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Regulation of the Growth of Cotton Bollworms by Metabolites from an Entomopathogenic Fungus *Paecilomyces cateniobliquus*

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Supporting Information

ABSTRACT: Chemical investigation of one entomopathogenic fungus *Paecilomyces cateniobliquus* YMF1.01799 led to the isolation and identification of six metabolites, which include three new compounds (**2**−**3**, and **5**) and three known metabolites. Their structures were established by spectroscopic studies such as 1D and 2D NMR and MS analysis. Insect growth experiments suggested that polyketide-derived compound **1** showed significant inhibitory effect on the growth of cotton bollworm *Helicoverpa armigera*, while terpenoid-derived metabolite **5** promoted the growth of the larvae. The findings revealed that the entomopathogenic fungus *P. cateniobliquus* could produce different types of metabolites to regulate growth of the insect.

KEYWORDS: *entomopathogenic fungus, Paecilomyces cateniobliquus, cateniobolins*

INTRODUCTION

The entomopathogenic fungi belonging to the genus *Paecilomyces* have been extensively studied as potential biological control agents against insects. Investigation of the chemical components of the genus *Paecilomyces* so far led to the isolation of more than 90 compounds.[1,2] Representative secondary metabolites are paecilotoxins (highly toxic linear peptides; also designated as leucinostatins) from *Paecilomyces lilacinus*,[3] paecilodepsipeptides (antimalarial and antitumor cyclohexadepsipeptide) from *Paecilomyces cinnamomeus*,[4] and paeciloquinones (anthraquinones, protein tyrosine kinase inhibitors) from *Paecilomyces carneus* P-177.[5] Many of these chemical compounds have been found to have a myriad of biological activities, including antibacterial, antifungal, antioxidative, antiviral, and antitumor activities.[2,6,7] However, studies with regard to the insecticidal activities of the natural compounds have been rarely reported and discussed. On the basis of chemoecological considerations, we recently embarked on the investigation of entomopathogenic deuteromycetes as a source for new bioactive secondary metabolites toward insects and nematodes.[8] Since the production of secondary metabolites can also be a strategy used by entomopathogenic fungi to infect or kill insects, the evaluation and commercial development of natural chemicals from these fungi have attracted considerable interest.

A preliminary experiment has indicated that the ethyl acetate fraction of the fermentation of one fungal strain, *Paecilomyces cateniobliquus* YMF1.01799, demonstrated good inhibitory activity toward the growth of cotton bollworms and nematodes.

Herein, we report the isolation of bioactive metabolites from the insect pathogenic fungus *P. cateniobliquus* and the evaluation of their possible biological roles.

MATERIALS AND METHODS

General Experimental Procedures. Column chromatography was performed on 200−300 mesh silica gel (Qingdao Marine Chemical Factory, P. R. China). Optical rotations were measured on a Horiba-SEAP-300 spectropolarimeter. UV spectroscopic data were measured on a Shimadzu-210A double-beam spectrophotometer. IR spectra of samples in KBr disks were recorded on a Bruker-Tensor-27 spectrometer with KBr pellets. NMR spectra were carried out on either a Bruker AM-400 or a DRX-500 spectrometer with respect to solvent as internal standard. MS were recorded on a VG-Auto-Spec-3000 spectrometer. Insect length and width evaluation was measured on an Image-Pro-ExPress (version 5.1.1.4 for Windows 2000/XP professional).

Strain and Cultivation. The strain *Paecilomyces cateniobliquus* was collected from Fengyang prefecture, and the isolate was deposited as YMF1.01799 in the strain collection of Laboratory for Conservation and Utilization of Bio-Resources & Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming, P. R. China. After the conidia had developed on PDA slants in test tubes at 25 °C, the strain was kept at −30 °C as a stock culture. The strain *Paecilomyces cateniobliquus* (YMF1.01799), cultured on a PDA medium for 6 days, was inoculated into 500 mL flasks each containing 200 mL of production medium consisting of beef extract 0.001%, peptone 0.002%, yeast extract 0.001%, and glucose 0.01%. The pH of the medium was neutral. The inoculated flasks were cultured on a rotary shaker (180 rpm) at 28 °C for 14 days.

