Antifungal Metabolites from the Plant Endophytic Fungus *Pestalotiopsis foedan*

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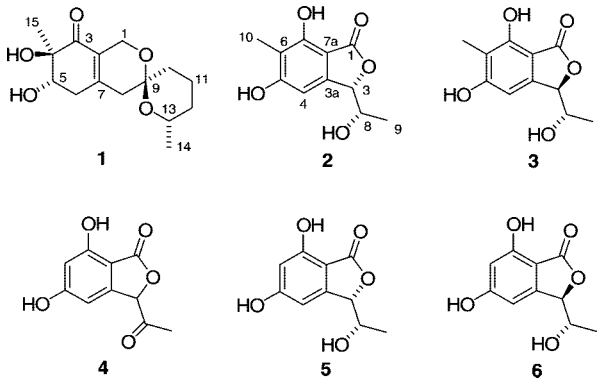
Pestafolide A (**1**), a new reduced spiro azaphilone derivative, and pestaphthalides A (**2**) and B (**3**), two new isobenzofuranones, have been isolated form solid cultures of an isolate of *Pestalotiopsis foedan*. The structures of these compounds were determined mainly by analysis of their NMR spectroscopic data. The relative configuration of **1**−**3** was assigned by analysis of ^{1}H NMR *J*-values and NOESY data, and the absolute configuration was determined by application of the CD excitation chirality and modified Mosher method. Compounds **1**−**3** showed modest antifungal activity.

Endophytic fungi that inhabit normal tissues of the host plants without causing apparent pathogenic symptoms have been demonstrated to be rich sources of bioactive natural products.[1–3] As one class of the most widely distributed endophytic fungi, *Pestalotiopsis* spp. are prolific producers of different types of bioactive metabolites.[4–12] During our ongoing chemical investigations of endophytic fungi as sources of new bioactive natural products, a subculture of an isolate of *Pestalotiopsis foedan* was grown in solid-substrate fermentation culture. Its organic solvent extract displayed antifungal activity against *Candida albicans* (ATCC 10231), *Geotrichum candidum* (AS2.498), and *Aspergillus fumigatus* (ATCC 10894). Bioassay-guided fractionation of this extract led to the isolation of one new reduced azaphilone derivative and two new isobenzofuranones that have been named pestafolide A (**1**) and pestaphthalides A (**2**) and B (**3**). Details of the isolation, structure elucidation, and stereochemical assignments of these compounds are presented here.



Results and Discussion

Pestafolide A (**1**) was assigned the molecular formula $C_{15}H_{22}O_5$ (five unsaturations) on the basis of HRESI analysis [*m*/*z* 305.1363 $(M + Na)^+$; Δ +0.4 mmu] and NMR data (Table 1). Analysis of the ^{1}H, ^{13}C, and HMQC NMR data for **1** revealed the presence of two methyl groups, six methylene units (one of which was oxygenated), two oxymethines, two oxygenated quaternary carbons, two olefinic carbons, and one ketone carbon (δ_C 199.6). These data accounted for all ^{1}H and ^{13}C resonances except two exchangeable protons and required compound **1** to be tricyclic. Analysis of the ^{1}H–^{1}H COSY NMR data led to the identification of two isolated proton spin-systems corresponding to the C-5–C-6 and C-10–C-14 subunits of structure **1**. HMBC correlations of the isolated methyl protons H_3-15 with the oxygenated quaternary carbon C-4, the oxymethine carbon C-5, and the ketone carbon C-3 indicated that C-4 was connected to C-3, C-5, and C-15. Correlations from H-5 to C-7 and from H-6 to C-2 and C-7 led to the connection of C-7 to both C-2 and C-6. Further HMBC correlations from H_2-1 to C-2 and C-3 revealed the connectivities of C-2 to both C-1 and the ketone carbon C-3, thereby completing the cyclohexenone subunit of **1**. HMBC correlations from H_2-1 to C-9 and from H-8 to C-2, C-6, C-7, and C-9 permitted completion of the dihydropyran moiety that was fused to the cyclohexenone unit at C2/C7. Correlations of H_2-10 with C-8 and of H_2-11 with C-9 established the connectivity between C-9 and C-10. Since no HMBC correlation was observed from H-13 to either C-4, C-5, or C-9, the location of the required ring remained to be determined. Treatment of pestafolide A (**1**) with acetic anhydride resulted in formation of a diacetate,[13] and the ^{1}H NMR spectrum of the product revealed two additional acetate methyl singlets at δ_H 2.01 and 2.11, respectively. The signal corresponding to H-5 (δ_H 4.00) was shifted downfield in the spectrum of the diacetate to δ_H 5.99, indicating that one hydroxy group was attached to C-5 in **1**. However, the remaining hydroxy

