Ten-Membered Lactones from *Phomopsis* sp., an Endophytic Fungus of *Azadirachta indica*

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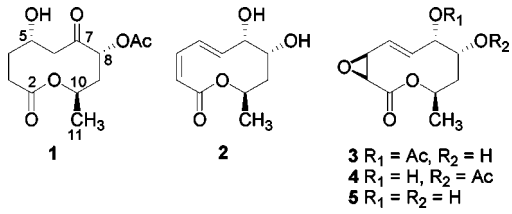
Four new 10-membered lactones (**1**–**4**) and one known one (**5**) were isolated from the broth extract of an endophytic fungus, *Phomopsis* sp., obtained from the stem of *Azadirachta indica*. Their structures were assigned by analysis of spectroscopic data, and the structures of **1** and **4** were also confirmed by X-ray analysis. Compounds **1**–**5** were tested for antifungal activity against several plant pathogens. Compound **4** demonstrated antifungal activity in the MIC value range 31.25–500 μg/mL.

Endophytic fungi are eukaryotic organisms that live inside plant tissues and are usually specific at the host species level.[1] They have proven to be a rich source of novel organic compounds with interesting biological activities and a high level of biodiversity.[2,3] Natural products from endophytic fungi have been observed to inhibit or kill a wide variety of harmful microorganisms including, but not limited to, phytopathogens, as well as bacteria, fungi, viruses, and protozoans that affect humans and animals.[4] In recent years, most work on endophytic fungi has been centered on plants in the temperate and tropical regions of the world.[5]

The Neem tree (*Azadirachta indica* A. Juss, Fam. Meliaceae), originally from India and Pakistan, is a fast-growing tree that is now common throughout large areas of the drier tropics. Almost all parts of this tree including leaves, bark, and seeds contain useful substances that can be used for medicinal purposes.[6] However, the best-known use of neem is for its insecticidal activities.[7,8] To date more than 300 natural products have been isolated from different parts of the tree.[9–11] Most of the active metabolites are tetranortriterpenoids, especially the azadirachtin analogues. Previous research on the isolation of endophytic fungi from neem grown in India has been performed,[12–14] but few of these are related to their metabolites.

Our study was performed on bioactive compounds produced by endophytic fungi of *A. indica* grown in Yuanjiang County, a tropical region in Yunnan Province, P. R. China. In the course of our investigations we have identified four new 10-membered lactones (**1**–**4**) and one known one (**5**) from the broth extract of an endophytic fungus *Phomopsis* sp. YM 311483, obtained from the stem of *A. indica*. Herein we describe the isolation and structural elucidation of these compounds and their *in vitro* antifungal activities on several plant pathogens. The culture broth of *Phomopsis* sp. YM 311483 was extracted with EtOAc, and this extract was subjected to column chromatography over silica gel, Sephadex LH-20, and RP-18 to afford compounds **1**–**5**.



Compound **1** was obtained as colorless crystals from a petroleum ether−acetone mixture. Its molecular formula was determined as $C_{12}H_{18}O_6$ by HRESIMS at *m*/*z* 259.1149 [M + H]$^+$ (calcd 259.1182) and NMR data. The IR spectrum of **1** showed intense carbonyl absorption bands at 1743 and 1702 cm^{-1} due to an ester group and a nonconjugated ketone, respectively. The ^{1}H NMR spectrum (Table 1) displayed a doublet methyl (δ 1.25), an acetoxyl methyl (δ 2.19), four nonequivalent methylenes, and three oxy protons (δ 4.51, 5.00, and 5.21). The ^{13}C NMR spectrum (Table 2) revealed 12 signals including two methyls (δ 20.3 and 21.3), four methylenes (δ 27.5, 28.0, 39.8, and 42.4), three oxygenated methines (δ 66.1, 67.1, and 76.3), two carboxyls (δ 170.9 and 172.2), and a ketone (δ 207.8). To accommodate four degrees of unsaturation, compound **1** was proposed to have a monocyclic skeleton and three carbonyl groups. From the ^{1}H−^{1}H COSY spectrum of **1**, a coupling sequence from H-3 to H-6 and from H-8 to H-11 could be established. HMBC correlations from C-2 (δ_C 172.2) to H-3 (δ_H 2.38 and 2.66), H-4 (δ_H 1.71 and 2.24), and H-10 (δ_H 5.21) and from C-7 (δ_C 207.8) to H-6 (δ_H 2.35 and 2.87), H-8 (δ_H 5.00), and H-9 (δ_H 2.09 and 2.39) indicated that compound **1** is a 10-memberd lactone ring with a ketone at C-7. The acetoxy group was attached to C-8, on the basis of HMBC correlations of the acetoxyl carbon at δ_C 170.9 with H-8 and the methyl group at δ_H 2.19. The orientation of the acetoxy group was concluded to be α from the coupling constants of H-8 (δ_H 5.00, dd, J = 1.0, 4.6 Hz) and its NOESY correlations between H-8 and both H-9a and H-9b. The coupling constants of H-6a (δ_H 2.87, dd, $J_{5,6a}$ = 11.3 Hz and $J_{6a,6b}$ = 17.9 Hz) revealed the α-orientation of the hydroxyl group at C-5. Thus, compound **1** was determined to be 8α-acetoxy-5α-hydroxy-7-oxodecan-9-olide. The relative stereochemistry of **1** was further demonstrated unambiguously by X-ray crystallographic analysis, which confirmed its proposed configuration. The three chiral carbons of **1** were definitely determined as 5*S**, 8*R**, and 10*R**, as reported in the X-ray structure shown in Figure 1.

