Discovery of Bacterial Fatty Acid Synthase Inhibitors from a *Phoma* Species as Antimicrobial Agents Using a New Antisense-Based Strategy[⊥]

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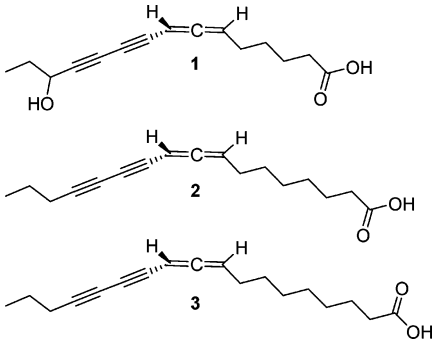
Fatty acids are essential for bacterial growth and viability, with the type II fatty acid synthesis (FAS II) pathway being a potential antibacterial target. A new, selective, and highly sensitive whole cell-based antisense strategy has been designed to screen for natural product inhibitors of FabH/F of the FAS II pathway using a high-throughput two-plate agar-based differential sensitivity assay ($FabF_2p$). An antisense assay along with the FASII enzyme prepared from *Staphylococcus aureus* was used for bioactivity-guided fractionation, leading to the isolation of phomallenic acids A−C (**1**−**3**) from a leaf litter fungus identified as *Phoma* sp. Compounds **1**−**3** exhibited minimum detection concentrations (MDC) of 0.63, 0.31, and 0.15 μg/mL in the $FabF_{2P}$ assay, IC_{50} values of 22, 3.4, and 0.77 μg/mL in the FASII enzyme assay, and minimum inhibitory concentrations (MIC) of 250, 7.8, and 3.9 μg/mL, respectively, against wild-type *S. aureus*. Phomallenic acid C (**3**), the analogue with the longest chain, exhibited the best overall activity within the phomallenic acids obtained and was superior to cerulenin and thiolactomycin, the two most studied and commonly used FabF inhibitors.

The emergence of drug resistance in the clinic is a major problem for all classes of antibiotics. The discovery of new antibiotics is urgently needed to overcome resistance. This may be best achieved through development of new methods/technologies to uncover novel mode of action leads that can be developed as antibacterial agents. The type II fatty acid synthesis (FAS II) pathway is essential for bacterial survival and has the potential to present novel targets for antibiotic discovery.[1−3] The bacterial FAS II is dissociated and individual enzymes are responsible for each reaction of the pathway, in stark contrast to human FAS, in which all reactions are conducted by a single multifunctional polypeptide.[4] Triclosan and isoniazid, two marketed narrow spectrum agents, target the FabI enzyme.[5,6] Two natural products, cerulenin and thiolactomycin, have been reported as inhibitors of the condensing enzymes, FabH and FabF/B.[7,8]

Antisense technology was recently used in *Staphylococcus aureus* for validation of essential targets. [9] We have applied this technology and developed a new high-throughput whole-cell agar-based screening method for the discovery of FabH/F inhibitors.[10] Screening of natural product extracts using this new assay led to the identification of a unique lead originating from a *Phoma* sp. strain, isolated from a leaf litter sample. Bioassay-guided fractionation of the extract led to the isolation of three new acetylenic allenes named phomallenic acids A−C (**1**−**3**). The isolation, structure elucidation, and biological activities of these compounds are reported herein.

Results and Discussion

Phomallenic acids A−C (**1**−**3**) were extracted from the fermentation broth of *Phoma* sp. (MF7018, CBS118751) with an equal volume of EtOAc. The organic phase was concentrated to give an oil that was chromatographed first on Sephadex LH-20 followed by preparative reversed-phase HPLC to afford compounds **1**−**3** (4−16 mg/L).