Table 1. NMR Spectroscopic Data for Pestafolide A (**1**) in $CDCl_3$

position	δ_H[a] (*J* in Hz)	δ_C[b], mult.	HMBC (H → C#)	NOESY[a]
1a	4.46, br, d (16)	57.2, CH_2	2, 7, 9	8b
1b	4.05, br, dd (16, 2.1)		3	13
2		127.4, qC		
3		199.3, qC		
4		77.3, qC		
5	4.00, dd (10, 5.8)	72.6, CH	3, 6, 7, 15	
6a	2.54, br, dd (18, 5.8)	36.1, CH_2	2, 5, 7, 8	8a
6b	2.38, br, d (18, 10)			8b, 15
7		150.7, qC		
8a	2.32, br, d (18)	41.3, CH_2	2, 6, 7, 9	6a, 10a, 10b
8b	2.20, br, d (18)			10b
9		95.2, qC		
10a	1.69, br, d (11)	33.9, CH_2	9, 11, 12	8a
10b	1.50, m		8	8b
11a	1.90, m	19.0, CH_2	10, 12, 13	
11b	1.61, m		9	
12a	1.61, m	32.3, CH_2	10, 11, 13, 14	
12b	1.18, m			
13	3.76, m	67.1, CH	11, 12, 14	1b
14-CH_3	1.09, d (6.0)	21.8, CH_3	12, 13	
15-CH_3	1.27, s	17.9, CH_3	3, 4, 5	6b

[a] Recorded at 400 MHz. [b] Recorded at 100 MHz.

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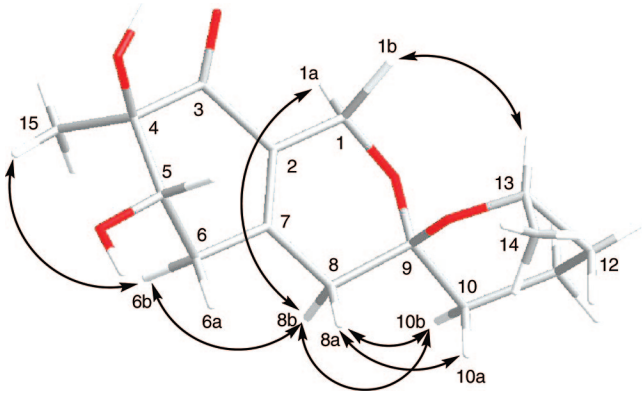


Figure 1. Key NOESY correlations of pestafolide A (**1**).

group in **1** could not be located by acetylation experiment, and C-13 could form an ether linkage with either C-4 or C-9 because of the lack of HMBC correlations from H-13 to any of these carbons. Although the ^{13}C chemical shift of C-9 (δ_C 95.2) matched well with that reported for a hemiketal carbon,[14–17] the different chemical environment for C-9 in **1** and those hemiketal carbons reported in the literature would not favor this assignment. In addition, geometric constraints would preclude linkage of C-13 to the oxygen at C-4.[18] Comparison of the ^{13}C NMR chemical shifts for C-4 (δ_C 77.3) and C-5 (δ_C 72.6) of **1** with those for corresponding carbons (δ_C 77.6 and 72.7, respectively) of the model compound monascusone A[19] revealed the connectivity of the two hydroxy groups to C-4 and C-5; therefore C-9 and C-13 must be attached to the same oxygen to complete the third six-membered ring in **1**.

The relative configuration of pestafolide A (**1**) was assigned by analysis of ^1H NMR *J*-values and NOESY data (Table 1). The large coupling constant (10 Hz) observed between H-5 and H-6b indicated that both protons were *trans* to each other with respect to the corresponding six-membered ring. NOESY correlations of H-6b with H_3-15 and H-8b and of H-8b with H-1a and H-10b placed these protons on the same face of the ring system, whereas correlation from H-1b to H-13 placed these two protons on the opposite face of the ring sytem. On the basis of these data, the relative configuration of pestafolide A was established as shown in **1** (Figure 1).