Extraction and Isolation. A 14-day-old fermentation broth of strain YMF 1.01799 (200 mL × 200 flasks) was filtered to separate the mycelia from the culture. The culture filtrate was concentrated in vacuo and partitioned with EtOAc (1000 mL × 3), and the organic part was evaporated to dryness to give an oily residue weighing 6.8 g. The EtOAc fraction was subjected to a silica gel column, developed with $CHCl_3$:MeOH (1:0, 15:1, 9:1, 4:1, 1:1 v/v) to give five fractions. Fractions were monitored by TLC, and spots were visualized by spraying with 10% H_2SO_4 in EtOH. The fraction of $CHCl_3$:MeOH (15:1, v/v) (0.08 g) was then chromatographed over a Sephadex LH-20 column, developed with acetone to give five subfractions.

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Subfraction 2 was repeatedly subjected to a silica gel column, developed with petroleum ether:acetone (8:1) to give compound **1** (42.5 mg). Subfraction 3 was subjected to a silica gel column, eluting with $CHCl_3$:acetone (9.5:1) to yield compound **3** (1.9 mg). Subfraction 4 was repeatedly chromatographed over a silica gel column washed with EtOAc:petroleum ether (4:1) to afford compounds **2** (3.2 mg) and **4** (2.1 mg). The rest of subfraction 4 was further separated on an RP_{18} column eluting with H_2O:MeOH (13%) to obtain **5** (13.1 mg), and the leftover was subjected to a silica gel column, developed with petroleum ether:acetone (6:1) to give compound **6**.

*Phomalactone (**1**).* Colorless oil: $[\alpha]_D^{23.5}$ +76.78° (*c* 0.21 MeOH); UV (MeOH) λ_{max} (log ε) 209.2 (3.79) nm; IR (KBr) ν_{max} 3420, 2969, 2940, 2919, 2857, 1722, 1629, 1449, 1381, 1163, 1101, 1076, 1035, 969, 935, 893, 830, 709, 643, 560 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz) see Table 2.

Table 1. ^1H Spectroscopic Data of Compounds 1−4[a]

	δ_H mult (*J*)			
no.	1	2	3	4
2	5.98 d, 9.9	2.58 m	2.75 dd, 17.4, 6.8 2.57 dd, 17.4, 2.3	6.20 dd, 6.0, 2.3
3	6.93 dd, 5.4, 9.9	2.25 m 2.11 m	4.60 m	7.50 dd, 6.0, 1.5
4	4.13 dd, 5.4, 3.1	4.46 dd, 12.8, 7.5	4.30 t, 4.1	5.02 brd, 4.5
5	4.73 dd, 7.1, 3.1	4.11 brt, 7.3	4.51 dd, 6.8, 4.1	4.40 brs
6	5.70 ddd, 15.5, 7.1, 1.5	5.54 ddd, 15.5, 7.5, 1.5	5.61 ddd, 15.3, 6.8, 1.0	5.52 ddd, 15.5, 7.5, 1.5
7	5.89 m	5.88 m	5.85 m	5.89 m
8	1.73 d, 6.2	1.76 d, 6.7	1.69 d, 6.2	1.76 d, 6.5

[a]Data were measured in acetone-d_6 at 400 MHz with reference to the solvent signals, δ in ppm and *J* in Hz.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1−4[a]

	δ_C			
no.	1	2	3	4
1	164.2 s	177.4 s	175.2 s	173.3 s
2	122.0 d	28.9 t	39.2 t	123.4 d
3	145.5 d	24.2 t	69.1 d	153.6 d
4	62.8 d	75.4 d	84.7 d	86.1 d
5	81.6 d	83.1 d	71.6 d	72.6 d
6	124.2 d	128.3 d	128.1 d	127.9 d
7	132.6 d	131.3 d	131.0 d	131.2 d
8	17.9 q	18.3 q	17.9 q	18.2 q

[a]Data were measured in acetone-d_6 at 100 MHz with reference to the solvent signals, δ in ppm.

*Catenioblin A (**2**).* Colorless oil: $[\alpha]_D^{23.5}$ +31.11° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 270.8 (1.84), 208.8 (2.79) nm; IR (KBr) ν_{max} 3443, 2962, 2939, 2920, 2858, 1772, 1673, 1451, 1419, 1379, 1289, 1265, 1189, 1126, 1081, 1035, 1001, 970, 918, 815, 648, 567, 539 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz) see Table 2; positive HRESI-MS *m*/*z* 179.0686 $[M + Na]^+$ (calculated for $C_8H_{12}O_3Na$, 179.0684).

