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Decaspirones A−E, Bioactive Spirodioxynaphthalenes from the Freshwater Aquatic Fungus *Decaisnella thyridioides*

Ping Jiao,[†] Dale C. Swenson,[†] James B. Gloer,*[,†] Jinx Campbell,[‡] and Carol A. Shearer[‡]

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, and Department of Plant Biology, University of Illinois, Urbana, Illinois 61801

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Decaspirones A−E (**1**−**5**), five new compounds related to the palmarumycins, were isolated from cultures of the freshwater aquatic fungal species *Decaisnella thyridioides*. The known compound palmarumycin CP_1 (**6**) was also obtained. The structures of **1**−**5** were determined by analysis of NMR and MS data, and their relative configurations were assigned by analysis of ^{1}H NMR *J*-values and NOESY data. The structure of the lead compound **1** was confirmed by X-ray crystallographic analysis. Compounds **1**−**5** possess a *trans* ring fusion not previously reported in members of this structural class. The absolute configuration of **1** was assigned using the modified Mosher method. Compounds **1**−**5** showed potent antifungal and antibacterial activity.

In our ongoing search for new bioactive fungal metabolites, we continue to investigate rarely studied ecological groups such as freshwater aquatic fungi. Prior investigations of freshwater fungi in our group have afforded a variety of bioactive metabolites.[1−4] In the course of this work, an extract showing potent antifungal activity was obtained from cultures of an isolate of *Decaisnella thyridioides* (Sacc. & Speg.) M. E. Barr (Pyrenulaceae) that was collected from submerged, decorticated wood in the Lemonweir River in Wisconsin. To our knowledge, no prior chemical study of any member of the genus *Decaisnella* has been reported. Investigation of this species afforded five new compounds containing dioxynaphthalene substructures, which were named decaspirones A−E (**1**−**5**), along with one related known compound (palmarumycin CP_1; **6**).[5] Decaspirones A−E belong to a growing family of fungal metabolites referred to as spirodioxynaphthalenes, which includes palmarumycins, cladospirones, and diepoxins.[5−11] These compounds display a wide range of biological activities, including antibacterial and antifungal effects, phospholipase D inhibitory activity, and antitumor activity. All of the spirodioxynaphthalene compounds reported to date incorporate a modified *cis*-decalin moiety. However, the decaspirones possess a *trans*-decalin system, making them the first examples of this class to incorporate a *trans* ring fusion. Details of the isolation, structure elucidation, and stereochemical assignments of decaspirones A−E (**1**−**5**) are presented here.

Results and Discussion

Solid-state fermentation cultures of *D. thyridioides* (A-00267-2A) were extracted with ethyl acetate, and the resulting crude extract showed significant antifungal activity in disk assays against a *Nectria* species, *Candida albicans*, and *Aspergillus flavus*. Fractionation of this extract led to the isolation of decaspirones A−E (**1**−**5**) and the known spironaphthalene compound palmarumycin CP_1 (**6**). The structure of palmarumycin CP_1 was established by comparison of NMR and MS data with literature values.[5]

Decaspirones A−E (**1**−**5**) were recognized as members of the spironaphthalene class by analysis of NMR data. The general characteristics of this class include the 1,8-dioxynaphthalene moiety linked with the second half of the molecule via a spiroketal carbon resonating at approximately δ_C 100. The NMR spectroscopic data for the dioxynaphthalene moiety and the bridging carbon atom of all five compounds were virtually identical, so the structure elucidation of these metabolites focused mainly on establishing the identities of the remaining portions of the molecules.

The molecular formula of decaspirone A (**1**) was determined as $C_{20}H_{16}O_5$ (13 degrees of unsaturation) by analysis of MS, as well

* To whom correspondence should be addressed. Tel: 319-335-1361. Fax: 319-335-1270. E-mail: james-gloer@uiowa.edu.
[†] University of Iowa.
[‡] University of Illinois.