The absolute configuration of pestafolide A (**1**) was determined by application of the CD excitation chirality method. The CD spectrum of **1** showed a positive Cotton effect at 217 ($\Delta\epsilon$ +43) nm and a negative Cotton effect at 248 ($\Delta\epsilon$ −24) nm, which was nearly identical to that of (*R*)-2-acetyl-3,6-dihdroxycyclohex-2-enone-4,[20] suggesting the *R* absolute configuration at C-4, which was exactly the same as that of monascusone A.[19] Therefore, the 4*R*, 5*S*, 9*S*, and 13*S* absolute configuration was assigned for pestafolide A (**1**).

The molecular formula of pestaphthalide A (**2**) was determined to be $C_{11}H_{12}O_5$ (six unsaturations) on the basis of HRESIMS analysis [*m*/*z* 247.0558 $(M + Na)^+$; Δ +0.1 mmu] and was supported by ^1H and ^{13}C NMR data (Table 2). Analysis of the ^1H, ^{13}C, and HMQC NMR data for **2** revealed the presence of two methyl groups, two oxymethines, six aromatic carbons (one protonated), and one carboxyl carbon. These data accounted for all but three exchangeable protons and required the presence of two rings for **2**. Analysis of ^1H–^1H COSY NMR data led to the identification of one isolated proton spin-system corresponding to the C-3–C-9 subunit of structure **2**. HMBC correlations of H_3-10 with three nonprotonated aromatic carbons C-5 (δ_C 164.7), C-6 (δ_C 112.7), and C-7 (δ_C 156.5) indicated that C-6 was connected to C-5, C-7, and C-10. Correlations from H-4 to C-3, C-5, C-6, and C-7a and from H-3 to C-1, C-3a, C-4, C-7a, and C-8 permitted completion of the 5,6,7-trisubstituted isobenzofuranone ring with C-5, C-7, and C-8 each bearing a hydroxy group. On the basis of these data, the gross structure of pestaphthalide A was characterized as 5,7-dihydroxy-3-(1-hydroxyethyl)-6-methyl-3*H*-isobenzofuran-1-one as depicted in **2**.

Table 2. NMR Spectroscopic Data for Pestaphthalides A (**2**) and B (**3**) in CD_3OD

	pestaphthalide A (**2**)		pestaphthalide B (**3**)	
position	$\delta_H{}^a$ (*J* in Hz)	$\delta_C{}^b$, mult.	$\delta_H{}^a$ (*J* in Hz)	$\delta_C{}^b$, mult.
1		173.5, qC		173.4, qC
3	5.32, d (2.4)	85.4, CH	5.23, d (4.3)	85.9, CH
3a		148.3, qC		148.2, qC
4	6.67, s	102.0, CH	6.71, s	102.3, CH
5		164.7, qC		164.7, qC
6		112.7, qC		112.8, qC
7		156.5, qC		156.8, qC
7a		104.9, qC		104.5, qC
8	4.19, qd (6.3, 2.4)	68.8, CH	3.98, qd (6.3, 4.3)	69.8, CH
9-Me	1.17, d (6.3)	18.5, CH_3	1.21, d (6.3)	18.0, CH_3
10-Me	2.08, s	7.8, CH_3	2.18, s	7.8, CH_3

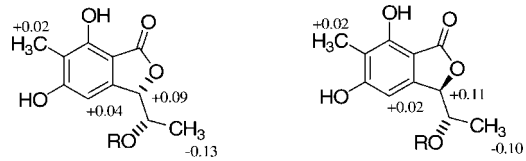
[a] Recorded at 400 MHz. [b] Recorded at 100 MHz.

Pestaphthalide B (**3**) was assigned the same molecular formula of $C_{11}H_{12}O_5$ as pestaphthalide A (**2**) on the basis of HRESIMS analysis [*m*/*z* 247.0557 $(M + Na)^+$] and NMR data (Table 2). Analysis of the ^1H and ^{13}C NMR data for **3** revealed the presence of nearly identical structural features to those found in **2**, except that the chemical shifts for the two oxymethines C-3 (δ_H/δ_C 5.32/85.4 in **2**; 5.23/85.9 in **3**) and C-8 (δ_H/δ_C 4.19/68.8 in **2**; 3.98/69.8 in **3**) were different, as well as the ^1H–^1H coupling constant observed between H-3 and H-8 (2.4 Hz in **2** and 4.3 Hz in **3**, respectively). These data implied that **3** was a stereoisomer of **2**, and this conclusion was supported by analysis of its COSY and HMBC data.

