM nicotinamide (experiment) or without it (control). The flasks were incubated on a rotating shaker at 150 rpm at 25 °C. After 14 d of cultivation, the culture medium was extracted with EtOAc. The EtOAc extracts of each condition were analyzed by reversed-phase HPLC on a Mightysil RP-18 column (250 x 20 mm, 5 μm, 1.0 mL/min) with acetonitrile and water (0-10 min: 80:20, 10-50 min: from 80:20 to 0:100, 50-60 min: 0:100). *C. cancroideum* was then cultivated in sixty 500 mL Erlenmeyer flasks containing 150 mL of PDB fermentation medium containing 50 μM nicotinamide. After this, 9.0 L of whole broth was filtered under suction to separate the broth filtrate and mycelia. The filtrate was extracted three times using ethyl acetate and concentrated under reduced pressure to obtain a dry extract (1.9 g). This extract was chromatographed on a Sephadex LH-20 column by eluting with MeOH to yield three fractions (F1-F3). Fraction F2 was purified using silica gel flash column with *n*-hexane-EtOAc (4:1–1:1) as the eluent to obtain **4** (26.2 mg) and three fractions (F2-1, F2-2 and F2-3). Fraction

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F2-1 (150.6 mg) was further purified using silica gel flash column by using $CHCl_3$-EtOAc (19:1) as an eluent to obtain **2** (20.4 mg). Compound **1** (1.8 mg), **3** (33.7 mg) and **5** (21.1 mg) were obtained from fraction F2-2 (574.2 mg) using silica gel flash column by using CHCl3-EtOAc (19:1 to 9:1) as an eluent.

For the labeling studies, *C. cancroideum* was cultivated in PDB medium (150 mL × 6) with [1-^{13}C] sodium acetate (final quantity: 30 mg for each flask), [1,2-^{13}C] sodium acetate (final quantity: 15 mg for each flask), or [$^{13}C_6$]-glucose (final quantity: 90 mg for each flask). The labeled compound was added periodically (after 7, 9, and 11 d) to the growing fungal culture, which was cultivated for 14 d. About 1 mg of labeled **2** and **3** were isolated from each culture medium.

Chaetophenol G (1). White amorphous; $[\alpha]D_{25}$ -29.0 (*c* 0.25, $CHCl_3$); UV (EtOH) λ_{max} nm (log ε): 304 (3.64), 266 (3.98), 222 (4.45); UV (CH_3CN) λ_{max} nm (log ε): 302 (3.63), 263 (3.99), 221 (4.53); CD (CH_3CN) λ_{max} nm (log ε): 308 (+0.14), 262 (-4.36), 240 (-0.18), 230 (-4.52), 214 (+4.90); IR (KBr) ν_{max} (cm^{-1}): 3421, 2926, 1782, 1733, 1617, 1507, 1422, 1217, 1100, 1014, 955; ^{1}H and ^{13}C NMR data are shown in Table 1; HREIMS: *m/z* 362.1347 $[M]^+$ (362.1366 calcd. for $C_{19}H_{22}O_7$).

(*R*)-MTPA ester of 1 (1a). Treatment of **1** (0.80 mg) with (*S*)-MTPACl (1.4 μL) in pyridine (80 μL) at room temperature afforded the corresponding (*R*)-MTPA ester **1a** (spot to spot). Colorless amorphous; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 (1H, s), 7.56-7.42 (5H, m), 6.35 (1H, s), 5.80 (1H, t, *J* = 9.1 Hz), 5.19 (1H, t, *J* = 6.2 Hz), 3.64 (3H, s), 3.46 (2H, m), 2.74 (1H, dd, *J* = 14.0, 9.1 Hz), 2.63 (1H, dd, *J* = 14.0, 9.1 Hz), 2.13 (3H, s), 1.86 (3H, s), 1.80 (3H, s), 1.07 (3H, s); HREIMS: *m/z* 578.1723 $[M]^+$ (578.1764 calcd. for $C_{29}H_{29}F_3O_9$).

Cancrolide A (2). Colorless amorphous; $[\alpha]D_{25}$ +40.8 (*c* 1.00, MeOH); UV (CH_3CN) λ_{max} nm (log ε): 232 (4.36), 221 (4.36); CD (CH3CN) λ_{max} nm (log ε): 249 (2.09), 219 (1.82), 202 (2.23); IR (KBr) ν_{max} (cm^{-1}): 3357, 2926, 1773, 1541, 1456, 1183, 1119, 993; ^{1}H and ^{13}C NMR data are shown in Table 2; HREIMS: *m/z* 234.1262 $[M]^+$ (234.1262 calcd. for $C_{14}H_{18}O_3$).