*Catenioblin B (**3**).* Colorless oil: $[\alpha]_D^{23.5}$ −2.72° (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 281.8 (2.61), 209.6 (3.19) nm; IR (KBr) ν_{max} 3425, 2960, 2931, 2873, 1777, 1729, 1601, 1451, 1384, 1288, 1203, 1123, 1075, 1030, 970, 910, 858, 796, 746, 669, 652, 569 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz) see Table 2; positive HRESI-MS *m*/*z* 195.0633 $[M + Na]^+$ (calculated for $C_8H_{12}O_4Na$, 195.0636).

*Musacin D (**4**).* Colorless oil: $[\alpha]_D^{23.5}$ −2.90° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 285.2 (2.84), 209.0 (3.52), 195.4 (2.94) nm; IR (KBr) ν_{max} 3426, 2960, 2931, 2873, 1729, 1628, 1602, 1462, 1452, 1384, 1287, 1122, 1175, 1040, 964, 942, 838, 745, 706, 651, 568 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz) see Table 2; positive ESI-MS *m*/*z* 177 $[M + Na]^+$.

*Catenioblin C (**5**).* Colorless oil: $[\alpha]_D^{23.5}$ −9.78° (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 270.2 (1.98), 207.0 (2.64) nm; IR (KBr) ν_{max} 3440, 2963, 2935, 2874, 1724, 1628, 1463, 1380, 1288, 1263, 1206, 1120, 1077, 1040, 920, 885, 802, 745, 531 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 0.97 (d, *J* = 6.8 Hz, H-1), 1.52 (overlap, H-2), 1.64 (overlap, H-4a), 1.49 (overlap, H-4b), 1.77 (overlap, H-5a), 1.48 (overlap, H-5b), 1.78 (overlap, H-6), 1.38 (overlap, H-8), 1.31−1.34 (2H, H-9), 1.62 (overlap, H-10a), 1.39 (overlap, H-10b), 2.49 (m, H-11), 1.07 (s, Me-13), 1.19 (s, Me-14), 1.12 (d, *J* = 6.8 Hz, Me-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 14.5 (q, C-1), 44.2 (d, C-2), 81.4 (s, C-3), 40.4 (t, C-4), 24.2 (t, C-5), 54.0 (d, C-6), 74.9 (s, C-7), 40.3 (t, C-8), 21.4 (t, C-9), 34.0 (t, C-10), 39.1 (d, C-11), 181.1 (s, C-12), 25.0 (q, C-13), 26.0 (q, C-14), 16.9 (q, C-15); negative HRESI-MS *m*/*z* 271.1904 $[M - H]^-$ (calculated for $C_{15}H_{28}O_4$, 271.1909).

*Cyclo(valyltryptophyl) (**6**).* Colorless powder: the ^1H NMR and ^{13}C NMR spectroscopic data were consistent with those of cyclo-(valyltryptophyl) reported in the literature.

Cotton Bollworm Bioassay. Second instars of *Helicoverpa armigera* Hubner reared on artificial diet (Henan Jiyuan Baiyun Industrial Company Limited, Beijing, P. R. China) were chosen as the test insect in our study. All bioassay was conducted under controlled environmental conditions (27 ± 1 °C, 65−70% RH). The larvae were caged individually to avoid cannibalism; 30 larvae were prepared for each tested concentration. Test samples (compounds **1**, **2**, **4**, and **5**) were dissolved in 600 μL of acetone, respectively, and then diluted with sterilized water containing 0.3% (v/v) Tween-20 to prepare a stock solution 200 μg/mL. The same amount of acetone dissolved in water containing 0.3% (v/v) Tween-20 was established as control. Five microliters of tested concentrations of 200, 100, 50, and 25 μg/mL of sample solution prepared by the stock solution was applied to the dorsal and ventral sides of second instar larvae, respectively.[9,10] The larvae were examined daily. Larvae were considered to be dead when their bodies were straight and they failed to move or respond to probing with a needle. Toxicity was evaluated according to the mean percentage of dead larvae.[10−13] The growth rates of larvae were quantitated in two ways, the mean total body length and head capsule width.[11,12] The size (length and width) of instar was measured by means of a graticule eyepiece fitted to a binocular microscope with substage illumination.

One-way analysis of variance (ANOVA) was used to test for significant differences between the mean larva lengths or widths achieved by the instars in each treatment. Paired values were subjected to the *t*-test to determine the significance with SPSS 17.0 for Windows.