Compound **2**, obtained as a colorless oil, was assigned the molecular formula $C_{10}H_{14}O_4$ from HRESIMS at *m*/*z* 199.0932 [M + H]$^+$ (calcd 199.0970). The IR spectrum showed an absorption band at 1707 cm^{-1} for an ester carbonyl. The ^{13}C NMR spectrum (Table 2) showed 10 resonances for a methyl (δ 20.0), a methlene (δ 36.5), three oxygenated methines (δ 67.9, 72.6, and 75.5), four olefinic methines (δ 126.1, 127.5, 136.4, and 139.6), and an ester carbonyl (δ 168.6). The ^{1}H NMR spectrum (Table 1) displayed a doublet methyl (δ 1.30), a nonequivalent methylene (δ 1.51 and 2.34), three oxy protons (δ 3.90, 4.32, and 5.31), and four olefinic protons (δ 5.71, 5.85, 6.08, and 6.61). The $J_{H\text{-}3,H\text{-}4}$ and $J_{H\text{-}5,H\text{-}6}$ values of 10.6 and 15.5 Hz revealed *Z*- and *E*-configurations of the two double bonds located at C-3 and C-5, respectively in **2**. The ^{1}H−^{1}H COSY and HMBC spectra demonstrated the connectivity sequence from H-2 to H-11. Compound **2** was therefore a 10-membered lactone showing similarities to multiplolide A (**5**).[15] The main difference was that the epoxide moiety at C-3 and C-4 in **5** was

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Table 1. ^{1}H NMR Data of Compounds **1**–**4** ($CDCl_3$, 500 MHz, δ in ppm, J in Hz)

position	1	2	3	4
3	2.66 dd, 11.9, 16.5 2.38 overlap	5.85 d, 10.6	3.66 d, 4.5	3.66 d, 4.7
4	2.24 dd, 13.2, 15.0 1.71 m	6.61 d, 10.6	3.77 m	3.81 m
5	4.51 m	6.08 d, 15.5	5.58 d, 15.6	5.84 d, 16.0
6	2.87 dd, 11.3, 17.9 2.35 brs	5.71 dd, 8.8, 15.5	5.93 dd, 2.1, 15.6	5.91 dd, 2.0, 16.0
7		4.32 m	5.63 m	4.63 m
8	5.00 dd, 1.0, 4.6	3.90 dd, 2.2, 8.3	4.15 dd, 2.1, 8.2	5.11 dd, 2.2, 8.3
9	2.39 overlap 2.09 ddd, 2.2,5.9,14.8	2.34 ddd, 3.4,8.2,16.0 1.51 dd, 3.3, 16.0	2.25 ddd, 3.2,8.1,15.9 1.40 dd, 2.7, 15.9	2.37 ddd, 3.5,8.3,16.1 1.21 dd, 2.8, 16.1
10	5.21 m	5.31 m	5.33 m	5.26 m
11	1.25 d, 6.3	1.30 d, 6.6	1.36 d, 6.7	1.40 d, 6.8
OAc	2.19 s		2.16 s	2.10 s

