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Metabolites from Carnivorous Fungus *Arthrobotrys entomopaga* and Their Functional Roles in Fungal Predatory Ability

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Supporting Information

ABSTRACT: The carnivorous fungus *Arthrobotrys entomopaga* (Drechsler) can develop adhesive knobs to capture nematodes. Chemical study on the culture medium of *A. entomopaga* producing adhesive knobs led to isolation of six trace amounts of metabolites, including two new metabolites, paganins A and B (**1** and **2**), blumenol A (**3**), talathermophilins A and B (**4** and **5**), and cyclo(glycyltryptophyl) (**6**). Compounds **3**−**6** were reported for the first time from carnivorous fungi. Compounds **1** and **2** promoted the formation of the predatory adhesive knobs with an increasing rate up to 118% at a concentration of 50 μM but showed moderate inhibitory activity at a concentration of 5 μM. Moreover, compounds **1** and **2** displayed strong inhibitory activities toward the formation of *A. entomopaga* conidiophores with inhibitory rates of 40−75%. Growth experiments suggested that compounds **1** and **2** could be involved in the regulation of the fungal predatory and reproductive abilities. Nematode chemotaxis bioassay indicated that compounds **1** and **3** displayed strong nematode-attracting abilities. These findings provided a new type of regulatory metabolite and support for the hypothesis that predators often evolve to respond to their metazoan prey.

KEYWORDS: *carnivorous fungus, Arthrobotrys entomopaga, paganins*

INTRODUCTION

The fungi belonging to the genus *Arthrobotrys* show a distinguishing feature under malnutrition, predatism, which is the ability to capture nematodes with the use of trapping devices.[1,2] They have been studied worldwide for their potential as biocontrol agents for their unique predatory habits.[3] *Arthrobotrys entomopaga* Drechsler is known as the only species in the genus *Arthrobotrys* that captures springtails in the order Collembola. This carnivorous fungus can develop adhesive knobs that are held aloft by a stalk-like basal cell, which has been postulated to contribute to the predatory activity of this organism.[4] However, studies with regard to secondary metabolites from this fungus have never been reported and discussed. On the basis of chemo-ecological considerations, we recently embarked on the investigation of entomopathogenic deuteromycetes as a source for new biological active secondary metabolites, which could be involved in the interaction between fungi and host. Our previous studies revealed that the production of secondary metabolites was a strategy used by entomopathogenic fungi to not only regulate, infect, or kill the host[5−7] but also influence the defensive morphological development of the producing organism.[8] The evaluation and commercial development of natural chemicals from these fungi have attracted considerable interest.[5−8]

Here, we reported the isolation of the secondary metabolites from the adhesive knob producing *A. entomopaga* CBS 642.8 and the evaluation of their possible ecological roles in the infection of nematodes.

MATERIALS AND METHODS

General Experimental Procedures. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography. Column chromatography was performed on 200−300 mesh silica gel (Qingdao Marine Chemical Factory, China). Optical rotations were measured on a Horiba-SEAP-300 spectropolarimeter. UV spectral data were obtained on a Shimadzu-210A double-beam spectrophotometer. IR spectra were recorded on a Bruker-Tensor-27 spectrometer with KBr pellets. NMR experiments were carried out on either a Bruker AV-400 or a DRX-500 spectrometer with TMS as internal standard. MS were recorded on a VG-Auto-Spec-3000 spectrometer. High-resolution ESIMS data were measured on a Bruker Bio-TOF III electrospray ionization mass spectrometer. X-ray diffraction was realized on a Bruker APEX DUO crystallography system. Compounds on plates were detected by spraying with 20% (w/v) H_2SO_4 and heating on a hot plate.