UPLC−MS/MS Analysis. The strain cultured on a PDA medium at 28 °C for 7 days was inoculated into 4 × 500 mL flasks containing 250 mL of the corn medium (corn 20%). The pH of the medium was neutral at 7. The strain was cultured at 28 °C, with 50 L/min aeration and stirring at 200 rpm for 9 days. The cultured larvae of *Helicoverpa armigera* (mixed stages) were obtained from culturing medium and sterilized by 1% NaOCl solution for 2 min. About 300 larvae were added into each flask. The analysis of compounds **1** and **5** in the culture broths was determined every 24 h. The culture broth of the liquid fermentation (1 L) was filtered to separate the mycelia from the culture. The culture filtrate was concentrated in vacuo to 50 mL and was exhaustively extracted overnight with ethyl acetate (1:1 v/v). The organic phase was concentrated under reduced pressure. The dried organic residue was then dissolved in 2 mL of methanol and filtered through 0.22 μm membranes, which was to be further analyzed using LC−MS/MS. All tests were repeated three times, and data obtained were statistically analyzed.

The UPLC−MS/MS analysis for the identification of compounds **1** and **5** was carried out on samples using a Waters Acquity Ultra Performance LC (Waters, Milford, MA), coupled to a tandem mass spectrometer (AB Sciex, Foster City, CA, USA), equipped with an ESI

ion source. The LC conditions with a UPLC BEH phenyl column (2.1 × 50 mm, 1.7 μm; Waters, Milford, MA, USA) were manually optimized based on the separation patterns of compounds and were as follows: a mobile phase A (0.1% formic acid in water) and a mobile phase B (0.1% formic acid in acetonitrile) delivered at 0.4 mL/min; gradient program of B (0 min, 10%; 1.0 min, 10%; 4.0 min, 30%; 5.0 min, 90%; 6.0 min, 90%; 6.2 min, 10%; 8.0 min, 10%). The column temperature was maintained at 40 °C. The mass spectrometer was operated in the negative ionization mode. Detection of the ions was performed in the multiple reaction monitoring (MRM) mode. The electrospray voltage was set to −4500 V, and capillary temperature was at 600 °C; curtain gas, 10 psi (69 kPa of max. 99.5% nitrogen); ion source gas 1 (nebulizer gas), 60 psi (414 kPa of nitrogen); ion source gas 2 (auxiliary gas), 20 psi (138 kPa of nitrogen). The MS parameters for analysis of **1** were quantitative ion 152.8/97.0 *m/z*, qualitative ion 152.8/109.0 *m/z*, declustering potential −33/−41 U/V, and collision energy −17/−13 U/eV, respectively. The MS parameters for analysis of **5** were quantitative ion 270.6/97.1 *m/z*, qualitative ion 270.6/253.2 *m/z*, declustering potential −83/−79 U/V, and collision energy −37/-40 U/eV, respectively. The equations of the calibration curves and correlation coefficients (R) for compounds **1** and **5** were $y = 138x - 332$ ($R = 0.9996$), and $y = 315x - 3.32$ e[4] ($R = 0.9998$), respectively.

RESULTS AND DISCUSSION

Compounds **1**−**4** were obtained as colorless oils in this study. Among them, the amount of compound **1** was overwhelming in *P. cateniobliquus* strain, and thus **1** was obtained as the first metabolite from the culture broth of the fungus. The structure of **1** was determined as a known metabolite phomalactone, on the basis of the ^{1}H NMR and ^{13}C NMR spectroscopic data (Tables 1 and 2), which were consistent with those reported in the literature.[14]