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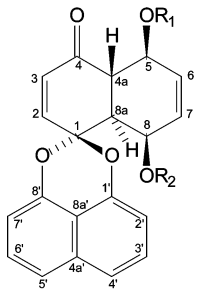
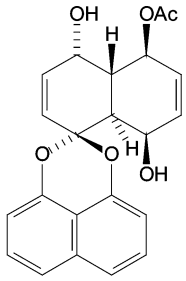


1 $R_1 = H$ $R_2 = H$
4 $R_1 = H$ $R_2 = OAc$
5 $R_1 = OAc$ $R_2 = H$



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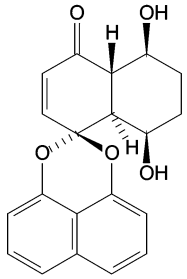

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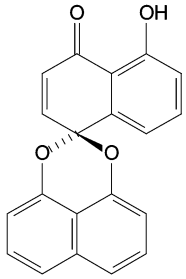

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as ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 2). Aside from a set of NMR signals characteristic of the dioxynaphthalene moiety, the ^{1}H and ^{13}C NMR spectra showed signals for a ketal carbon, two 1,2-disubstituted olefin units, a ketone moiety, four contiguous sp^3 methine units (two of which were oxygenated), and two hydroxy groups.

The connectivities among these units were determined by analysis of ^{1}H−^{1}H COSY and HMBC data. COSY data revealed that one of the olefins was isolated, while the other was linked to the methine units to form a dioxycyclohexene spin-system that corresponded to the C4a−C8a substructure in **1**. HMBC correlations of H-2, H-3, and H-4a to ketone carbonyl C-4, and of H-2 and H-3 to C-4a, linked C-4 to the C-2/C-3 double bond and to C-4a of the cyclohexene ring. Correlations of H-3, H-4a, H-8a, and H-8 to ketal carbon C-1 (δ_C 97.9) enabled the connection of the C-2/C-3 double bond and C-8a of the cyclohexene ring to C-1. The dioxynaphthalene unit must be linked to C-1 via the two oxygen atoms from the ketal moiety, thereby completing the gross structure of **1** as shown.

A 13-Hz coupling constant between the two bridgehead protons H-4a and H-8a indicated the presence of a *trans* ring fusion. However, the orientation of the two hydroxy groups at C-5 and C-8 could not be established with confidence on the basis of J_{H-H} values. NOESY data were consistent with proposal of the relative configuration shown in **1**, but any ambiguity was resolved upon X-ray crystallographic diffraction analysis of a crystal of **1** obtained from acetonitrile solution. The X-ray data confirmed the presence of the *trans*-decalin ring system (Figure 1) and revealed that the C-5 and C-8 hydroxy groups have a *syn*-relative orientation.

The molecular formula of decaspirone B (**2**) was determined to be $C_{22}H_{20}O_6$ (13 degrees of unsaturation) on the basis of EIMS and NMR data. The presence of signals for one additional oxygenated methine group and an acetate unit in the ^{1}H NMR spectrum relative to that of **1** suggested reduction of the ketone carbonyl C-4 and acetylation of one of the hydroxy groups. Changes in the ^{13}C NMR spectrum were also consistent with these proposed differences. ^{1}H−^{1}H COSY correlations confirmed the presence of the new extended spin-system and were used to locate the acetate group at C-5, since the H-5 signal (δ 5.92) was shifted downfield by 1.4 ppm relative to that of compound **1**. ^{1}H and ^{13}C NMR chemical shift assignments were confirmed by analysis of HMQC data. The relative configuration was assigned to match that of compound **1** on the basis of close similarities among the coupling constants of the signals for H-4a, H-8a, H-4, and H-8 for both compounds. This conclusion was confirmed, and the relative configuration of the new stereocenter at C-4 was assigned by analysis of NOESY data (Figure 2). Correlations of H-4 to H-4a and to the methyl singlet of the acetate unit led to placement of the C-4 hydroxy group *anti* to the acetate and hydroxy groups at C-5 and C-8. The resulting pseudoequatorial orientation of H-4 is consistent with the smaller vicinal coupling ($J = 4.4$ Hz) observed between H-4 and H-4a, in comparison to the *J*-value observed between H-4a and H-5.