The ESIMS analysis of phomallenic acid A (**1**) produced a molecular ion at *m*/*z* 269 corresponding to $[M + Na]^+$. The ^{13}C NMR and ^1H NMR spectra of **1** displayed 15 carbon and 16 proton signals (Table 1), respectively, helping in the assignment of the empirical formula $C_{15}H_{18}O_3$. The presence of three methines (two olefinic type), five methylenes, and one methyl group were determined from the DEPT spectrum. The HMQC spectrum indicated that the two methines at δ_H 5.55 and 5.48 were bonded to carbons at δ_C 94.7 and 75.4, and the third methine proton (δ_H 4.3) was connected to an oxygen-bearing carbon (δ_C 64.0). The ^1H NMR spectrum showed a methyl triplet (δ_H 0.97, $J = 7.5$ Hz), indicating it to be a terminal methyl group. The IR spectrum exhibited absorption bands for an allene (ν_{max} 2229 cm^{-1}) and acetylene (ν_{max} 1946 cm^{-1}) with the characteristic UV of a conjugated bis-acetylenic allene at λ_{max} 238, 251, 265, and 281 nm. This structural group assignment was supported by the appearance of corresponding acetylenic allene chemical shifts in the ^{13}C NMR spectrum of **1** at δ_C 94.7 (C-6), 215.0 (C-7), 75.4 (C-8), 72.8 (C-9), 73.5 (C-10), 68.8 (C-11), and 84.4 (C-12).

Correlations from $^1H-^1H$ COSY, TOCSY, and HMBC NMR experiments (Figure 1) enabled the establishment of the final structure of **1**. The correlations from these experiments established the linkage of the carboxyl carbon to the allene separated by four methylene groups. The COSY correlations of the remaining methylene group to the methyl and the methine groups helped to connect them together. The oxymethine proton H-13 (δ_H 4.30) exhibited HMBC correlations to the acetylenic carbons at δ_C 84.4 (C-12) and 68.8 (C-13), thus linking the methine carbon to one

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Table 1. NMR Spectroscopic Assignments of Phomallenic Acids A−C (**1**−**3**) in CD_3CN

	1		**2**		**3**	
position	δ_C	δ_H, mult., *J* in Hz	δ_C	δ_H, mult., *J* in Hz	δ_C	δ_H, mult., *J* in Hz
1	175.2		175.1		175.2	
2	33.9	2.27, t, 7.5	34.2	2.25, t, 7.5	34.2	2.25, t, 7.5
3	24.9	1.57, m	25.5	1.55, m	25.6	1.55, m
4	28.7	1.48, m	30.0	1.30, m	29.6	1.30, m
5	28.2	2.08, dq, 6.5, 3.0	30.0	1.30, m	29.6	1.30, m
6	94.7	5.55, q, 6.5	29.4	1.30, m	29.6	1.30, m
7	215.0		28.5	2.05, dq, 6.5, 3.0	29.3	1.40, m
8	75.4	5.48, dtd, 6.5, 3.0, 1.0	94.6	5.52, q, 6.5	28.5	2.04, dq, 6.5, 3.0
9	72.8		215.1		94.7	5.52, q, 6.5
10	73.5		75.5	5.45, dtt, 6.5, 3.0, 1.0	215.0	
11	68.8		69.4		75.5	5.43, dtt, 6.5, 3.0, 1.0
12	84.4		75.0		69.4	
13	64.0	4.30, brt, 6.5	65.5		74.9	
14	31.4	1.65, m	85.0		65.6	
15	9.7	0.97, t, 7.5	21.6	2.30, brt, 7.0	85.1	
16			22.5	1.52, m	21.7	2.30, brt, 7.0
17			13.7	0.95, t, 7.5	22.4	1.52, m
18					13.6	0.95, t, 7.5

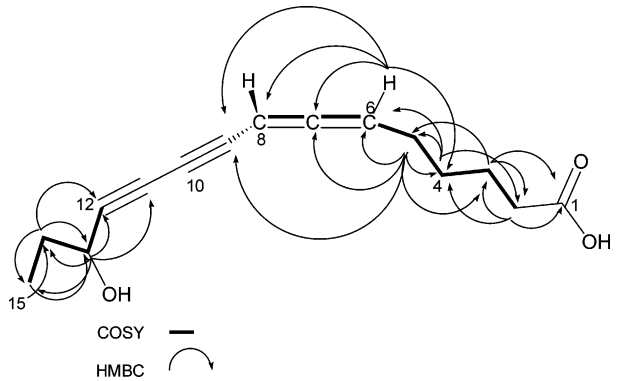

Figure 1. Key COSY and HMBC NMR correlations of phomallenic acid A (**1**).

side of the acetylene. The allene proton H-6 (δ_H 5.55) showed HMBC correlations to the acetylenic carbon at δ_C 72.8 (C-9) and methylene carbons C-4 and C-5 as well as the two allene carbons C-7 (δ_C 215.0) and C-8 (δ_C 75.4). These correlations placed the allene between acetylene C-9 and the methylene C-5 and established the complete structure of **1**. Surprisingly, the other allene methine H-8 (δ_H 5.48) did not show any HMBC correlations but did exhibit a COSY correlation to the methine H-6 (δ_H 5.55) as well as to methylene protons at C-5. These assignments were corroborated by TOCSY data that exhibited the contiguous correlations of H-8 to H-2.