HRESI-MS of compound **2** indicated a molecular formula of $C_8H_{12}O_3$. The ^{1}H NMR spectrum of **2** (Table 1) revealed the presence of one secondary methyl at δ_H 1.76 (3H, d, J = 6.7 Hz), two oxygenated methines at δ_H 4.46 (1H, dd, J = 12.8, 7.5 Hz) and 4.11 (1H, brt, J = 7.3 Hz) respectively, and two olefinic methines with highfield shifted signals at δ_H 5.54 (1H, ddd, J = 15.5, 7.5, 1.5 Hz) and 5.88 (1H, m) respectively. The ^{13}C NMR and DEPT spectra of **2** (Table 2) showed 8 carbon signals, which were classified as one methyl, two methylenes, two oxygen-bearing methines, two olefinic methines, and one carbonyl group of an ester. The above data suggested that compound **2** was a phomalactone type compound. Comparison of the NMR data of **2** with those of compound **1** revealed that the difference was that **2** had two methylene groups at δ_C 28.9 t and 24.2 t instead of two olefinic methines in **1**, and the chemical values of the carbonyl group at δ_C 164.2 (s, C-1) and the oxymethine at δ_C 62.8 (d, C-4) in **1** were both shifted in the low field at δ_C 177.4 (s) and 75.4 (d) respectively, in **2**. This indicated that the double bond between C-2 and C-3 in **1** was reduced in **2**. Further support came from analysis of correlations observed in the ^{1}H−^{1}H COSY and HMBC spectra. The proton signal of the methylene at δ_H 2.58 (2H, m, H_2-2) showed a ^{1}H−^{1}H COSY relation with H_2-3 at δ_H 2.25 and 2.11 (each 1H, m), and ^{1}H−^{13}C long-range correlations with C-1, C-3 and C-4 at δ 177.4 (s), 24.2 (t) and 75.4 (d) respectively, demonstrating that the two methylene groups were present between C-1 and C-4. All physicochemical data are in full agreement with the proposed structure of **2** as described. Unambiguous assignments of the NMR data of **2** were achieved through 2D NMR experiments. Thus, compound **2** was determined as a dihydro derivative of **1** as described in Figure 1 and named catenioblin A.

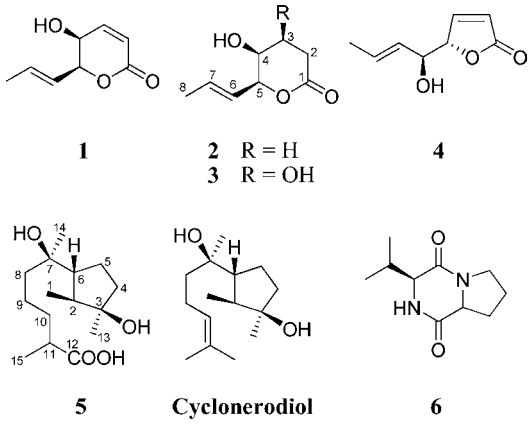

Figure 1. Structures of compounds **1**−**6** and cyclonerodiol.

HRESI-MS of compound **3** indicated a molecular formula of $C_8H_{12}O_4$, suggesting that **3** possessed one more oxygen atom than **2**. The UV and IR spectra of **3** were almost identical to those of **2**, suggesting its similar structural properties to **2**. Comparison of the NMR data of **3** with those of compound **2** (Tables 1 and 2) revealed that **3** was very similar to **2**. The only difference between them was that **3** had one more hydroxyl group than **2**. The methylene group (δ_C 24.2 t, δ_H 2.25 and 2.11) in **2** was replaced by an oxygen-bearing methine group (δ_C 69.1 d, δ_H 4.60) in **3** (Tables 1 and 2). The chemical value of the C-2 at δ_C 28.9 t and the C-4 at δ_C 75.4 d in **2** were downfield shifted to δ_C 39.2 t and 84.7 d in **3**, further demonstrating that the extra hydroxyl group was attached to C-3 in **3**. All the above data suggested that compound **3** was C-3 hydroxylated analogue of compound **2**. The small vicinal coupling constant between H-3 and H-4 (J = 4.1 Hz) indicated a *cis* configuration at C3/C4 with one hydrogen atom in a quasibisequatorial conformation and the other in a quasibisaxial conformation, which established the stereochemistry of the hydroxyl at C-3 as that of the hydroxyl group at C-4. The beta-configuration of 3-OH was also evident from the upfield shifted C-5 due to gamma-gauche effect. All physicochemical data are in full agreement with the proposed structure of **3** as described (Figure 1), given trivial name catenioblin B.

The structure of **4** was determined as musacin D previously reported from *Streptomyces griseoviridis* (FH-S 1832), on the basis of the ^{1}H NMR and ^{13}C NMR spectroscopic data, which were in full agreement with those reported in the literature.[15]

HRESI-MS of compound **5** indicated a molecular formula of $C_{15}H_{28}O_4$. The ^{1}H NMR spectrum of **5** gave the proton signals at δ_H 0.95−2.5 and revealed the presence of two doublets at δ_H 1.12 and 0.97 (each 3H, d, J = 6.8 Hz) for two secondary methyls, and two singlets at δ_H 1.19 and 1.07 (each 3H, s) for two tertiary methyls. The ^{13}C NMR and DEPT spectra of **5** showed 15 carbon signals, which were classified as four methyls, five methylenes, three methines, two oxygen-bearing quaternary carbons, and a carboxylic group. Comparison of the NMR data of **5** with those of a known sesquiterpenoid cyclonerodiol reported from *Gibberella fujikuroi*, *Fusarium culmorum*, *Trichothecium roseum*, and *Trichoderma polysporum*[16] demonstrated that these two compounds were very similar, and the differences between them occurred at the end of the side chain. The olefinic methine, olefinic quaternary carbon, and one methyl group were substituted by one methylene at δ_C 34.0 (t), one methine at δ_C 39.1 (d), and a carboxyl group at δ_C 181.1 (s) respectively, in **5**. The proton signal at δ_H 2.49 of the