The relative configuration of compounds **2** and **3** was assigned by NOED data. Upon irradiation of H-3, enhancement was observed for H-8 in the NOE difference spectrum of **2**, suggesting a *cis* relationship between H-3 and H-8, whereas enhancement was observed for H_3-9 in the spectrum of **3**, indicating a *trans* relationship between H-3 and H-8.

The absolute configuration at C-3 for pestaphthalides A (**2**) and B (**3**) was again determined by application of the CD excitation chirality method. The CD spectrum of **3** showed the same chirality as that reported for spirolaxine,[21] and the CD curve of **2** displayed the opposite chirality of that for spirolaxine and **3**, suggesting the 3*S* absolute configuration for **2** and the 3*R* absolute configuration for **3**. On the basis of the relative configuration established for **2** and **3** by NOED experiments, the absolute configuration at C-8 for these was assigned as *S* for these compounds. Considering the relative configuration established by NOED data, the absolute configuration at C-8 for **2** and **3** was assigned as *S*. As confirmation, the absolute configuration at C-8 was also assigned by application of the modified Mosher method.[22] Treatment of **2** with (*S*)-MTPACl and (*R*)-MTPACl afforded the *R*-MTPA ester (**2a**) and *S*-MTPA ester (**2b**), respectively. The difference in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the diastereomeric esters **2b** and **2a** was calculated in order to assign the absolute configuration at C-8 (Figure 2). Calculations for all of the relevant signals suggested the *S* absolute configuration at C-8 in **2**. In a similar fashion, the absolute configuration at C-8 was also determined to be *S* in **3** (Figure 2). The above assignments were further supported by comparison of the ^1H NMR data and specific rotation values of **2** and **3** with those reported for their closest related synthetic analogues **5** and **6**.[23]

Pestafolide A (**1**) and pestaphthalides A (**2**) and B (**3**) were evaluated for antifungal activity against *Candida albicans* (ATCC 10231), *Geotrichum candidum* (AS2.498), and *Aspergillus fumigatus* (ATCC 10894) in agar diffusion assays. Pestafolide A (**1**) displayed antifungal activity against *Aspergillus fumigatus* (ATCC 10894), affording a zone of inhibition of 10 mm at 100 μg/disk.



2a R = (*R*)-MTPA **3a** R = (*R*)-MTPA
2b R = (*S*)-MTPA **3b** R = (*S*)-MTPA

Figure 2. $\Delta\delta$ values (in ppm) $= \delta_S - \delta_R$ obtained for (*R*)- and (*S*)-MTPA esters **2a**, **2b**, **3a**, and **3b**.

Pestaphthalide A (**2**) showed activity against *Candida albicans* (ATCC 10231), causing a zone of inhibition of 13 mm, and pestaphthalide B (**3**) showed activity against *Geotrichum candidum* (AS2.498) with a 11 mm zone of inhibition when tested at the same level (fluconazole: 18–28 mm zones of inhibition for *C. albicans*, *A. fumigatus*, and *G. candidum* at 100 μg/disk).

Pestafolide A (**1**) possesses the same spiro azaphilone skeleton as that appearing in decipinin A and the daldinins.[18,24–26] However, pestafolide A differs significantly from these known compounds by having a tetrahydro-1*H*-isochromen-8(5*H*)-one moiety instead of the common 3,4-dihydro-7*H*-isochromene-6,8-dione unit and by the absence of a substituent next to the ketal carbon on the tetrahydropyran ring. The tetrahydroisochromenone partial structure in **1** resembles the dihydroisochromenone core structure presented in monascusone A,[19] but **1** differs from monascusone A by the presence of an additional tetrahydropyran ring spirally joined to the tetrahydroisochromenone moiety at C-9. Isobenzufuranoes have been isolated frequently from microbial sources,[21,27–32] such as acetophthalidin (**4**),[32] which is the most closely related natural product to pestaphthalides A (**2**) and B (**3**). However, **2** and **3** differ from **4** by having a methyl group at C-6 and a hydroxyethyl substituent at C-3. Acetophthalidin derivatives **5** and **6** were prepared due to instability of acetophthalidin,[23] and these two synthetic analogues closely resembled **2** and **3**, with the only difference of the methyl group presented at C-6 for **2** and **3**. Pestafolide A (**1**) and pestaphthalides A (**2**) and B (**3**) are the first secondary metabolites to be reported from *P. foedan*.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. The CD spectra were recorded on a JASCO J-815 spectropolarimeter, using CH_3OH as solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^1H and ^{13}C NMR data were acquired with Varian Mercury-400 and -500 spectrometers using solvent signals ($CDCl_3$; δ_H 7.26/δ_C 77.7) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer. HRESIMS and EIMS data were obtained using a Bruker APEX III 7.0 T spectrometer and APEX II FT-ICR, respectively.