Decaspirone C (**3**) was assigned the molecular formula $C_{20}H_{18}O_5$ on the basis of NMR and HREIMS data. Two additional hydrogens in the molecular formula relative to that of **1** and analysis of the ^{1}H NMR spectrum suggested that the C-6/C-7 double bond in **1** was reduced in **3**. This was consistent with the ^{13}C NMR data, which included two new methylene carbon signals at δ_C 26.9 and 30.2 in place of the C-6 and C-7 olefin signals in the spectrum of **1**. Proton and carbon shift assignments were made by analysis of

Table 1. ^{1}H NMR Data [δ_H (mult.; J_H)] for Decaspirones A−E (**1**−**5**; 300 MHz, $CDCl_3$)

position	1	2	3	4	5
2	6.88 (d, 10)	5.93 (d, 10)	6.77 (d, 10)	6.95 (d, 10)	6.77 (d, 10)
3	6.05 (d, 10)	6.07 (dd, 10, 5.1)	5.99 (d, 10)	6.03 (d, 10)	6.02 (d, 10)
4		4.25 (br t, 4.6)			
OH-4		3.23 (br s)			
4a	3.42 (dd, 13, 9.0)	2.88 (ddd, 14, 8.3, 4.4)	3.35 (dd, 13, 9.1)	3.37 (dd, 13, 9.2)	3.73 (dd, 13, 8.6)
5	4.52 (br d, 9.0)	5.92 (ddd, 8.3, 3.2, 1.8)	3.97 (ddd, 11, 9.1, 5.0)	4.54 (br d, 9.2)	5.80 (ddd, 8.6, 2.8, 1.6)
OH-5	4.16 (br s)		4.73 (br s)	4.23 (br s)	
6	5.91 (dd, 10, 2.3)	5.86 (dd, 10, 3.2)	α 1.88 (br d, 13) β 2.12 (br q, 13)	6.00 (dd, 10, 2.3)	5.88 (dd, 10, 2.8)
7	6.06 (ddd, 10, 5.5, 2.0)	6.26 (ddd, 10, 5.8, 1.8)	α 1.53 (br t, 13) β 2.03 (br d, 13)	5.95 (ddd, 10, 5.3, 1.9)	6.21 (ddd, 10, 5.9, 1.6)
8	4.90 (dd, 5.5, 2.6)	4.90 (dd, 5.8, 2.5)	4.73 (m)	6.04 (dd, 5.3, 2.8)	4.95 (dd, 5.9, 2.2)
OH-8	3.42 (br s)	3.90 (br s)	3.44 (br s)		3.53 (br s)
8a	2.69 (dd, 13, 2.6)	2.62 (dd, 14, 2.5)	2.46 (dd, 13, 1.6)	2.80 (dd, 13, 2.8)	2.78 (dd, 13, 2.2)
2′[a]	6.97 (d, 7.5)	6.90 (d, 7.6)	6.97 (d, 7.6)	6.85 (d, 7.6)	6.97 (d, 7.6)
3′[b]	7.44 (t, 7.6)	7.41 (t, 7.6)	7.44 (t, 7.6)	7.38 (t, 7.6)	7.45 (t, 7.6)
4′[c]	7.57 (d, 8.4)	7.53 (d, 8.4)	7.57 (d, 8.4)	7.52 (d, 8.4)	7.58 (d, 8.4)
5′[c]	7.58 (d, 8.4)	7.51 (d, 8.4)	7.57 (d, 8.4)	7.54 (d, 8.4)	7.58 (d, 8.4)
6′[b]	7.50 (t, 7.6)	7.47 (t, 7.6)	7.48 (t, 7.6)	7.48 (t, 7.6)	7.50 (t, 7.6)
7′[a]	7.04 (d, 7.6)	6.95 (d, 7.6)	7.02 (d, 7.6)	7.01 (d, 7.6)	7.05 (d, 7.6)
OAc		2.18 (s)		2.17 (s)	2.17 (s)

[a −c]Assignments for positions with identical superscripts are interchangeable.