The structures of phomallenic acids B and C (**2** and **3**) were elucidated analogously to the structure determinations of **1**. Compound **2** differs from **1** by the lack of the hydroxy group at C-13 and contains a pair of additional methylenes located between the allene and carboxyl group. Phomallenic acid C (**3**) contains an extra methylene carbon between the allene and carboxyl group as compared to **2**. Like many acetylenic allenes, these compounds are somewhat unstable to concentration, storage, and acidic pH but can be easily lyophilized to provide solvent-free compounds.

The absolute configuration of acetylenic allenes may be deduced from the sign of the optical rotation of the compound as it applies to Lowe's rule.[11] Compounds with a positive optical rotation (dextrorotatory) are assigned the *S* configuration, and those with a negative rotation (levorotatory) are assigned the *R* configuration. Since all three phomallenic acids (**1**−**3**) exhibited negative optical rotations, they were assigned as *R* and, hence, have the same configuration as certain other acetylenic allenes [e.g., antibiotic 07F275,[12] cepacin A,[13] and scorodonin[14]]. These known acetylenic allenes have been reported to exhibit antibacterial activities against

Table 2. Biological Activities of Phomallenic Acids A−C (**1**−**3**) and Cerulenin

assay	cerulenin	**1**	**2**	**3**
$FabF_2p$ (MDC, μg/mL)[a]	32−64	0.63	0.31	0.15
FASII (IC_{50}, μg/mL)	1.5	22	3.40	0.77
MIC (*S. aureus* MB2865, μg/mL)	64−128	250	12.5	3.90

[a] MDC (minimum drug concentration at which a zone formation was observed in the sensitive strain and a 5 mm zone differential was maintained against nonsensitized strain).

S. aureus with MIC values of 2−4, 0.2, and >50 μg/mL, respectively.[12−14]

Phomallenic acids A−C (**1**−**3**) inhibited the fatty acid pathway (FAS II)[2] with IC_{50}'s of 22, 3.4, and 0.77 μg/mL, respectively (Table 2). These compounds are significantly more sensitive (lower MDC) for inhibition of the sensitized strain compared to the wild-type strain. In the $FabF_{2P}$ assay these compounds are significantly more potent than cerulenin and thiolactomycin (data not shown).

Compounds **1**−**3** were also evaluated against a small panel of microorganisms and compared with thiolactomycin (Table 3). Phomallenic acid A (**1**) was the least effective (MIC 250 μg/mL) of the three, but its activity was comparable to thiolactomycin. Phomallenic acids B (**2**) and C (**3**) showed better activity and exhibited MIC values of 12.5 and 3.9 μg/mL against both wild-type *S. aureus* and methicillin-resistant *S. aureus* (MRSA), and their antibacterial activity was 5−32-fold better than cerulenin and thiolactomycin. These compounds did not show any activity against other Gram-positive (e.g., *Enterococcus faecalis*, *E. faecium*) or Gram-negative (e.g., *E. coli*) organisms. However, they did inhibit the growth of membrane-permeabilized *E. coli envA* (*lpxC*) with MIC values in the range 12.5−31.3 μg/mL, indicating that these compounds cannot penetrate the cell membrane of wild-type *E. coli*. The inhibition of bacterial cell growth and fatty acid biosynthesis correlated well, suggesting that the inhibition of bacterial growth is by the inhibition of fatty acid biosynthesis. Overall, it appears that chain length plays a role in the manifestation of the activity of these compounds. A longer chain appears to be better than a shorter chain (e.g., **3** versus **2**), and the polar groups may have significant detrimental effects (e.g., **2** versus **1**). Further details of the biological activities of **1**−**3** have been published.[10]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Beckman DU-70 spectrophotometer. IR spectra were recorded with a Perkin-Elmer Spectrum One FT-IR spectrophotometer. All NMR spectra were recorded with a Varian Unity 500 (^{1}H, 500 MHz; ^{13}C,

Table 3. MIC Values of Phomallenic Acids A−C and Thiolactomycin against Selected Microorganisms

compound	*S. aureus* MB2865	*S. aureus* COL (MRSA)	*E. faecalis* CL8516 (VS)[a]	*E. faecium* CL5791 (VR)[b]	*E. coli* WT[c]	*E. coli* envA
1	250	250	>250	>250	>250	31.3
2	12.5	12.5	>100	>100	>100	25.0
3	3.9	3.9	>100	>100	>100	12.5
thiolactomycin	64−128	64−128	>250	>250	>250	>250

[a] VS: vancomycin sensitive. [b]VR: vancomycin resistant. [c]WT: wild type.