Table 2. ^{13}C NMR Data of Compounds **1**−**4** ($CDCl_3$, 125 MHz, δ in ppm)[a]

position	1	2	3	4
2	172.2 qC	168.6 qC	167.5 qC	167.6 qC
3	28.0 CH_2	126.1 CH	55.3 CH	55.3 CH
4	27.5 CH_2	139.6 CH	54.6 CH	54.9 CH
5	66.1 CH	127.5 CH	119.0 CH	119.5 CH
6	42.4 CH_2	136.4 CH	130.2 CH	132.5 CH
7	207.8 qC	75.5 CH	75.1 CH	70.8 CH
8	76.3 CH	72.6 CH	67.9 CH	71.5 CH
9	39.8 CH_2	36.5 CH_2	36.4 CH_2	32.8 CH_2
10	67.1 CH	67.9 CH	68.5 CH	68.5 CH
11	20.3 CH_3	20.0 CH_3	18.1 CH_3	17.9 CH_3
OAc	170.9 qC		171.2 qC	170.3 qC
	21.3 CH_3		21.2 CH_3	21.6 CH_3

[a] The assignments were based on DEPT and HMQC experiments.

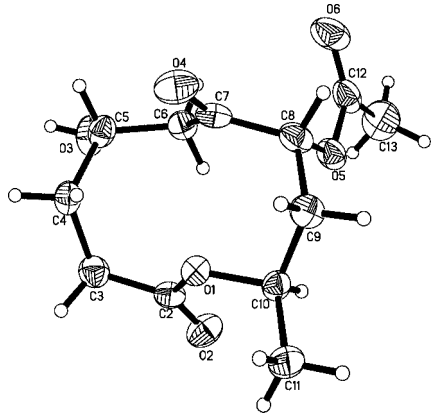

Figure 1. X-ray crystal structure of compound **1**.

substituted by a double bond in **2**. This was confirmed by HMBC correlations of the ester carbonyl carbon C-2 (δ_C 168.6) with H-3 (δ_H 5.85, d, J = 10.6 Hz), H-4 (δ_H 6.61, d, J = 10.6 Hz), and H-10 (δ_H 5.31, m). The configurations at the chiral centers C-7, C-8, and C-10 were determined as *S*, *R*, and *R*, respectively, which were identical to that of multiplolide A, as supported by its ^{1}H NMR, ^{1}H−^{1}H COSY, and NOESY spectra. Therefore, the structure of compound **2** was determined as 7α,8α-dihydroxy-3,5-decadien-10-olide.

Compound **3**, obtained as colorless crystals, had the molecular formula $C_{12}H_{16}O_6$ according to HRESIMS at *m*/*z* 257.1032 $[M + H]^+$ (calcd 257.1025), in which the molecular ion was 42 mass units greater than that of multiplolide A (**5**). The ^{1}H and ^{13}C NMR spectra of **3** were very similar to those of multiplolide A,[15] except for the additional signals at δ_C 171.2 (qC) and 21.2 (CH_3) in the ^{13}C NMR spectrum and at δ_H 2.16 (s) in the ^{1}H NMR spectrum. These differences showed that **3** had an acetoxy group instead of a hydroxyl group in multiplolide A. The HMBC spectrum displayed correlations of both the methyl signals at δ_H 2.16 and H-7 at δ_H 5.63 with the carbonyl carbon at δ_C 171.2. Therefore, the acetoxy group was attached to C-7, and the structure of compound **3** was determined to be 7α-acetoxymultiplolide A.

Compound **4**, obtained as colorless crystals, possessed the same molecular formula of $C_{12}H_{16}O_6$ as compound **3** on the basis of HRESIMS at *m*/*z* 257.1021 $[M + H]^+$ (calcd 257.1025). Careful comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **4** with those of compound **3** and multiplolide A revealed that **4** also had an acetoxy group that differed in substitution position compared to **3**. The HMBC experiment showed that the methyl signals at δ_H 2.10 (s) and H-8 at δ_H 5.11 (dd, J = 2.2, 8.3 Hz) were correlated with the carbonyl carbon at δ_C 170.3. Thus, the acetoxy group was placed at C-8, and the structure of compound **4** was determined as 8α-acetoxymultiplolide A. The orientation of the epoxide moiety was confirmed to be β by X-ray crystallographic analysis of **4** (Figure 2). This experiment also provided the stereochemistry at C-3 (*S*), C-4 (*S*), C-7 (*S*), C-8 (*R*), and C-10 (*R*), which is the same as compound **3**.