Strain and Cultivation. The strain of *A. entomopaga* CBS 642.8 was isolated from soil in Gejiu, Yunnan, in the People's Republic of China, in 2004, and identified as *A. entomopaga* by the morphological features of the conidiophores and the submerged hyphae and rates of growth. The adhesive knob, ovoid or ellipsoid in shape, was aloft on a stalk-like basal cell tapering upward. A portion of the adhesive knob was surrounded by a droplet of colorless, mucilaginous material. The group of adhesive knobs arose primarily only from a web-shaped network of hyphae, in which each mesh of the net was mainly composed of straight-growing short hyphal branches and branches that changed their direction abruptly to anastomose with other branches at angles approaching a right angle. In older cultures the network grew bigger and was composed of procumbent adhesive knobs and stalks, in addition to the branches of hyphae. The isolates were all deposited in the strain collection of the Laboratory for Conservation and Utilization of Bioresources and the Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University. After the conidia had

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Table 1. NMR Spectroscopic Data for Compounds 1 and 2 (400 MHz)

	1[a]		2[b]	
position	δ_C	δ_H mult (J in Hz)	δ_C	δ_H mult (J in Hz)
1	123.1, C		125.0, C	
2	156.0, C		156.7, C	
3	112.6, CH	6.87 d (1.5)	113.9, CH	6.76 brs
4	147.3, C		144.4, C	
5	117.2, CH	6.72 dd (7.6, 1.5)	118.7, CH	6.70 d (7.7)
6	131.2, CH	6.98 d (7.8)	131.9, CH	6.99 d (7.7)
1-Me	16.0, CH_3	2.15 s	16.3, CH_3	2.15 s
1′	69.8, CH	4.71 m	76.7, CH	4.60 q (6.4)
2′	26.3, CH_3	1.33 d (6.8)	22.7, CH_3	1.40 d (6.4)
1″			100.9, CH	4.87 brs
2″			73.1, CH	3.82 brs
3″			72.9, CH	3.63 dd (9.4, 3.4)
4″			74.5, CH	3.31 s
5″			70.4, CH	3.37 m
6″			18.0, CH_3	1.03 d (6.0)
2-OH		8.15 brs		
1′-OH		4.07 brs		

[a]Recorded in acetone-d_6. [b]Recorded in CD_3OD.

developed on PDA slants in test tubes at 25 °C, the strains were kept at −30 °C as stock cultures. A characteristic fragment (GenBank, accession no. AY965758) was amplified by PCR using the species-specific primers ITS4 and ITS5 and the genome DNA of CBS 642.8 as template.

Extraction and Isolation. The strain *A. entomopaga* CBS 642.8 cultured on a PDA medium at 28 °C for 7 days was inoculated into 500 mL flasks each containing 250 mL of production medium consisting of potato (peel off) 20% and glucose 2%. The pH of the medium was natural. The inoculated flasks were cultured on a rotary shaker (180 rpm) at 28 °C for 14 days. Eighty liters of fermentation broth of strain CBS 642.8 was filtered to separate the mycelia from the culture. The culture filtrate was concentrated in vacuo and partitioned with ethyl acetate (1000 mL × 5), and the organic fraction was evaporated to dryness to give 7 g of residue.

The EtOAc extract was fractionated by silica gel columns using $CHCl_3$/MeOH gradient elution to yield five fractions (A−E) according to TLC analysis. Fraction B (700 mg) was subjected to Sephadex LH-20 washing with methanol to yield four subfractions (B1−B4). Subfraction B3 (200 mg) was loaded onto a silica gel column using $CHCl_3$/Me_2CO (20:1) to give **1** (2.5 mg). Subfraction B4 was loaded onto a Sephadex LH-20 gel column with methanol to yield four fragments (B4a−B4d). Fragment B4a was further fractionated by a Sephadex LH-20 gel column washing with acetone to yield three subfragments (B4a1−B4a3). Subfragment B4a2 was further chromatographed on MPLC eluting with petroleum ether/Me_2CO (6:1) to give **6** (2.0 mg). Subfragment B4b was further subjected to MPLC eluting with $CHCl_3$/MeOH (80:1) to yield two subfragments (B4b1 and B4b2). Subfragment B4c1 was loaded onto a Sephadex LH-20 gel column with acetone to yield **5** (4.0 mg). Fraction C (550 mg) obtained on elution with $CHCl_3$/MeOH (10:1) was subjected to a Sephadex LH-20 gel column with $CHCl_3$/MeOH (1:1) as eluent to yield three subfractions (C1−C3). Subfraction C3 was further chromatographed on a Sephadex LH-20 gel column with acetone as eluent to yield three fragments (C3a−C3c). Fragment C3a was chromatographed on MPLC with $CHCl_3$/MeOH (10:1) as eluent to yield **2** (2.0 mg). Fraction D (230 mg) was chromatographed over a Sephadex LH-20 column eluting with methanol to yield three subfractions (D1−D3). Subfraction D2 was further loaded onto MPLC eluting with $CHCl_3$/isopropanol (24:1) to give two fragments (D2a and D2b). Fragment D2b was subjected to a Sephadex LH-20 gel column with acetone as eluent to yield **4** (2.0 mg).