125 MHz) spectrometer in CD_3CN. $^1H-^1H$ COSY, DEPT, TOCSY, HMQC, and HMBC spectra were measured using standard Varian pulse sequences. Chemical shifts are given in δ (ppm) with CD_3CN (^1H, 1.93 and ^{13}C, 1.39 ppm) as internal standard. LRMS data were recorded on an Agilent 1100 MSD with ESI ionization. HRMS were obtained with a Bruker 7T BioApex II FTMS. An Agilent HP1100 instrument was used for analytical HPLC.

Fungal Material. The producing organism (MF7018, CBS 118751) is a fungus that was isolated from a culture of a leaf litter sample collected in the Reunion Islands (France) using a particle-washing method previously described.[15] The fungus was identified as *Phoma* sp. (anamorphic Pleosporales, Ascomycetes) by morphological analysis.[16] The strain produced colonies on PDA (Difco) with moderate growth rate, mycelium greenish gray and texture floccose to velvety. Conidiomata consisted of brown pycnidia scattered throughout the colony surface. Conidia were ellipsoid to cylindrical in shape, hyaline, 4−6 × 2−3 μm in size. Sequence analysis of the internal transcribed spacers of the ribosomal DNA[17] indicated a 87% similarity with a number of GenBank sequences from isolates belonging to the genus *Lophiostoma* Ces. & De Not. (Pleosporales). The result from sequence analysis confirmed the morphological characterization but did not allow the identification of the producing strain at the species level.

Fermentation. A seed culture of *Phoma* sp. (MF7018) was prepared by inoculation of frozen mycelia agar plugs in a 250 mL Erlenmeyer flask containing 60 mL of KFA seed medium of the following composition (g/L): corn steep powder, 2.5; tomato paste, 40.0; oat flour, 10.0; glucose, 10.0; agar, 4.0; and trace elements solution, 10 mL (in g/L, $FeSO_4 \cdot H_2O$, 1.0; $MnSO_4 \cdot 4H_2O$, 1.0; $CuCl_2 \cdot 2H_2O$, 0.025; $CaC_{l2} \cdot 2H_2O$, 0.1; H_3BO_3, 0.056; $(NH_4)_6MoO_{24} \cdot 4H_2O$, 0.019; $ZnSO_4 \cdot 7H_2O$, 0.2). The pH was adjusted to 6.8 by addition of a solution of NaOH. The seed culture was incubated at 22 °C on a gyratory shaker (220 rpm) for 5−8 days, resulting in a pH of 5.8. The production medium (CYS80) was formulated as follows (g/L): sucrose, 80; corn meal (yellow), 50; and yeast extract, 1. No pH adjustment was made prior to inoculation. Fermentation flasks (500 mL unbaffled flasks containing 120 mL of medium) were inoculated with 4% vegetative seed growth and were incubated at 22 °C for 14 days at 70% humidity with shaking at 220 rpm. The final pH of the broth at harvest was 4.4.

Extraction and Isolation. One liter of the fermentation broth was extracted with 1 L of EtOAc and concentrated to dryness under reduced pressure to produce 1 g of an oil. This material was dissolved in MeOH (10 mL) and charged to a 550 mL Sephadex LH-20 column (2.54 × 500 cm), eluted with MeOH at 5 mL/min. Ten-milliliter fractions were collected, and the active compounds eluted in fractions 31−35. These fractions were pooled and concentrated to dryness, affording 132 mg of a gum, which was dissolved in 1.2 mL of MeOH and chromatographed on a reversed-phase HPLC (Atlantis 30 × 100 mm, 21 × 2500 mm), eluted at a flow rate of 10 mL/min with 50 min linear gradient of 25−80% aqueous acetonitrile. Lyophilization of fractions eluting at 21 min afforded phomallenic acid A (**1**, 16 mg/L), that at 34 min afforded phomallenic B (**2**, 4 mg/L), and that at 38 min afforded phomallenic C (**3**, 12 mg/L) as oils.