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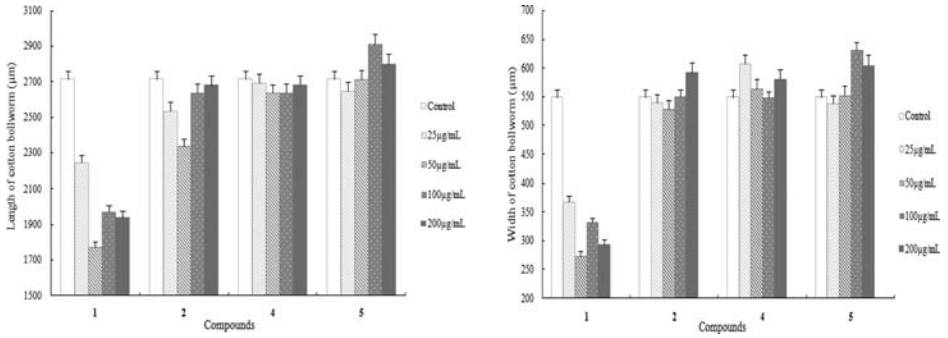

Figure 2. The growth of *Helicoverpa armigera* according to the concentrations (μg/mL) of compounds **1**, **2**, **4**, and **5** at 48 h.

methine in **5** showed $^{1}H,^{13}C$ long-range correlations with one methylene at δ_C 21.4, one methyl at δ_C 16.9, the methylene at δ_C 34.0, and the carboxylic group at δ_C 181.1, which indicated that the double bond between C-10 and C-11 in cyclonerodiol was saturated in **5** and one of the methyl groups at the end of the side chain in cyclonerodiol was replaced by a carboxyl group in **5**. All physicochemical data are in full agreement with the proposed structure of **5** as described. Unambiguous assignments of the NMR data of **5** were achieved through 2D NMR ($^{1}H-^{1}H$ COSY, HSQC, HMBC, and ROESY) experiments. Finally, the structure of compound **5** was determined as described in Figure 1, and named as catenioblin C.

Previous reports displayed that compound **1**, which was also found in other fungi, e.g. *Nigrospora sphaerica*, and the entomopathogenic fungi *Hirsutella thompsonii* var. *synnematosa*, showed the mortality of nematode *Meloidogyne incognita* reaching 84% in 96 h at the concentration of 500 mg/L.[17] However, no study involving its biological activity toward insects was reported.

Cotton bollworm *H. armigera* is a major pest on a wide range of crops in Europe, Africa, Asia, and Australia. 50% of all insecticides used in China and India are used to control this pest, which has resulted in insecticide resistance occurring in some situations. Farmers spend up to 40% of their annual income to buy chemicals to curb *H. armigera*.[9] In our study, compounds **1**, **2**, **4**, and **5** were evaluated for their activities toward cotton bollworm *H. armigera*.

Our preliminary trial indicated that cotton bollworm *H. armigera* larvae were not sensitive to topical treatments of compound **1** applied to 10 μg to 1 mg. Since similar cases have been reported for methomyl insecticides,[18] an alternative bioassay using tested solutions in sterile water was modified and applied.[10] In our experiments, all the compounds showed no obvious lethal activities toward the bollworms, since the mean corrected mortality rate of *H. armigera* for all the compounds was not more than 10% at concentrations of 25–200 μg/mL. However, it was observed that the *H. armigera* larvae treated with compound **1** showed significant growth retardation after 48 h at concentrations of 25–200 μg/mL. At a concentration of 25 μg/mL, compound **1** displayed strong inhibitory effect on the overall growth of the *H. armigera* larvae. It was observed that the tested larvae were almost 50% smaller in the size than those without treatment (Figure 2). The weights of the tested larvae were only one-third of those without treatment at a concentration of 25 μg/mL. Compound **2** showed moderate activity toward the length of the larvae at a concentration of 100 μg/mL. Surprisingly, larval growth was significantly promoted by exposures to compound **5** at concentrations of 25–200 μg/mL. The *H. armigera* larvae treated with compound **5** at a concentration of 200 μg/mL grew almost a third bigger in size than those without treatment (Figure 2).