From spectroscopic data analysis compound **5** was identified as multiplolide A, a metabolite produced by the fungus *Xylaria multiplex*,[15] but the relative configuration of the epoxide moiety was not assigned. During our study we confirmed that the epoxide moiety in **5** possessed the same β-orientation as in **3** and **4** through comparison of their NMR data. To the best of our knowledge, this is the first report of 10-membered ring lactones to be isolated from the fungal genus *Phomopsis*.

Compounds **1**–**5** were evaluated for their antifungal activity against seven plant pathogens, *Aspergillus niger*, *Botrytis cinerea*, *Fusarium avenaceum*, *Fusarium moniliforme*, *Helminthosporium maydis*, *Penicillium islandicum*, and *Ophiostoma minus*, using the dose-dependent paper-disk diffusion method.[16] While all five compounds showed weak antifungal activities (Table 3), compound **4** was the most potent, with MIC values in the range 31.25−500 μg/mL. Interestingly, compound **4** was more active than compound

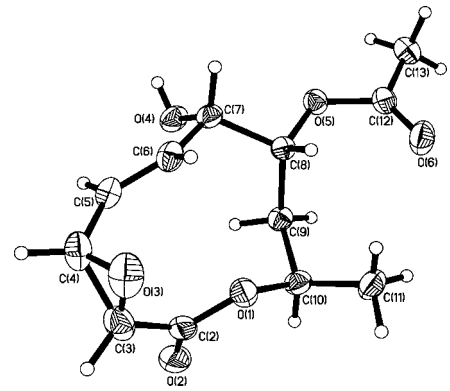

Figure 2. X-ray crystal structure of compound **4**.

Table 3. Antifungal Activities of Compounds **1**−**5** against Phytopathogenic Fungi *in Vitro*

organism	MIC values (μg/mL)					
	1	**2**	**3**	**4**	**5**	nystatin[a]
Aspergillus niger YM 3029	125	125	125	125	250	15.63
Botrytis cinerea YM 3061	250	500	–[b]	62.5	–[b]	31.25
Fusarium avenaceum YM 3065	–[b]	–[b]	–[b]	–[b]	–[b]	62.5
Fusarium moniliforme YM 3067	–[b]	–[b]	–[b]	500	–[b]	62.5
Helminthosporium maydis YM 3076	500	500	–[b]	500	500	31.25
Penicillium islandicum YM 3104	250	–[b]	–[b]	250	250	15.63
Ophiostoma minus YM3429	–[b]	–[b]	–[b]	31.25	–[b]	31.25

[a] Positive control. [b] Value >500 μg/mL.

3 even though their structures differed only in the position of the acetoxy substituent.

Experimental Section

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus and are uncorrected. Optical rotations were measured with a HORIBA SEPA-300 polarimeter. UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-500 spectrometer (500 and 125 MHz for ^1H and ^{13C} NMR, respectively), using TMS as an internal standard. HRESIMS were recorded on an Agilent G3250AA LC/MSD TOF spectrometer. X-ray crystallographic data were collected on a Bruker APEX II diffractometer. Column chromatography was performed on silica gel (Qingdao Marine Chemical Factory, China), Sephadex LH-20 (Amersham Pharmacia Biotech), and RP-18 silica gel (40–63 μm, Merck). Precoated silica gel plates (Qingdao Marine Chemical Factory, China) were used for TLC. Detection was done by spraying the plates with 5% H_2SO_4, followed by heating.