Table 2. ^{13}C NMR Data (δ_C) for Decaspirones A−E (**1**−**5**; 100 MHz, $CDCl_3$)

position	1	2	3	4	5
1	97.9	100.3	99.2	98.5	98.8
2	143.3	125.7	142.5	145.8	141.2
3	130.4	132.7	131.3	130.4	131.5
4	201.6	61.6	204.1	202.0	197.7
4a	43.9	36.8	46.8	45.5	41.8
5	68.9	70.3	71.6	69.5	69.0
6	131.5	129.0	26.9	133.7	129.3
7	128.3	131.9	30.2	125.9	130.9
8	61.8	62.1	64.3	62.3	61.9
8a	45.6	42.0	49.8	45.6	47.3
1′[a]	145.8	146.6	146.4	146.4	146.2
2′[b]	109.7	110.0	111.1	109.2	110.4
3′[c]	127.2	127.6	128.0	127.7	127.8
4′[d]	121.5	121.8	122.5	121.7	122.4
4a′	133.9	131.9	134.8	133.9	134.6
5′[d]	121.3	121.2	122.1	121.5	121.9
6′[c]	127.5	128.1	128.3	128.0	128.2
7′[b]	110.3	110.6	110.5	110.6	110.0
8′[a]	146.1	147.5	146.8	147.0	146.6
8a′	113.4	113.9	114.0	113.4	114.0
$OCOCH_3$		21.7		21.8	21.6
$OCOCH_3$		172.8		170.4	170.9

[a−d]Assignments for positions with identical superscripts are interchangeable.

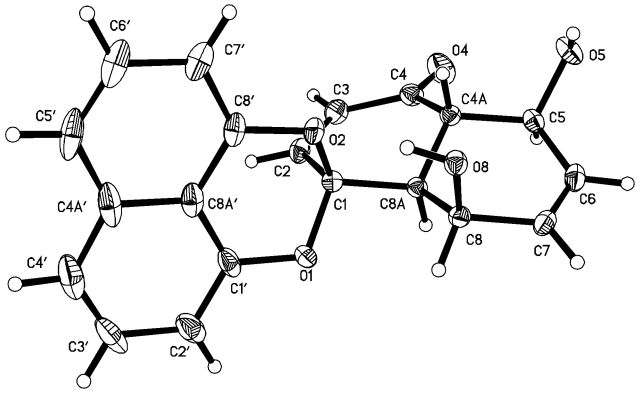

Figure 1. X-ray crystal structure of decaspirone A (**1**).

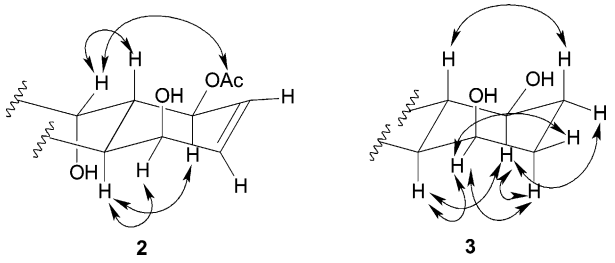

Figure 2. Key NOESY correlations for decaspirones B (**2**) and C (**3**).

HMQC and HMBC experiments. The relative configuration was determined to be the same as that of **1** by analysis of NOESY data (Figure 2) and ^1H NMR coupling constants.

Decaspirones D (**4**) and E (**5**) were both readily identified as different monoacetylated products of compound **1**. Both compounds showed a molecular ion peak at *m*/*z* 378, suggesting a molecular formula of $C_{22}H_{18}O_6$, and the structures were assigned by analysis of ^1H, ^{13}C, and COSY NMR data. In each case, the replacement of one hydroxy group signal with an acetate methyl singlet in the ^1H NMR spectrum indicated acetylation of one of the hydroxy groups. As a result, the H-8 signal in compound **4** was shifted downfield to δ 6.04 (δ 4.90 in **1**), while in **5**, the H-5 signal moved downfield significantly compared with that of **1** (δ 5.80 vs δ 4.52). ^1H and ^{13}C NMR assignments were established by analysis of HMQC data.

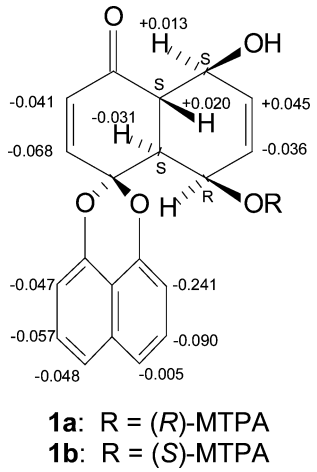

Figure 3. $\Delta\delta$ values (in ppm) ($= \delta_S - \delta_R$) obtained for (*S*)- and (*R*)-MTPA esters **1a** and **1b**.