It is very interesting to note that an entomopathogenic fungus could produce different types of metabolites which exhibited opposite effects on the growth and development of insect larvae. During the course of the metabolic profiling of *P. cateniobliquus*, we noted that compound **1** was the predominantly richest component in the extract, and its amount in the cultural broth of *P. cateniobliquus* could reach as high as 13.03 mg/L. Compound **2** ranked second with a content of 3.75 mg/L. Compounds **4** and **5** were just at contents of 0.08 and 0.05 mg/L respectively, in the cultural broth of *P. cateniobliquus*. The data suggested that *P. cateniobliquus* could produce much more metabolites with an inhibitory activity toward the growth of the insect larvae.

Another experiment was performed to test the influence of larvae on the contents of **1** and **5** in *P. cateniobliquus*. The fungus *P. cateniobliquus* was cultivated in the nutrient poor medium CML for 10 days and then added with larvae. The exact yields of compounds **1** and **5** in the culture broths of *P. cateniobliquus* cultivated in the media were quantitatively determined with UPLC–ESI/MS/MS analysis. It is obvious to note that larvae could induce the large accumulation of **1**, but cause the decrease of compound **5** in the cultural broth of the entomopathogenic fungus *P. cateniobliquus* (Figure 3).

In conclusion, by chemical metabolite profiling we have obtained six metabolites including four phomalactone-type metabolites with two being new, one new sesquiterpenoid and one cyclodipeptide, from a fermentation of an entomopathogenic fungus *P. cateniobliquus* and solved their structures by 2D NMR spectroscopy. Biological evaluation of these compounds

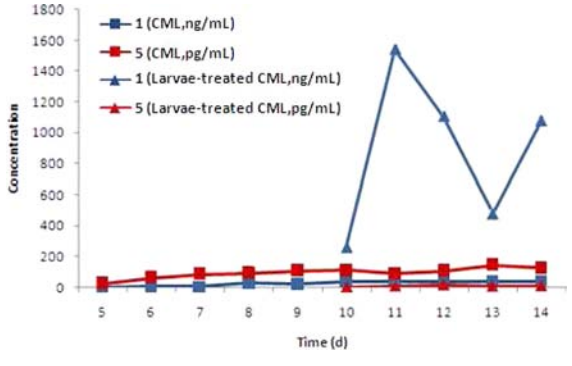

Figure 3. UPLC–MS/MS quantitative analysis of **1** and **5** in extracts of *P. cateniobliquus* in CML, and larva treated CML at the 10th day.

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revealed that the major metabolite, polyketide-derived compound **1**, showed significant growth retardation effect on *H. armigera* larvae after 48 h, while terpenoid-derived metabolite **5** promoted the growth of the larvae. This is the first time that direct evidence has been found that an entomopathogenic fungus could regulate the growth of insects by producing different types of compounds. These results suggest that insect growth might be under complex positive and negative control by entomopathogenic fungi. The accumulation of compound **1** in *P. cateniobliquus* once the fungus was exposed to *H. armigera* larvae suggested that compound **1** would be involved in the infective process of the entomopathogenic fungus *P. cateniobliquus*. Our study would help reveal the biological functions of secondary metabolites of this agriculturally important biological agent.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1**−**5** and 2D NMR spectra of compound **5** and table showing comparison of mean lengths and widths achieved by *H. armigera* second instars treated with compounds with those of controls (*t*-test). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Xu, L.; He, Z.; Xue, J.; Chen, X.; Wei, X. Resorcylic acid lactones from a *Paecilomyces* fungus. *J. Nat. Prod.* **2010**, *73*, 885−889.

(2) Putri, S. P.; Kinoshita, H.; Ihara, F.; Igarashi, Y.; Nihira, T. Farinomalein, a maleimide-bearing compound from the entomopathogenic fungus *Paecilomyces farinosus*. *J. Nat. Prod.* **2009**, *72*, 1544−1546.

(3) Otoguro, K.; Ui, H.; Ishiyama, A.; Arai, N.; Kobayashi, M.; Takahashi, Y.; Masuma, R.; Shiomi, K.; Yamada, H.; Omura, S. *In vitro* antimalarial activities of the microbial metabolites. *J. Antibiot.* **2003**, *56*, 322−323.