Phomallenic acid A (1): oil; $[\alpha]^{23}_D$ −170 (*c* 0.4, MeOH); UV (MeOH) λ_{max} 212 (22 878), 238 (3395), 251 (5928), 265 (8302), 281 (6540) nm; IR ν_{max} (ZnSe) 2932, 2860, 2229, 1946, 1710, 1457, 1407, 1231,1096 cm^{-1}; ^{13}C and ^1H NMR, see Table 1; ESIMS *m/z* 269 $[M + Na]^+$, 229 $[M - H_2O + H]^+$; HRAPCIMS *m/z* 493.2600 $[2M + H]^+$, [calcd for $2(C_{15}H_{18}O_3)$+H, 493.2585].

Phomallenic acid B (2): oil; $[\alpha]^{23}_D$ −140 (*c* 0.4, MeOH); UV (MeOH) λ_{max} 212 (23 555), 237 (4322), 250 (6787), 265 (9380), 280 (7742) nm; IR ν_{max} (ZnSe) 2931, 2857, 2229, 1946, 1710, 1457, 1409, 1275, 1091 cm^{-1}; ^{13}C and ^1H NMR, see Table 1; ESIMS *m/z* 276 $[M + NH_4]^+$, 259 $[M + H]^+$; HRAPCIMS *m/z* 517.3331 $[2M + H]^+$, [calcd for $2(C_{17}H_{22}O_2)$+H, 517.3313].

Phomallenic acid C (3): oil; $[\alpha]^{23}_D$ −160 (*c* 0.4 MeOH); UV (MeOH) λ_{max} 212 (26 394), 237 (5238), 250 (8544), 265 (12040), 280 (9945) nm; IR ν_{max} (ZnSe) 2928, 2855, 2229, 1946, 1710, 1457, 1412, 1277, 1091 cm^{-1}; ^{13}C and ^1H NMR, see Table 1; low-resolution ESIMS *m/z* 290 $[M + NH_4]^+$, 273 $[M + H]^+$; HRAPCIMS *m/z* 545.3641 $[2M + H]^+$, [calcd for $2(C_{18}H_{24}O_2)$+H, 545.3625].

FabF$_{2P}$ Assay. *Staphyloccus aureus* cells (RN450) carrying plasmid S1-1941 bearing antisense to FabF (*fabF* AS-RNA strain) or vector (control strain) were inoculated from a frozen vial source into a tube containing 3 mL of Miller's LB broth (Invitrogen) plus 34 μg/mL chloramphenicol. Tubes were incubated at 37 °C at 220 rpm for 18−20 h and kept at room temperature until use. Miller's LB broth was supplemented with 1.2% Select agar (Invitrogen), 0.2% glucose, 15 μg/mL chloramphenicol, and 50 mM xylose (only for the antisense strain). The OD_{600} of the culture was measured and 1:1000 of OD 3.0 inoculated. Next, 100 mL was poured into each NUNC plate, well-caster templates were placed into the agar, and the agar was allowed to solidify. Then, 20 μL of test samples was added to the wells, the plates were incubated at 37 °C for 18 h, and zones of inhibition were measured.[10] MDC (minimum detection concentration) values were determined by 2-fold serial dilution.

FAS II Assay. The assay was performed in a phospholipid-coated 96-well flash plate. Briefly, 1.26 μg of the partially purified protein from *S. aureus* containing all the required fatty acid synthesis enzymes was preincubated with a serial dilution of natural products at room temperature for 20 min in 50 μL of buffer containing 100 mM sodium phosphate (pH 7.0), 5 mM EDTA, 1 mM NADPH, 1 mM NADH, 150 μM DTT, 5 mM β-mercaptoethanol, 20 μM *n*-octanoyl-CoA (or lauroyl-CoA), 4% Me_2SO, and 5 μM ACP. The reaction was initiated by addition of 10 μL of water-diluted [2-^{14}C]-malonyl-CoA, giving a final concentration of 4 μM malonyl-CoA with total counts of about 10 000 cpm. The reaction was incubated at 37 °C for 90 min. The reaction was terminated by adding 100 μL of 14% perchloric acid. The plates were sealed and incubated at room temperature overnight and counted for 5 min using a Packard TopCount NXT scintillation counter. Further details of the assay are described in ref 2.