On the basis of similarities of the $^1H-^1H$ coupling constant values with those observed for **1**, compounds **4** and **5** were proposed to have the same relative configurations at C-4a, C-5, C-8, and C-8a as in **1**.

The absolute configuration of decaspirone A (**1**) was assigned by application of the modified Mosher method.[12,13] One equivalent of *S*-MTPA Cl was added to a sample of **1** in an effort to obtain a mono-*R*-MTPA ester. Some selectivity for acylation of the C-8 hydroxy group was obtained, as indicated by NMR analysis, and the resulting product (**1a**) was isolated by reversed-phase HPLC. The process was then repeated with the *R*-MTPA Cl isomer to form the mono-*S*-MTPA ester (**1b**). The differences in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the two diastereomeric esters **1b** and **1a** were calculated in order to assign the absolute configuration at C-8 (Figure 3). The particularly dramatic effect for one of the protons *ortho* to the ketal linkage (H-2′) is consistent with its placement on the same face of the modified decalin system as the MTPA ester unit. Calculations for all of the relevant signals except one (H-7) suggested the *R* absolute configuration at C-8. It is possible that the presence of the nearby 1,8-dioxynaphthalene moiety could cause this apparent anomalous result for the H-7 signal, since some interaction between the MTPA phenyl group and the naphthalene moiety might occur to perturb the favored conformations from those adopted by typical MTPA esters. This concept was investigated using molecular modeling techniques (Spartan 02), but the results were inconclusive. Ultimately, the 5*S*, 8*R*, 4a*S*, and 8a*S* absolute configuration was proposed for compound **1** on the basis of the vast majority of the $\Delta\delta$ results summarized in Figure 3. The absolute configurations of compounds **2**−**5** were presumed to be analogous to that of **1**.

Decaspirones A−E (**1**−**5**) are new members of the so-called spirodioxynaphthalene class with a *trans*-fused decalin ring system that is unprecedented among members of this class.[14,15] These compounds seem likely to be somewhat more stable than their *cis*-ring-fused counterparts, and most of them contain a proton α to a ketone moiety. However, there was no evidence of epimerization during the isolation process, as the major components were very abundant in the extract and were evident in the NMR spectrum prior to any separation efforts. In addition, the only prior literature examples with similar functionality contain *cis* ring fusions, and none of the corresponding *cis* analogues were detected in the *D. thyridioides* extract, even as minor analogues. Biogenetically, both the naphthalene and the decalin units are presumably derived from the polyketide pathway.[16] Palmarumycin CP_1 (**6**) can be considered as an aromatized dehydration product of decaspirone A.

In standard antibacterial disk assays, compounds **1**−**5** all showed significant activity against *Bacillus subtilis* (ATCC 6051) when tested at 50 μg/disk, causing inhibition zones of 39, 19, 34, 30,

and 30 mm, respectively. Compounds **1**, **3**, and **4** were active against *Staphylococcus aureus* (ATCC 29213), causing zones of inhibition of 41, 28, and 30 mm, respectively, at 100 μg/disk. Compounds **2** and **5** were inactive against *S. aureus* at this level. Compounds **1**, **4**, and **5** also showed activity against *Candida albicans* (ATCC 14053) at 100 μg/disk, affording inhibition zones of 30, 13, and 17 mm, respectively, while compounds **2** and **3** were inactive in this assay. None of the compounds showed significant activity against *Escherichia coli* (ATCC 25922) at this level.

Due to sample limitations, only compounds **1** and **3** were evaluated for activity against *Aspergillus flavus* (NRRL 6541) and *Fusarium verticillioides* (NRRL 25457). Assays at 200 μg/disk indicated that both of them have significant activity against *A. flavus* (inhibition zones of 20 and 15 mm, respectively) and *F. verticillioides* (inhibition zones of 20 and 26 mm, respectively). Upon further evaluation, compound **1** displayed MIC values of approximately 10 and 5 μg/mL against *A. flavus* and *F. verticillioides*, respectively. Compound **3** was less active than **1**, affording an MIC value of 25 μg/mL against *F. verticillioides* and >25 μg/mL against *A. flavus* (growth of *A. flavus* was 27% that of controls at the 25 μg/mL level).