Isolation of the Endophytic Fungus. The fungus was isolated from surface-sterilized fresh stems of an apparently healthy *Azadirachta indica* specimen collected in January 2006 in Yuanjiang County, Yunnan Province, P. R. China. The stems were cut into small rods (about 10 cm in length) and rinsed in running tap H_2O followed by successive surface sterilization in 75% EtOH and 0.2% $HgCl_2$ for 1 min each. The stem rods were rinsed three times in sterilized distilled H_2O and cleaved aseptically into small segments, which were deposited on a Petri dish containing PDA medium (200 g potato, 20 g dextrose, and 15 g agar in 1 L of H_2O, supplemented with 100 mg/L penicillin to suppress bacterial growth) and incubated at 28 °C. The germinating hypha tips were observed and transferred to new PDA plates and then subcultured until pure cultures were obtained. The endophytic fungi were deposited in Yunnan Institute of Microbiology, Kunming, P. R. China. One of the fungal strains, YM 311483, was identified as a *Phomopsis* sp. by comparing its morphological characteristics[17] and molecular identification. Genomic DNA was purified from fungal mycelium using the cetyltrimethylammonium bromide (CTAB) extraction method[18] and then subject to ribosomal internal transcribed spacer (ITS) analysis.[19] The nucleotide sequence data of ITS1-5.8S-ITS2 of *Phomopsis* sp. YM 311483 is deposited in GenBank with accession number EU256482. A GenBank search for sequences similar to its ITS region revealed *Phomopsis* sp. OOW7 and *Phomopsis columnaris* as the closest match, each with a 96% sequence identity. These results suggested that the endophytic fungus YM 311483 belongs to the genus *Phomopsis*.

Fermentation, Extraction, and Isolation. The fungus was cultured in 500 mL Erlenmeyer flasks (×200) containing 100 mL of PDB medium at 200 rpm at 28 °C for 6 days on a rotary shaker. The culture broth was filtered to remove mycelia. The filtrate was concentrated under reduced pressure to 5 L and then exhaustively extracted with EtOAc (3 × 5 L). After removal of the solvent in vacuum, the resulting residue (14.5 g) was subjected to column chromatography on Si gel eluted by a gradient of $CHCl_3$/Me_2CO from 1:0 to 0:1 (v/v) to afford six fractions. Fraction 2 (1.8 g) was rechromatographed on a Si gel column, eluted with petroleum ether/EtOAc (8:2, 7:3, 6:4) to provide three fractions. Fraction 2-1 was subjected to gel filtration on Sephadex LH-20 (Me_2CO) followed by crystallization from petroleum ether/Me_2CO to yield **1** (95 mg). Fraction 2-2 was purified by RP column chromatography (MeOH/H_2O gradient system, 20−60% MeOH) to give **3** (137 mg) and **4** (346 mg). Fraction 3 (1.6 g) was chromatographed on a silica gel column, eluted with petroleum ether/Me_2CO (8:2, 7:3), and then purified by RP column chromatography (MeOH/H_2O gradient system, 25−50% MeOH) to afford **2** (35 mg) and **5** (183 mg).

8α-Acetoxy-5α-hydroxy-7-oxodecan-10-olide (1): colorless crystals; mp 141−143 °C; $[\alpha]^{21}_D$ +56.2 (*c* 0.48, $CHCl_3$); UV (MeOH) λ_{max} end absorption at 210 nm; IR (KBr) ν_{max} 3536, 3345, 2995, 2881, 1743, 1702, 1381, 1361, 1283, 1228, 1051, 932, 860, 665 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (pos.) *m*/*z* 259.1149 $[M + H]^+$ (calcd for $C_{12}H_{19}O_6$ 259.1182).

7α,8α-Dihydroxy-3,5-decadien-10-olide (2): colorless oil; $[\alpha]^{21}_D$ +5.8 (*c* 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 255.3 (2.76) nm; IR (KBr) ν_{max} 3471, 3407, 3021, 2936, 1707, 1447, 1386, 1258, 1164, 1102, 1057, 981, 957 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (pos.) *m*/*z* 199.0932 $[M + H]^+$ (calcd for $C_{10}H_{15}O_4$ 199.0970).

7α-Acetoxymultiplolide A (3): colorless crystals; mp 116−118 °C; $[\alpha]^{21}_D$ +17.4 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} end absorption at 210 nm; IR (KBr) ν_{max} 3522, 3423, 2985, 2937, 1723, 1435, 1368, 1238, 1061, 913, 866, 827 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (pos.) *m*/*z* 257.1032 $[M + H]^+$ (calcd for $C_{12}H_{17}O_6$ 257.1025).