The strain *A. entomopaga* CBS 642.8 cultured on a PDA medium at 28 °C for 7 days was inoculated into 500 mL flasks each containing 250 mL of YGP culture medium (0.5% yeast extract, 1% glucose, 0.1% peptone). The inoculated flasks were cultured on a rotary shaker (180 rpm) at 28 °C for 14 days. A 55 L portion of fermentation broth of strain CBS 642.8 was filtered to separate mycelia from the culture. The culture filtrate was concentrated in vacuo and partitioned with ethyl acetate (1000 mL × 6). The EtOAc extract was evaporated to dryness under vacuum to afford the crude extract (4.7 g). This gum was loaded onto a silica gel column using $CHCl_3$/Me_2CO and $CHCl_3$/MeOH gradient elution to yield six fractions. Fraction B (150 mg) was subjected to MPLC washing with $CHCl_3$/isopropano (7:1) to yield three subfractions (B1−B3). Subfraction B2 (8.0 mg) was further subjected to MPLC eluting with $CHCl_3$/Me_2CO (4:1) to yield **3** (2.0 mg).

Two hundred microliters of 2 M HCl and 2.5 mg of compound **2** were put in a vial (CEM Discover) and dissolved in 200 μL of MeOH. The vial was heated under microwave irradiation (CEM Discover) for 10 min. The reaction was cooled with 2 mL of water and partitioned with $CHCl_3$ (3 mL × 3), and the organic fraction was evaporated to dryness to give 1 mg of compound **1**.

*Paganin A (**1**):* colorless oil; $[\alpha]_D^{23.5}$ +23.51° (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 206.0 (3.96), 215.8 (3.88), 276.6 (3.38); IR (KBr) ν_{max} 3456, 3240, 3027, 2976, 2929, 2863, 1621, 1594, 1527, 1455, 1428, 1395, 1371, 1330, 1306, 1260, 1194, 1163, 1119, 1086, 1075, 1012, 994, 943, 872, 817, 756, 724, 689, 642, 610, 569, 540, 480 cm $^{-1}$; ^{1}H NMR and ^{13}C NMR, see Table 1; negative HRESI-MS *m*/*z* 151.0759 $[M - H]^-$, calculated for $C_9H_{11}O_2$. Crystal data: $C_9H_{12}O_2$, M = 152.19, monoclinic, a = 8.47140(10) Å, b = 5.92450(10) Å, c = 8.51030(10) Å, α = 90.00°, β = 110.3010(10)°, γ = 90.00°, V = 400.590(9) Å^3, T = 100(2) K, space group $P21$, Z = 2, μ(Cu Kα) = 0.711 mm^{-1}, 3598 reflections measured, 1221 independent reflections (R_{int} = 0.0247). The final R_1 value was 0.0277 ($I > 2\sigma(I)$). The final $wR(F^2)$ value was 0.0733 ($I > 2\sigma(I)$). The final R_1 value was 0.0282 (all data). The final $wR(F^2)$ value was 0.0738 (all data). The goodness of fit on F^2 was 1.110. Flack parameter = −0.1(2). The Hooft parameter is 0.05(8) for 420 Bijvoet pairs.

*Paganin B (**2**):* colorless oil; $[\alpha]_D^{23.5}$ −30.83° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 206.8 (3.98), 215.8 (3.90), 276.6 (3.48); IR (KBr) ν_{max} 3406, 2973, 2929, 1721, 1622, 1593, 1520, 1453, 1423, 1384, 1281, 1261, 1168, 1124, 1097, 1070, 1048, 1014, 981, 938, 912, 878, 837, 809, 742, 653, 640, 579, 546, 476 cm $^{-1}$; ^{1}H NMR and ^{13}C NMR, see Table 1; negative HRESI-MS *m*/*z* 297.1338 $[M - H]^-$, calculated as 297.1338 for $C_{15}H_{21}O_6$; positive HRESI-MS *m*/*z* $[M + Na]^+$, calculated for $C_{15}H_{22}O_6Na$.