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Rudolph automatic polarimeter, model AP III. UV spectra were recorded with a Varian Cary III UV−visible spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained using Bruker DPX-300 and DRX-400 spectrometers, respectively. HMQC and HMBC data were obtained using a Bruker AMX-600. HPLC was carried out using a Beckman System Gold HPLC instrument with a model 166 detector. Other general procedures and instrumentation have been described previously.[17]

Fungal Material. Strain A-00267-2A was collected from submerged, decorticated wood in the Lemonweir River, Wisconsin, and was identified as *Decaisnella thyridioides* (Sacc. & Speg.) M. E. Barr (Pyrenulaceae, Melanommatales, Loculoascomycetes, Ascomycota). A voucher specimen and a subculture of the isolate employed in this work were deposited in the University of Illinois Department of Plant Biology fungal collection with the accession number A-00267-2A.

Ascospores were subcultured onto 250 g of rice in five 2-L Erlenmeyer flasks (50 g each) and incubated at 25 °C under 12 h light/12 h dark conditions for five weeks. The fermentation mixture was broken up with a spatula and extracted twice with EtOAc (2 × 500 mL). The combined EtOAc extract was filtered and evaporated to afford 2.3 g of crude extract.

Extraction and Isolation. The crude EtOAc extract was partitioned between CH_3CN and hexanes. The resulting CH_3CN fraction (1.7 g) was fractionated by Sephadex LH-20 chromatography using a hexanes/CH_2Cl_2/acetone solvent system to afford 20 fractions. Fraction 2 (398 mg) was subjected to silica gel column chromatography (CH_2Cl_2/MeOH gradient elution), yielding 10 fractions. Subfraction A (25 mg) contained one major component (palmarumycin CP_1; **6**). Subfraction B (31 mg) was further separated by reversed-phase HPLC (MeCN/H_2O, 50−60% over 20 min) on a Beckman Ultrasphere 5-μm C_8 column (250 × 10 mm) at a flow rate of 2 mL/min with UV detector at 215 nm to afford compounds **3** (13 mg), **4** (1.1 mg), and **5** (1.4 mg). Compound **1** precipitated from a CH_3CN solution of Sephadex LH-20 column fraction 3 (426 mg). After the solid (116 mg) was filtered, the filtrate was processed by reversed-phase HPLC under the same conditions as above to obtain compound **2** (1.9 mg) and an additional sample of compound **1** (40 mg).

Decaspirone A (1): colorless crystals (from CH_3CN); mp 141−142 °C; $[\alpha]^{25}_D$ +309 (*c* 0.56, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (5.0); 296 (3.9) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; selected HMBC data, H-2 → C-1, 3, 4, 4a, 8, 8a; H-3 → C-1, 4, 4a; H-4a → C-1, 4, 5, 6, 8, 8a; H-5 → C-4, 4a, 6, 7; H-6 → C-4a, 5, 7, 8; H-7 → C-5, 6, 8, 8a; H-8 → C-4a, 6, 7; H-8a → C-1, 4a, 5, 8; EIMS *m*/*z* 336 (M^+; 70), 318 [$(M - H_2O)^+$; 64], 197 (58), 160 (65), 131 (87), 58 (100); ESIMS *m*/*z* 337 $[M + H]^+$, 354 $[M + NH_4]^+$, 695 $[2M + Na]^+$; HREIMS *m*/*z* 336.0982 (calcd for $C_{20}H_{16}O_5$, 336.0998).

Decaspirone B (2): colorless glass; $[\alpha]^{25}_D$ +171 (*c* 0.085, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.5); 300 (3.6) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; COSY, H-2 ↔ H-3; H-3 ↔ H-4; H-4 ↔ H-4a; H-4a ↔ H-5; H-5 ↔ H-6; H-6 ↔ H-7; H-7 ↔ H-8; H-8 ↔ H-8a; H-8a ↔ H-4a; HREIMS *m*/*z* 380.1248 (calcd for $C_{22}H_{20}O_6$, 380.1260).