8α-Acetoxymultiplolide A (4): colorless crystals; mp 125−127 °C; $[\alpha]^{21}_D$ +22.5 (*c* 0.36, $CHCl_3$); UV (MeOH) λ_{max} end absorption at 210 nm; IR (KBr) ν_{max} 3505, 2987, 2957, 1726, 1436, 1369, 1279, 1156, 1100, 1007, 965, 821 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (pos.) *m*/*z* 257.1021 $[M + H]^+$ (calcd for $C_{12}H_{17}O_6$ 257.1025).

X-ray Crystallography of 1. Crystal data of **1**:[20] $C_{12}H_{18}O_6 \cdot H_2O$, M_r = 276.28, orthorhombic, crystal dimensions 0.29 × 0.17 × 0.10 mm, space group *P*2(1)2(1)2(1), a = 7.1270(8) Å, b = 13.9423(16) Å, c = 14.4961(16) Å, α = β = γ = 90°, V = 1440.4(3) Å^3, Z = 4, D_{calc} = 1.274 mg/m^3, $F(000)$ = 592, μ = 0.105 mm^{-1}, T_{min}/T_{max} = 0.97/0.99, 12 531 collected reflections, 3453 unique reflections (R_{int} = 0.0427), goodness of fit on F^2 = 1.024, final R_1, wR_2 [$I > 2\sigma(I)$] = 0.0498/0.0905, R_1, wR_2 (all data) = 0.0889/0.1061. The data were collected on a Bruker APEX II diffractometer at 298(2) K, with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) using $\omega-2\theta$ scans in the range θ = 2.03−28.42°. The structure was solved by the direct method using SHELXS-97[21] and then refined by full-matrix least-squares on F^2 using SHELXL-97 package software.[22] The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in the idealized positions and refined using a riding model.

X-ray Crystallography of 4. Crystal data of **4**:[20] $C_{12}H_{16}O_6$, M_r = 256.25, orthorhombic, crystal dimensions 0.31 × 0.22 × 0.15 mm, space group *P*2(1)2(1)2(1), a = 7.5444(7) Å, b = 9.8302(9) Å, c = 16.6997(15) Å, α = β = γ = 90°, V = 1238.5(2) Å^3, Z = 4, D_{calc} = 1.374 mg/m^3, $F(000)$ = 544, μ = 0.111 mm^{-1}, T_{min}/T_{max} = 0.97/0.98, 10 630 collected reflections, 2927 unique reflections (R_{int} = 0.0235), goodness of fit on F^2 = 1.024, final R_1, wR_2 [$I > 2\sigma(I)$] = 0.0365/0.0832, R_1, wR_2 (all data) = 0.0440/0.0879. The data were collected on a Bruker APEX II diffractometer at 298(2) K, with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) using $\omega-2\theta$ scans in the range θ = 2.40−28.32°. The structure was solved by the direct method using SHELXS-97[21] and then refined by full-matrix least-squares on F^2 using SHELXL-97 package software.[22] The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in the idealized positions and refined using a riding model.

Antifungal Activity. The antifungal bioassay was performed by the paper-disk diffusion method.[16] The phytopathogenic fungi were cultured in PDA medium at 28 °C. Fungal spores were diluted with melted PDA medium to obtain 1 × 10^6 spores/mL and then poured into a Petri plate. Each test compound was dissolved in 50% aqueous DMSO, serially double diluted from 500 to 15.63 μg/mL, and added to the sterilized paper disk (6 mm in diameter) under aseptic conditions. The dried paper disks were placed onto the agar medium Petri dish and cultured at 28 °C for 72 h, at which time the inhibition zones were measured. Nystatin and DMSO were used as positive and negative controls, respectively. The results are

expressed in terms of minimal inhibitory concentration (MIC) (in μg/mL). Three repetitions were conducted for each sample tested.

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Note Added after ASAP Publication: Compounds **2** and **4** mentioned in our article posted on March 13, 2008, were reported just before the time of our submission of the manuscript, and thus, they are not new as claimed. They are identical to compounds reported by Tan et al. in 2007 {Tan, Q.; Yan, X.; Lin, X.; Huang, Y.; Zheng, Z.; Song, S.; Lu, C. *Helv. Chim. Acta* **2007**, *90*, 1811–1816]. In this regard, compound **2**, named 7α,8α-dihydroxy-3,5-decadien-10-olide, is identical to 3,4-deoxy-3,4-didehydromultiplolide A, and compound **4**, named 8α-acetoxy-multiplolide A, is identical to 8-*O*-acetylmultiplolide A, as reported by this other group. The authors apologize for any inconvenience. This note was added on April 8, 2008.