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Blumenol A (3): colorless powder; the ^{1}H NMR and ^{13}C NMR spectroscopic data were consistent with those of blumenol A reported in the literature.[9]

Talathermophilin A (4): yellow solid; the ^{1}H NMR and ^{13}C NMR spectroscopic data were consistent with those of talathermophilin A reported in the literature.[10]

Talathermophilin B (5): yellow solid; the ^{1}H NMR and ^{13}C NMR spectroscopic data were consistent with those of talathermophilin B reported in the literature.[10]

Cyclo(glycyltryptophyl) (6): colorless powder; the ^{1}H NMR and ^{13}C NMR spectroscopic data were consistent with those of cyclo(valyltryptophyl) reported in the literature.[11]

Nematode Chemotaxis Bioassay. In nature, the nematode larval form that participates in the transmigration is the dispersal fourth stage larva. The *Caenorhabditis elegnns* individuals were treated with 1% NaOCl solution to obtain a synchronous population, and synchronized populations of selected larval stages (L4) were washed twice with M9 buffer (KH_2PO_4, 3 g; Na_2HPO_4, 6 g; NaCl, 5 g; H_2O to 1 L) by centrifugation and prepared for the test.

The nematode-attracting ability (AA) of the tested compounds was determined as described in the literature.[12−14] The preference of *C. elegans* for samples was measured in a binary- or multiple-choice assay. In a binary-choice assay, animals migrate toward one of two ends on opposite sides of T-glass tubing containing 9 mL of 1.5% agar as described in the literature.[14] The tested compound was absorbed with a small piece of filter paper (6 mm × 4 mm) to allow gradual vaporization of sample during the test. The filter with the absorbed sample was placed on one side of the agar in the T-tubing (sample side). The nematodes (ca. 300 nematodes) were placed in the middle of the T-tubing, and excess water was removed with a small cotton ball. After airing for different time intervals in a dark chamber at 26 °C, the nematodes, which had moved away from the center to either side of the T-tubing, were collected individually with a pipet from each side and counted under a microscope (Olympus CX-37, Tokyo, Japan; 40×). In a multiple-choice assay, preferences were tested in a partially enclosed six-arm maze made from polydimethyl siloxane (PDMS) elastomer resting on the surface of an agar plate. A decision area in the center of the maze was connected by slender channels to six small chambers, each containing one concentration of the sample. Animals were placed in the open decision area and approached chambers through the channels. The attracting activity was determined with the following equation.

$$\mathrm{AA}\ (\%) = \frac{(N_s - N_c)}{N_c} \times 100\%$$

where N_s is the number of the nematodes on the sample side and N_c that on the control side.[14] This experiment was conducted twice with three replicates each.

Fungal Growth Bioassay. The fungal growth bioassay for the tested compounds was determined as described in the literature.[8,15,16] *A. entomopaga* CBS 642.8 was maintained on potato dextrose agar (PDA) slants and transferred every 3 months. To prepare spores for the experiments, *A. entomopaga* was cultured at 28 °C on PDA medium for 2 weeks. Five milliliters of sterile water was used to harvest *A. entomopaga* from 2-week-old cultures on PDA. The spores were washed three times with sterile distilled water before use. A 5 mm diameter disk of *A. entomopaga* was placed on PDA medium in a 9 cm Petri dish and incubated in darkness at a constant temperature of 28 °C for a week. For the bioassays, fungal spore suspensions were prepared by washing the cultures with 5 mL of sterile water. The suspension was concentrated to 1 mL by centrifuge at 6000 rpm. An aqueous suspension (2×10^5 spores per milliliter) was prepared for inoculation. Test samples were dissolved in 500 μL of acetone, respectively, and then diluted with sterilized water to prepare a stock solution 500 μg/mL. The same amount of acetone dissolved in water was established as control. One hundred microliters of sample solution prepared by the stock solution was added to a clean 6 cm Petri dish, which contained 5 mL of autoclaved agar medium (2 g of agar water, 100 mL). One hundred microliters of the fungal spore suspension was then transferred to each Petri dish and gently mixed. The final concentrations of tested sample solutions were 5, 25, and 50 μM. All dishes were incubated at 28 °C for a week. The germination of spores was assessed at 6, 8, 12, and 32 h exposure periods using a normal binocular microscope. At each time point during the experiment, the percentage of spore germination was determined microscopically by counting 100 spores from triplicate dishes. After we confirmed that >95% of the spores converted into hyphal form, the differentiation assay was performed and the bioassay of the morphological transitions to conidiophores and adhesive knobs was assessed. The percentage of hypha to conidiophores and adhesive knobs was counted using a normal binocular microscope, and three visions in each Petri dish were picked at random for counting. All of the tests were repeated three times, and morphology results were quantified as the numbers of germ tubes, conidiophores, and adhesive knobs observed (mean % ± SD) after a specified time period.[8]