(4) Isaka, M.; Palasarn, S.; Lapanun, S.; Sriklung, K. Paecilodepsipeptide A, an antimalarial and antitumor cyclohexadepsipeptide from the insect pathogenic fungus *Paecilomyces cinnamomeus* BCC 9616. *J. Nat. Prod.* **2007**, *70*, 675−678.

(5) Petersen, F.; Fredenhagen, A.; Mett, H.; Lydon, N. B.; Delmendo, R.; Jenny, H.-B.; Peter, H. H. Paeciloquinones A, B, C, D, E and F: new potent inhibitors of protein tyrosine kinases produced by *Paecilomyces carneus*. *J. Antibiot.* **1995**, *48*, 191−198.

(6) Cheng, Y.; Schneider, B.; Riese, U.; Schubert, B.; Li, Z.; Hamburger, M. (+)-*N*-Deoxymilitarinone A, a neuritogenic pyridone alkaloid from the insect pathogenic fungus *Paecilomyces farinosus*. *J. Nat. Prod.* **2006**, *69*, 436−438.

(7) Lang, G.; Blunt, J. W.; Cummings, N. J.; Cole, A. L. J.; Munro, M. H. G. Paecilosetin, a new bioactive fungal metabolite from a New Zealand isolate of *Paecilomyces farinosus*. *J. Nat. Prod.* **2005**, *68*, 810−811.

(8) Niu, X.-M.; Wang, Y.-L.; Xue, H.-X.; Li, N.; Wei, L.-X.; Mo, M.-H.; Zhang, K.-Q. Nematodetoxic aurovertin-type metabolites from a root-knot nematode parasitic fungus *Pochonia chlamydosporia*. *J. Agric. Food Chem.* **2010**, *58*, 828−834.

(9) Lammers, J. W.; MacLeod, A. Report of a pest risk analysis *Helicoverpa armigera* (Hübner, 1908). Plant protection service (NL) and central science laboratory (UK) joint pest risk analysis for *Helicoverpa armigera*. **2007**, Aug. (http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/helicoverpa.pdf).

(10) Shinde, S. S.; Kamtikar, V. N.; Muley, S.; Nimbalkar, R. K. LC50 for insecticides against second instar larvae of cotton bollworm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) in Maharashtra. *J. Ecobiotechnol.* **2011**, *3*, 22−24.

(11) Mortimer, M. R.; Mill, G. J. Susceptibility of larval and juvenile instars of the sand crab, *Portunus pelagicus* (L.), to sea water contaminated by chromium, nickel or copper. *Aust. J. Mar. Freshwater Res.* **1994**, *45*, 1107−1121.

(12) Brødsgaard, H. F. Insecticide resistance in European and African strains of western flower thrips tested in a new residue-on-glass test. *J. Econ. Entomol.* **1994**, *87*, 1141−1146.

(13) Bhakuni, R. S.; Shukla, Y. N.; Tripathi, A. K.; Prajapati, V.; Kumar, S. Insect growth inhibitor activity of arjunolic acid isolated from *Cornus capitata*. *Phytother. Res.* **2002**, *16*, 68−70.

(14) Krasnoff, S.; Gupta, S. Identification of the antibiotic phomalactone from the entomopathogenic fungus *Hirsutella thompsonii* var. *synnematosa*. *J. Chem. Ecol.* **1994**, *20*, 293−302.

(15) Schneider, A.; Spath, J.; Breiding-Mack, S.; Zeeck, A. New cineromycins and musacins obtained by metabolite pattern analysis of *Streptomyces griseoviridis* (FH-S 1832). *J. Antibiot.* **1996**, *49*, 438−446.

(16) Cutler, H. G.; Jacyno, J. M.; Phillips, R. S.; VonTersch, R. L.; Cole, P. D.; Montemurro, N. Cyclonerodiol from a novel source, *Trichoderma koningii*: plant growth regulatory activity. *Agric. Biol. Chem.* **1991**, *55*, 243−244.

(17) Kim, J.; Choi, G.; Park, J.; Kim, H.; Cho, K. Activity against plant pathogenic fungi of phomalactone isolated from *Nigrospora sphaerica*. *Pest Manage. Sci.* **2001**, *57*, 554−559.

(18) Reissig, W. H.; Stanley, B. H.; Hebding, H. E. Azinphosmethyl resistance ad weight-related response of oliquebanded leafroller larvae to insecticides. *J. Econ. Entomol.* **1986**, *79*, 329−333.