Supporting Information Available: X-ray crystallographic data for **1** and **4** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Petrini, O. In *Endophytic Fungi in Grasses and Woody Plants: Systematics, Ecology and Evolution*; Redlin, S. C., Carris, L. M., Eds.; APS Press: Saint Paul, MN, 1996; pp 87–93.
(2) Schulz, B.; Boyle, C.; Draeger, S.; Aust, H. J.; Römmert, A. K.; Krohn, K. *Mycol. Res.* **2002**, *106*, 996–1004.
(3) Schulz, B.; Boyle, C. *Mycol. Res.* **2005**, *109*, 661–686.
(4) Strobel, G.; Daisy, B.; Castillo, U.; Harper, J. *J. Nat. Prod.* **2004**, *67*, 257–268.
(5) Rodrigues, K. F.; Petrini, O. In *Biodiversity of Tropical Micro-fungi*; Hyde, K. D., Ed.; Hong Kong University Press: Hong Kong, 1997; pp 57–69.
(6) National Research Council. *Neem: A Tree for Solving Global Problems*; National Academy Press: Washington DC, 1992; p 60.
(7) Isman, M. B.; Koul, O.; Luczenski, A.; Kaminski, Z. *J. Agric. Food Chem.* **1990**, *38*, 1406–1411.
(8) Koul, O.; Isman, M. B.; Ketkar, C. M. *Can. J. Bot.* **1990**, *68*, 1–11.
(9) Govindachari, T. R. *Curr. Sci.* **1992**, *63*, 117–122.
(10) Kumar, C. S. S. R.; Srinivas, M.; Yakkundi, S. *Phytochemistry* **1996**, *43*, 451–455.
(11) Morgan, E. D.; Wilson, I. D. In *Comprehensive Natural Products Chemistry*; Mori, K., Ed.; Elsevier: Amsterdam, 1999; Vol. 8, Chapter 5, pp 324–331.
(12) Rajagopal, K.; Suryanarayanan, T. S. *Curr. Sci.* **2000**, *78*, 1375–1378.
(13) Mahesh, B.; Tejesvi, M. V.; Nalini, M. S.; Prakash, H. S.; Kini, K. R.; Subbiah, V.; Shetty, H. S. *Curr. Sci.* **2005**, *88*, 218–219.
(14) Verma, V. C.; Gond, S. K.; Kumar, A.; Kharwar, R. N.; Strobel, G. *Microb. Ecol.* **2007**, *54*, 119–125.
(15) Boonphong, S.; Kittakoop, P.; Isaka, M.; Pittayakhajonwut, D.; Tanticharoen, M.; Thebtaranonth, Y. *J. Nat. Prod.* **2001**, *64*, 965–967.
(16) Barry, A. L.; Coyle, M. B.; Thornsberry, C.; Gerland, E. H.; Howkinson, R. W. *J. Clin. Microbiol.* **1979**, *10*, 885–889.
(17) Wei, J. C. *Handbook of Identification of Fungi*; Shanghai Science & Technology Press: Shanghai, 1979; p 424.
(18) Rogers, S. O.; Bendich, A. J. In *Plant Molecular Biology Manual D1*; Gelvin, S. B., Schilperoort, R. A., Eds.; Kluwer Academic Publishers: Dordrecht, 1994: pp 1–8.
(19) White, T. J.; Bruns, T. D.; Lee, S. B.; Taylor, J. W. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M. A., Gelfand, D. H., Sninsky, J. J., White, T. J., Eds.; Academic Press: New York, 1990; pp 315–322.
(20) Crystallographic data for compounds **1** (CCDC 680128) and **4** (CCDC 680129) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
(21) Sheldrick, G. M. *Acta Crystallogr., Sect. A* **1990**, *46*, 467–473.
(22) Sheldrick, G. M. *SHELXTL, v. 5, Reference Manual*; Siemens Energy and Automation, Inc., 1997.

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