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

RESULTS AND DISCUSSION

Compound **1** was isolated as a colorless oil. It exhibited a quasi-molecular ion peak at *m/z* 151 $[M - H]^-$ in the negative ESIMS and was assigned to a molecular formula of $C_9H_{11}O_2$, which was confirmed by HRESIMS and NMR spectrometric data (Table 1). The ^{1}H NMR spectrum of **1** (Table 1) displayed one methyl attached to a double bond at δ_H 2.15 (3H, s), one secondary methyl at δ_H 1.33 (3H, d, J = 6.8 Hz), and three olefinic protons accounting for a 1,2,4-trisubstituted phenyl ring at δ_H 6.72 (1H, dd, J = 7.6, 1.5 Hz), 6.87 (1H, d, J = 1.5 Hz), and 6.98 (1H, d, J = 7.8 Hz). Another three protons at δ_H 4.07 (1H, d, J = 3.8 Hz), 4.71 (1H, m), and 8.15 (1H, s) were also observed. The ^{13}C NMR spectrum (Table 1) of **1** demonstrated nine carbon resonances for one tertiary methyl group, one secondary methyl group, one oxymethine, three olefinic methines, and three olefinic quaternary carbons. The HSQC spectrum of **1** enabled the assignment of all the carbons linked with the protons except for the two protons at δ_H 4.07 (1H, d, J = 3.8 Hz) and 8.15 (1H, s), indicating that there were two hydroxyl groups in **1**. The connectivities of these partial groups and the positions of the substituents were determined by ^{1}H−^{13}C long-range correlations detected in the HMBC spectrum. The correlations of the methyl at δ_H 2.15 (3H, s) with the carbons at δ_C 156.0 (s), 123.1 (s), and 131.2 (d) and of the proton for one hydroxyl group at δ_H 8.15 (1H, s) with the carbon at δ_C 156.0 (s) (Figure 2) suggested that the methyl group and one hydroxyl group were attached to C-1 and C-2 of the phenyl ring, respectively. The ^{1}H−^{13}C long-range cross-link of the proton at δ_H 4.07 (1H, d, J = 3.8 Hz) for the second hydroxyl group with the oxygenated methine carbon at δ_C 69.8 (d) and of the corresponding proton at δ_H 4.71 with the carbons at δ_C 147.3 (s), 117.2 (d), 112.6 (d), and 26.3 (q) in the HMBC spectrum indicated that one 1-hydroxyethyl moiety was attached to C-4 of the phenyl ring. Thus, compound **1** was characterized as 5-(1-hydroxyethyl)-2-methylphenol. Detailed analysis revealed that the optical rotation value of compound **1** was positive and consistent with those compounds which share similar stereocenters, such as (*R*)-1-(4-isobutylphenyl)ethanol from a marine sponge *Phycopszs* sp. collected from the Tuticorin coast, India,[17] (*R*)-2-hydroxy-6-methylphenyl)-ethanol from leaves of *Juniperus occidentalis* collected in Oregon, USA,[18] and (*R*)-1-(4-methylphenyl)ethanol and (*R*)-1-(4-hydroxyphenyl)ethanol.[19] Thus, **1** was determined to have the same absolute *R* configuration as those of the above three

compounds. A single crystal of **1** was obtained after several recrystallizations. X-ray crystallographic analysis using anomalous dispersion with copper radiation was performed (CCDC 931794), confirming the NMR spectroscopic structure elucidation and the absolute stereochemistry of C-1′ as *R* in **1** (Figure 3). All of the physicochemical data are in full agreement with the proposed structure of **1** as described (Figure 1), given trivial name paganin A.