Fungal Material. The culture of *P. foedan* was isolated by one of authors (L.G.) from the branches of an unidentified tree near Dongzai, Hainan Province, on April 2, 2005. The isolate was identified (by L.G.) and assigned the accession number L436 in the culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. The agar plugs were used to inoculate 250 mL Erlenmeyer flasks, each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for 5 days. Fermentation was carried out in four 500 mL Fernbach flasks each containing 75 g of rice. Spore inoculum was prepared by suspension in sterile distilled H_2O to give a final spore/cell suspension of 1×10^6/mL. Distilled H_2O (100 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min.[33] After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented rice substrate was freeze-dried and extracted with MEK (3 × 500 mL), and the organic solvent was evaporated to dryness under vacuum to afford 5.0 g of crude extract. The crude extract was fractionated by silica gel VLC using petroleum ether−EtOAc gradient elution. The fraction (100 mg) that was eluted with 25% EtOAc was further separated by Sephadex LH-20 column chromatography (CH_2Cl_2−*n*-C_6H_{14}, 4:1) to afford a subfraction of 30 mg, and purification of this fraction by semipreparative reversed-phase HPLC (Kramosil C_{18} column; 10 μm; 10 × 250 mm, 2 mL/min) afforded pestafolide A (**1**; 1.9 mg, t_R 26.0 min; 45% MeOH in H_2O over 2 min, 45–53% over 40 min). The fraction (150 mg) that was eluted with 40% EtOAc was fractionated again by Sephadex LH-20 column chromatography using the same solvent system as described above to give a subfraction of 40 mg, and further purification by semipreparative reversed-phase HPLC afforded pestaphthalides A (**2**; 2.0 mg, t_R 30.3 min; 30% MeOH in H_2O over 5 min, 30–60% over 60 min) and B (**3**; 2.3 mg, t_R 32.8 min; same gradient as in purification of **2**).

Pestafolide A (1): colorless oil; $[\alpha]_D$ −19 (*c* 0.05, CH_3OH); UV (CH_3OH) λ_{max} 203 (ϵ 18 600), 255 (ϵ 15 800) nm; CD (*c* 1.0 × 10^{-4} M, CH_3OH) λ_{max} ($\Delta\epsilon$) 248 (−24), 217 (+43) nm; IR (neat) ν_{max} 3413 (br), 2937, 1681, 1434 cm^{-1}; ^1H, ^{13}C NMR, HMBC, and NOESY data, see Table 1; HRESIMS obsd *m/z* 305.1363 (M + Na)$^+$, calcd for $C_{15}H_{22}O_5$ Na, 305.1359.

Pestaphthalide A (2): brown oil; $[\alpha]_D$ +51 (*c* 0.05, CH_3OH); UV (CH_3OH) λ_{max} 215.5 (ϵ 13 800), 256 (ϵ 10 400) nm; CD (*c* 1.0 × 10^{-4} M, CH_3OH) λ_{max} ($\Delta\epsilon$) 226 (+13), 290 (−2.8) nm; IR (neat) ν_{max} 3363 (br), 2927, 1717, 1612 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS obsd *m/z* 247.0558 (M + Na)$^+$, calcd for $C_{11}H_{12}O_5$ Na, 247.0557.

Pestaphthalide A (3): brown oil; $[\alpha]_D$ −41 (*c* 0.05, CH_3OH); UV (CH_3OH) λ_{max} 214 (ϵ 17 500), 255 (ϵ17 500) nm; CD (*c* 1.0 × 10^{-4} M, CH_3OH) λ_{max} ($\Delta\epsilon$) 222 (−4.5), 260 (+0.3) nm; IR (neat) ν_{max} 3360, 2924, 1717, 1631 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS obsd *m/z* 247.0557 (M + Na)$^+$, calcd for $C_{11}H_{14}O_3$ Na, 247.0557.