Antibiotic Assay (MIC). The MIC (minium inhibitory concentration) against each of the strains was determined as previously described.[2,10] Cells were inoculated at 10^5 colony-forming units/mL followed by incubation at 37 °C with a 2-fold serial dilution of compounds in the grwoth medium for 20 h. MIC is defined as the lowest concentration of antibiotic inhibiting visible growth.

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Supporting Information Available: ^1H NMR, ^{13}C NMR, and UV spectroscopic data of phomallenic acids A−C (**1**−**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Heath, R. J.; White, S. W.; Rock, C. O. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 695−703.
(2) Kodali, S.; Galgoci, A.; Young, K.; Painter, R.; Silver, L. L.; Herath, K. B.; Singh, S. B.; Cully, D.; Barrett J. F.; Schmatz, D.; Wang, J. *J. Biol. Chem.* **2005**, *280*, 1669−1677.
(3) Khandekar, S. S.; Daines, R. A.; Lonsdale, J. T. *Curr. Prog. Pept. Sci.* **2003**, *4*, 21−29.
(4) Heath, R. J.; White, S. W.; Rock, C. O. *Prog. Lipid Res.* **2001**, *40*, 467−497.
(5) Heath, R. J.; Yu, Y. T.; Shapiro, M. A.; Olson, E.; Rock, C. O. *J. Biol. Chem.* **1998**, *273*, 30316−30320.
(6) Banerjee, A.; Dubanu, E.; Quemard, A.; Balasubramanian, V.; Um, K.; Wilson, T.; Collins, D.; de Lisle, G.; Jacobs, W. J. *Science* **1994**, *263*, 227−230.

(7) Sato, Y.; Normura, S.; Kamio, Y.; Omura, S.; Hata, T. *J. Antibiot.* **1967**, *20*, 344−348.
(8) Noto, M.; Oishi, H.; Endo, H.; Okazaki, H. *J. Antibiot.* **1982**, *35*, 401−410.
(9) Forsyth, R. A.; Haselbeck, R. J.; Ohlsen, K. L.; Yamamoto, R. T.; Xu H.; Trawick, J. D.; Wall, D.; Wang, L.; Brown-Driver, V.; Froelich, J. M.; Kedar, K. G.; King, P.; McCarthy, M.; Malone, C.; Misiner, B.; Robbins, D.; Tan, Z.; Zhu, Z.-Y.; Carr, G.; Mosca, D. A.; Zamudio, C.; Foulkes, J. G.; Zyskind, J. W. *Mol. Microbiol.* **2002**, *53*, 1387−1400.
(10) Young, K.; Jayasuriya, H.; Ondeyka, J. G.; Herath, K.; Zhang, C.; Kodali, S.; Galgoci, A.; Painter, R.; Brown-Driver, V.; Yamamoto, R.; Silver, L. L.; Zheng, Y.; Ventura, J. I.; Sigmund, J.; Ha, S.; Basilio, A.; Vicente, F.; Tormo, J. R.; Pelaez, F.; Youngman, P.; Cully, D.; Barrett, J. F.; Schmatz, D. M.; Singh, S. B.; Wang, J. *Antimicrob. Agents Chemother.* **2006**, *50* (2), 519−526.
(11) Lowe, D. *J. Chem. Soc., Chem. Commun*. **1965**, 411−413.
(12) Schlingmann, G.; Milne, L.; Pearce, C. J.; Borders, D. B.; Greenstein, M.; Maiese, W. M.; Carter, G. T. *J. Antibiot.* **1995**, 48, 375−379.
(13) Parker, W. L.; Rathnum, M. L.; Seiner, V.; Trejo, W. H.; Principle, P. A.; Sykes, R. B. *J. Antibiot*. **1984**, *37*, 431−440
(14) Anke, T.; Kupka, J.; Schramm, G.; Steglich, W. *J. Antibiot.* **1980**, 33, 463−467.
(15) Bills, G. F.; Polishook, J. D. *Mycologia* **1994**, *86*, 187−198.
(16) Sutton, B. C. *The Coelomycetes*; Commonwealth Mycological Institute: Kew, UK, 1980.
(17) Arenal, F.; Platas, G.; Peláez, F. *Mycol. Res.* **2000**, *104*, 301−303.

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