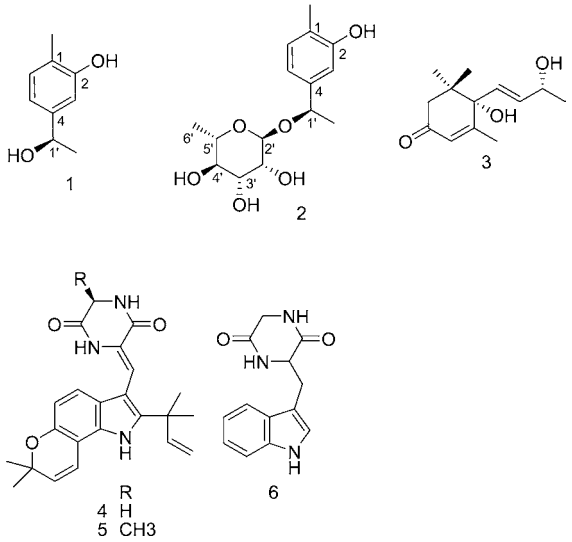

Figure 1. Compounds **1**−**6** from *A. entomopaga*.

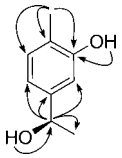

Figure 2. Key $^{1}H-^{13}C$ long-range correlations of **1**.

Compound **2** was isolated as a colorless solid. It exhibited a quasi-molecular ion peak at *m*/*z* 297 $[M - H]^-$ in the negative ESIMS and was assigned to a molecular formula of $C_{15}H_{21}O_6$, which was confirmed by HRESIMS and NMR spectrometric data (Table 1). Comparison of the NMR data of **2** with those of **1** revealed that the two compounds were alike except for the appearance of one additional sugar in **2**. All the above data suggested that **1** was an aglycone of **2**. The sugar signals in the ^{13}C NMR of **2** at δ_C 100.9 (d), 74.5 (d), 73.1 (d), 72.9 (d), 70.4 (d), and 18.0 (q) are comparable with those reported for an α-6-deoxy-L-mannopyranosyl moiety.[20−23] The outstanding change of the C-1′ signal from δ_C 69.8 (d) in **1** to δ_C 76.7 (d) in **2** gave a hint that the sugar moiety was attached to C-1′. This was further confirmed by the $^{1}H-^{13}C$ long-range correlation between the anomeric proton at δ_H 4.87 of the sugar and C-1′ at δ_C 76.7 (d) in the HMBC spectrum. Acid hydrolysis of **2** with 2 M HCl afforded an α-6-deoxy-L-mannopyranosyl moiety and **1**. Accordingly, **2** was established as (*R*)-5-(1-(α-6-deoxy-L-mannopyranosyl)ethyl)-2-methylphenol in Figure 1 and named paganin B.

Compound **3** was elucidated as blumenol A on the basis of the NMR data and comparison with the data reported in the literature.[9] Blumenol A is a major secondary metabolite initially isolated from the leaves of *Annona glabra* L. (Annonaceae), commonly known as pond apple, which is a tropical tree distributed mainly in the Americas and in Southeast Asia, and used in traditional medicine as an insecticide and a parasiticide. Compound **3** was reported to display inhibitory activity against a panel of six human solid tumor cell lines.[9] However, studies with regard to the compound from a fungus and its biological activity related to nematode has not been reported and discussed.

Compounds **4**−**6** were elucidated as talathermophilins A and B (**4** and **5**) and cyclo(glycyltryptophyl) (**6**) on the basis of the NMR data and comparison with the data reported in the literature.[10,11] Previous study indicated that these compounds were obtained from the thermophilic fungus *Talaromyces thermophilus* and did not show any inhibitory activity toward nematodes. Compounds **4**−**6** were reported for the first time from carnivorous fungi.