Decaspirone C (3): colorless glass; $[\alpha]^{25}_D$ +89 (*c* 0.58, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.8); 297 (3.8) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; selected HMBC data, H-2 → C-3, 4, 8, 8a; H-3 → C-1, 4a, 5; H-4a → C-1, 4, 5, 6, 8, 8a; H-5 → C-4, 6; H-6 → C-4a, 5; H-7 → C-8, 8a; H-8 → C-1, 4a, 5, 6; H-8a → C-1, 4, 4a, 5, 7, 8; HREIMS *m*/*z* 338.1155 $[M]^+$ (calcd for $C_{20}H_{18}O_5$, 338.1154).

Decaspirone D (4): colorless glass; $[\alpha]^{25}_D$ +102 (*c* 0.055, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.6); 276 (3.7) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; COSY, H-2 ↔ H-3; H-4a ↔ H-5; H-5 ↔ H-6; H-6 ↔ H-7; H-7 ↔ H-8; H-8 ↔ H-8a; H-8a ↔ H-4a; EIMS *m*/*z* 378 (M^+; 5.1), 377 (14), 270 (11), 158 (46), 113 (100).

Decaspirone E (5): colorless glass; $[\alpha]^{25}_D$ +171 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (4.6); 297 (3.7) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; COSY, H-2 ↔ H-3; H-4a ↔ H-5; H-5 ↔ H-6; H-6 ↔ H-7; H-7 ↔ H-8; H-8 ↔ H-8a; H-8a ↔ H-4a; EIMS *m*/*z* 378 (M^+; 2.1) 301 (7.5), 131 (9.1), 115 (100).

X-ray Crystallographic Analysis of Decaspirone A (1).[18] A needle obtained from CH_3CN solution (0.37 × 0.14 × 0.12 mm) was found to have crystallized in the orthorhombic class, space group $P2_12_12_1$ with cell dimensions a = 7.9766(8) Å, b = 10.4205(10) Å, c = 38.098(4) Å. Crystallographic data were collected on a Nonius Kappa CCD diffractometer (Mo Kα radiation, graphite monochromator) at 190(2) K (cold N_2 gas steam) using the standard CCD techniques, yielding 39 938 data. Lorentz and polarization corrections were applied. A correction for absorption using the multiscan technique also was applied ($T6_{max}$ = 0.9879, $T6_{min}$ = 0.9633). Equivalent data were averaged, yielding 4114 unique data (R_{int} = 0.41, 3577 $F > 4\sigma(F)$, Friedel pairs averaged). The computer programs from the HKL package were used for data reduction. The preliminary model of the structure was obtained using XS, a direct method program. Least-squares refining of the model versus the data was performed with the XL computer program. Illustrations were made with the XP program, and tables were made with the XCIF program. All are in the SHELXTL V6.1 package. There are two independent molecules in the asymmetric unit that are joined via hydrogen bonds to form a dimer. No further restraints or constraints were imposed on the refinement model. The final refinement gave R_1 = 0.0391, wR_2 = 0.0940.

Preparation of (*R*)-MTPA Ester 1a and (*S*)-MTPA Ester 1b. To a solution of **1** (12 mg, 0.036 mmol) in CH_2Cl_2 (2 mL) were added (*S*)-MTPA Cl (9.0 mg, 0.036 mmol) and DMAP (one crystal). After stirring at ambient temperature for 24 h, saturated aqueous $NaHCO_3$ was added, and the resulting mixture was extracted with CH_2Cl_2 (3 × 1 mL). The combined organic layers were evaporated to dryness and subjected to reversed-phase HPLC (Alltech Apollo 5-μm C_{18} column, 250 × 10 mm; flow rate 2 mL/min, UV detection at 215 nm, eluted with CH_3CN/H_2O, 40−100% over 30 min) to afford **1a** (7.2 mg): white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, J = 8.3 Hz, H-4′ or 5′), 7.49 (t, J = 7.6 Hz, H-3′ or 6′), 7.48 (d, J = 8.3 Hz, H-5′ or 4′), 7.28 (t, J = 7.6 Hz, H-6′ or 3′), 7.04 (d, J = 7.5 Hz, H-2′ or 7′), 6.99 (d, J = 10 Hz, H-2), 6.60 (d, J = 7.5 Hz, H-7′ or 2′), 6.25 (ddd, J = 10, 5.4, 2.2 Hz, H-7), 6.06 (dd, J = 10, 2.5 Hz, H-6), 6.02 (m, H-8), 6.02 (d, J = 10 Hz, H-3), 4.53 (br d, J = 9.3 Hz, H-5), 4.09 (br s, 5-OH), 3.31 (dd, J = 13, 9.3 Hz, H-4a), 2.94 (dd, J = 13, 2.8 Hz, H-8a); EIMS *m*/*z* 552 (M^+; 23), 318 (4), 189 (100).