Acetate of 2 (2a). Treatment of **1** (1.0 mg) with acetic anhydride (50 μL) in pyridine (100 μL) at room temperature afforded the corresponding acetate **2a** (spot to spot). Colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.32 (1H, dt, *J* = 16.7, 10.1 Hz, H-9), 6.16 (1H, dd, *J* = 15.5, 10.1 Hz, H-8), 6.13 (1H, dd, *J* = 15.2, 10.4 Hz, H-3), 6.03 (1H, ddq, *J* = 15.0, 10.4, 1.2 Hz, H-3), 5.71 (1H, dq, *J* = 15.0, 6.7 Hz, H-2), 5.42 (1H, dd, *J* = 15.2, 8.3 Hz, H-5), 5.27 (1H, dd, *J* = 8.9, 7.2 Hz, H-13), 5.20 (1H, d, *J* = 16.7 Hz, Ha-10), 5.10 (1H, d, *J* = 10.1 Hz, Hb-10), 4.65 (1H, ddd, *J* = 13.9, 8.9, 4.3 Hz, H-11), 3.07 (1H, ddd, *J* = 8.3, 7.4, 5.7 Hz, H-6), 2.49 (1H, ddd, *J* = 13.9, 8.9, 4.3 Hz, Ha-12), 2.24 (1H, dt, *J* = 13.9, 8.5 Hz, Hb-12), 1.75 (3H, dd, *J* = 6.7, 1.2 Hz, H_3-1); HREIMS: *m/z* 276.1349 $[M]^+$ (276.1362 calcd. for $C_{15}H_{20}O_3$).

Cancrolide B (3). White amorphous; $[\alpha]D_{25}$ +10.6 (*c* 1.00, $CHCl_3$); UV (EtOH) λ_{max} nm (log ε): 268 (2.72), 215 (4.04); IR (KBr) ν_{max} (cm^{-1}): 3446, 2969, 2930, 1742, 1671, 1456, 1340, 1077, 1032, 968, 936; ^{1}H and ^{13}C NMR data are shown in Table 2; HRFABMS: *m/z* 197.1177 $[M+H]^+$ (197.1178 calcd. for $C_{11}H_{17}O_3$).

(*S*)-MTPA ester of 3 (3a). Treatment of **3** (0.60 mg) with (*R*)-MTPACl (1.2 μL) in pyridine (30 μL) at room temperature afforded the corresponding (*S*)-MTPA ester **3a** (spot to spot). Colorless amorphous; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51-7.40 (5H, m), 5.51 (H-7), 5.35 (H-2), 5.24 (H-7), 4.62 (Ha-11), 4.52 (Hb-11), 3.44 (OMe), 3.06 (Ha-6), 2.90 (Hb-6), 2.67 (Ha-3), 2.52 (Hb-3), 1.65 (H-9), 1.35 (H_3-1); HREIMS: *m/z* 412.1496 $[M]^+$ (412.1498 calcd. for $C_{21}H_{23}F_3O_5$).

(*R*)-MTPA ester of 3 (3b). Treatment of **3** (0.60 mg) with (*R*)-MTPACl (1.2 μL) in pyridine (30 μL) at room temperature afforded the corresponding (*S*)-MTPA ester **3a** (spot to spot). Colorless amorphous; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50-7.35 (5H, m), 5.46 (H-7), 5.33 (H-2), 5.19 (H-7), 4.48 (Ha-11), 4.21 (Hb-11), 3.57 (OMe), 2.94 (Ha-6), 2.63 (Hb-6), 2.59 (Ha-3), 2.46(Hb-3), 1.65 (H-9), 1.43 (H_3-1); HREIMS: *m/z* 412.1496 $[M]^+$ (412.1498 calcd. for $C_{21}H_{23}F_3O_5$); HREIMS: *m/z* 412.1521 $[M]^+$ (412.1498 calcd. for $C_{21}H_{23}F_3O_5$).

ECD calculations

The conformational distributions of (2*R*,2′*R*,3*S*)-**1** and (2*R*,2′*R*,3*R*)-**1** were calculated using an MMFF Monte Carlo search using the Spartan'10 software. The lower energy conformers within 10 kcal/mol from the most stable were further fully optimized at the DFT/B3LYP/6-311G(d,p) level of theory as implemented in a Gaussian09 program. For the resultant conformers within 1.9 kJ/mol from the most stable, ECD spectra were calculated using a Gaussian09 program by means of the TDDFT approach, the B3LYP functional, and the 6-311G(d,p) basis set. Final spectra were obtained by Boltzmann-weighed average of the spectra of each conformer, and presented based on the velocity-representation rotational strengths by using Gaussian band shapes and 0.15 eV half-width at 1/e of peak height.

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