Nematode chemotaxis bioassay for **1**−**3** was carried out according to the literature.[12−14] Compound **1** showed strong nematode-attracting activity at doses of 0.2−0.5 mg within 8 h, with the highest attracting activity of 275% (Figure 4). At a dose of 0.5 mg within 4 h, compound **3** was observed to show the highest attracting activity of 306% (Figure 5). Compound **2**

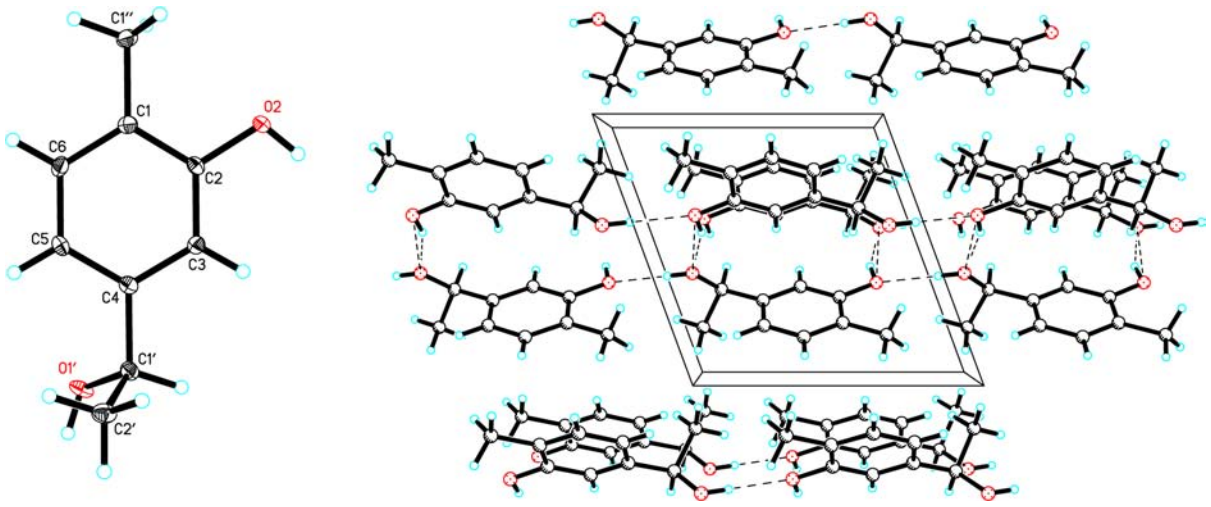

Figure 3. Single X-ray crystallographic structure of paganin A (**1**).

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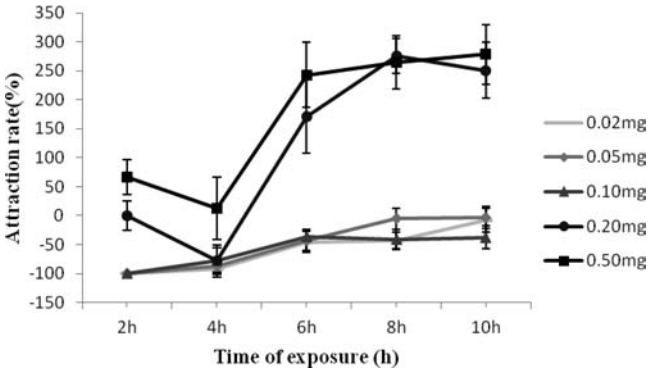

Figure 4. Attracting activity of compound **1** for the L4 larvae of nematode *C. elegans*.

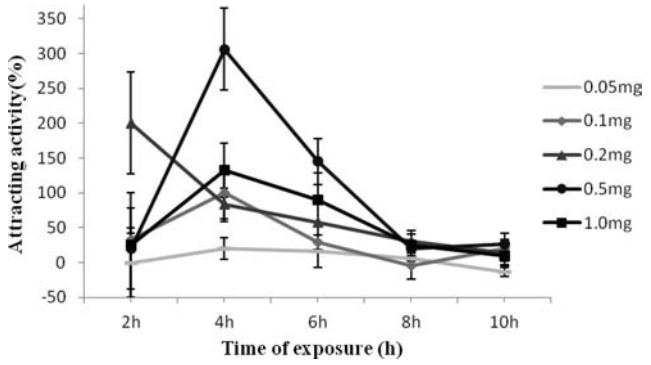

Figure 5. Attracting activity of compound **3** for the L4 larvae of nematode *C. elegans*.

did not show any attracting activity. The result indicated that compounds **1** and **3** could be involved in subverting the nematode olfactory chemotaxis system to make the carnivorous fungus attain access to its host. Compounds **1** and **3** thus serve as fungus-associated molecular patterns that might be recognized by nematodes and perhaps other insects.