In a similar fashion, another sample of compound **1** (7.4 mg, 0.022 mmol), (*R*)-MTPA Cl (5.6 mg, 0.022 mmol), and DMAP (one crystal) in CH_2Cl_2 (1.5 mL) were mixed together, allowed to react at room temperature for 24 h, and processed as described above for **1a** to afford **1b** (2.6 mg): white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 8.3 Hz, H-4′ or 5′), 7.48 (d, J = 8.3 Hz, H-5′ or 4′), 7.44 (t, J = 7.6 Hz, H-3′ or 6′), 7.19 (t, J = 7.6 Hz, H-6′ or 3′), 6.99 (d, J = 7.5 Hz, H-2′ or 7′), 6.91 (d, J = 10 Hz, H-2), 6.37 (d, J = 7.5 Hz, H-7′ or 2′), 6.22 (ddd, J = 10, 5.3, 2.1 Hz, H-7), 6.09 (dd, J = 10, 2.5 Hz, H-6), 6.06 (m, H-8), 5.98 (d, J = 10 Hz, H-3), 4.55 (br d, J = 9.1 Hz, H-5), 4.15 (br s, OH-5), 3.33 (dd, J = 13, 9.1 Hz, H-4a), 2.92 (dd, J = 13, 2.8 Hz, H-8a).

MIC Determinations. *Aspergillus flavus* (NRRL 6541) was grown in Roux bottles containing potato dextrose agar (PDA) for 14 days (25 °C). A conidial spore suspension (propagule density 10^6/mL in sterile distilled H_2O) prepared from the Roux bottle cultures served as the inoculum. Hyphal fragments and conidium-bearing structures were removed by filtering through a double layer of sterile cheese cloth.

Compounds were evaluated in 96-well plates with a growth area of 0.32 cm^2 and volume of 370 μL (BD Primaria Clear 96-well microtest plate no. 353872, Becton-Dickinson) at concentrations of 1, 3, 5, 10, and 25 μg/mL. Appropriate amounts of test compound in 10 μL of MeOH were added to each of eight replicate wells and evaporated to dryness. Eight replicate wells received only 10 μL of MeOH and served as controls. Potato dextrose broth (PDB) was seeded with *A. flavus* conidia, giving a final conidial suspension of approximately 4×10^4/mL PDB. A small quantity of MeOH (10 μL) was added to each well to solubilize the test compound, and then 200 μL of PDB containing ca. 8000 *A. flavus* conidia was added to each test well. The plates were incubated for 48 h at 25 °C and examined at 8−16 h intervals using a plate reader (Dynatech MR 5000 with BioLinx Version 2.0 Assay Management Software; Dynatech Laboratories, Inc.) for evidence of inhibition of fungal growth in the wells. A minimum inhibitory concentration (MIC) was assigned to the lowest treatment concentration for which no fungal growth was observed. Nystatin was used as a positive control and gave an MIC value of approximately 10 mg/mL using this protocol. An analogous process was used for the assay against *Fusarium verticillioides* NRRL 25457.

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Supporting Information Available: X-ray data table for **1**, ^{1}H NMR spectra for compounds **1**−**5**, and ^{13}C NMR spectra for compounds **1**, **2**, and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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(14) Upon nearing completion of the manuscript, we became aware that another group had independently isolated and characterized (from a different fungal source) a series of four other compounds that are very close analogues of those described here. Because of the similarities in the structures, a mutual decision was made to name them all as decaspirones and to submit the corresponding manuscripts simultaneously to the *Journal* (see ref 15).
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(18) Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 284776). 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 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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