Acetylation of Pestafolide A (1). A solution of pestafolide A (1.0 mg), 4-*N*,*N*-(dimethylamino)pyridine (catalytic amount), and acetic anhydride (0.5 mL) was stirred at room temperature for 24 h. The resulting solution was dried to afford the diacetate (0.8 mg): ^1H NMR ($CDCl_3$, 400 MHz) δ 5.99 (1H, dd, *J* = 10, 6.5 Hz, H-5), 4.30 (1H, br d, *J* = 16 Hz, H-1a), 4.13 (1H, br d, *J* = 16 Hz, H-1b), 3.78 (1H, m, H-13), 2.50 (1H, m, H-6a), 2.34 (1H, m, H-6b), 2.14 (1H, m, H-8a), 2.11 (3H, s, CH_3COO−), 2.08 (1H, m, H-8b), 2.01 (3H, s, CH_3COO−), 1.90 (1H, m, H-11a), 1.70 (1H, m, H-10a), 1.60 (1H, m, H-12a, H-11b), 1.50 (1H, m, H-10b), 1.25 (3H, s, 15-Me), 1.17 (1H, m, H-12b), 1.09 (3H, d, *J* = 6.5, 14-Me); ESIMS *m/z* [M + Na]$^+$ 389.2.

Preparation of (*R*)-MTPA Ester (2a) and (*S*)-MTPA Ester (2b). A solution of **2** (1.0 mg, 0.005 mmol) in CH_3OH was transferred to a clean NMR tube, and then the solvent was completely removed under vacuum. Pyridine-d_5 (0.5 mL) and (*S*)-MTPACl (4.5 μL, 0.025 mmol) were quickly added into the NMR tube, and all contents were mixed thoroughly by shaking the NMR tube carefully. The reaction was performed at room temperature, and the solution was allowed to stand for 24 h. ^1H NMR data of the *R*-MTPA ester (**2a**) were obtained without purification: ^1H NMR (pyridine-d_5, 400 MHz) δ 6.99 (1H, s, H-4), 5.70 (1H, dq, *J* = 7.5, 2.6 Hz, H-8), 5.36 (1H, br s, H-3), 2.32 (3H, s, 10-Me), 1.35 (3H, d, *J* = 7.5 Hz, 9-Me); EIMS *m/z* 440 (M$^+$; 3%), 189 (88%), 105 (100%), 77 (68%).

In a similar fashion, compound **2** (1.0 mg, 0.005 mmol), (*R*)-MPTACl (4.5 μL, 0.025 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 24 h and processed as described above for **2a** to afford **2b**: ^1H NMR (pyridine-d_5, 400 MHz) δ 7.01 (1H, s, H-4), 5.64 (1H, m, H-8), 5.45 (1H, br s, H-3), 2.36 (3H, s, 10-Me), 1.22 (3H, d, *J* = 7.5 Hz, 9-Me); EIMS *m/z* 440 (M$^+$; 16%), 206 (55%), 179 (100%).

Preparation of (*R*)-MTPA Ester (3a) and (*S*)-MTPA Ester (3b). The preparation of **3a** and **3b** followed the same procedure as described above for **2a** and **2b**. Compound **3a**: ^1H NMR (pyridine-d_5, 400 MHz) δ 7.03 (1H, s, H-4), 5.92 (1H, m, H-8), 5.57 (1H, br s, H-3), 2.51 (3H, s, 10-Me), 1.52 (3H, d, *J* = 7.5 Hz, 9-Me); EIMS *m/z* 440 (M$^+$; 13%), 189 (100%), 105 (38%). Compound **3b**: ^1H NMR (pyridine-d_5, 400 MHz) δ 7.05 (1H, s, H-4), 5.85 (1H, m, H-8), 5.68 (1H, br s, H-3), 2.53 (3H, s, 10-Me), 1.42 (3H, d, *J* = 7.5 Hz, 9-Me); EIMS *m/z* 440 (M$^+$; 9%), 189 (100%), 105 (29%).

Antifungal Bioassays. Antifungal bioassays were conducted according to a literature procedure.[34] The yeasts *Candida albicans* (ATCC 10231) and *Geotrichum candidum* (AS2.498) were grown on Sabouraud dextrose agar, and the fungus *Aspergillus fumigatus* (ATCC 10894) was grown on potato dextrose agar. Test compounds were absorbed onto individual paper disks (6 mm diameter) at 100 μg/disk and placed on the surface of the agar. The assay plates were incubated at 25 °C for 48 h and examined for the presence of a zone of inhibition.

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, and CD spectra of pestafolide A (**1**) and pestaphthalides A (**2**) and B (**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

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