The effects of paganins on spore germination and the formation of adhesive knobs and conidiophores of *A. entomopaga* were evaluated according to the literature.[8,15,16] Compounds **1** and **2** showed strong inhibitory activities toward the formation of fungal conidiophores with inhibitory rates of 65−75% at a concentration of 25 μM (Table 2; Table S1 in the Supporting Information). In the morphological bioassay of hypha to predatory adhesive knobs, it was interesting to note that the addition of compounds **1** and **2** to *A. entomopaga* generally stimulated the formation of adhesive knobs at a high concentration of 50 μM, whereas both compounds showed negative effects on the formation of knobs at a low concentration of 5 μM. Compound **1** demonstrated the strongest promoting activity with an increasing rate up to 118% at a concentration of 50 μM, whereas compound **2** displayed the highest inhibitory activity with an inhibiting rate up to 44% at a concentration of 5 μM. In addition, at a concentration of 50 μM, compound **1** displayed strong activity against spore germination of *A. entomopaga* with inhibitory rates of 55−75% within 12 h, and compound **2** showed inhibitory activity with rates of 40−45% within 8 h (Table 3; Table 2S in the Supporting Information). The concentrations of the tested compounds were within the ranges of physiological contents detected in the fungal fermention cultures of *A. entomopaga*. The evidence obtained here indicated that paganins produced by *A. entomopaga* were capable of regulating the fungal morphological transitions from hyphae growth form to adhesive knobs and conidiophores, respectively. This suggested that a carnivorous fungus could produce small molecular factors to regulate its reproductive and predatory abilities.

Table 3. Effect of Compounds 1 and 2 (50 μM) on the Spore Germination of *A. entomopaga*

	germination rates (mean % ± SD[a])			
	6 h	8 h	12 h	32 h
control[b]	8 ± 1	29 ± 3	44 ± 4	72 ± 6
1	2 ± 1**	9 ± 2*	21 ± 3**	60 ± 3*
2	4 ± 2	15 ± 4	43 ± 5	71 ± 4

[a]SD, standard deviation. *, significantly different ($P < 0.05$); **, significantly different ($P < 0.01$). [b]For the control, no compounds was added.

Compounds **1**−**3** were evaluated in our general bioactivity profiling programs, for example, antimicrobial and nematicidal testing.[5] None of compounds showed any antibacterial activity against *Bacillus subtilis* or nematicidal toxicity toward the individuals of the free-living nematode *Panagrellus redivevus* at concentrations reaching 400 μg/mL.

On the basis of these results, we propose that a carnivorous fungus emits volatile compounds that attract nematodes and

Table 2. Effect of Compounds 1 and 2 on the Morphology of *A. entomopaga*

		morphology results (mean % ± SD[a]) with			
		conidiophores		adhesive knobs	
	concn (μM)	56 h	64 h	96 h	102 h
control[b]	0	55 ± 11	74 ± 4	43 ± 4	80 ± 15
1	5	34 ± 8	54 ± 9*	32 ± 6*	62 ± 5
	25	14 ± 4*	29 ± 4***	65 ± 4*	78 ± 10
	50	26 ± 7	38 ± 5**	94 ± 2**	113 ± 19
2	5	51 ± 8	49 ± 5**	24 ± 3*	70 ± 8
	25	15 ± 4*	44 ± 6**	66 ± 4**	86 ± 14
	50	41 ± 7	59 ± 7	71 ± 12*	122 ± 12*

[a]SD, standard deviation. *, significantly different ($P < 0.05$); **, significantly different ($P < 0.01$); ***, significantly different ($P < 0.001$). [b]For the control, no compounds was added.

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produces morphological regulatory compounds to stimulate the formation of the predatory devices. That is, the fungus has subverted the nematode olfactory chemotaxis system to attain access to the nematodes and then used the trap device to eventually kill the hosts.[13] The mechanism action of these fungal metabolites in the fungal biology in our study needs further study.

In conclusion, by chemical metabolite profiling, we have identified two new phenyl-type metabolites and four other known compounds from a large-scale fermentation of a carnivorous fungus *A. entomopaga* and solved their structures by 2D NMR spectroscopy. Biological evaluation of these compounds revealed that small molecular phenylethanol compounds displayed significant morphological regulatory activity and improved fungal attracting ability for nematode larvae. These findings have implications for developmental signaling by the fungus.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR, ^{13}C NMR, and 2D NMR spectra of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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