Decaspirones F−I, Bioactive Secondary Metabolites from the Saprophytic Fungus *Helicoma viridis*

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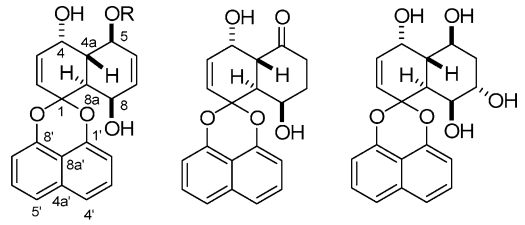
Received August 3, 2006

Decaspirones F−H (**1**−**3**), three new spirobisnaphthalene derivatives, and one new non-spirobisnaphthalene, decaspirone I (**4**), have been isolated from cultures of an isolate of the saprophytic fungus *Helicoma viridis*. The structures of these compounds were determined mainly by analysis of their NMR spectroscopic data. The absolute configuration of decaspirone F (**1**) was established by X-ray crystallographic analysis of its mono-bromobenzoate derivative. In standard disk assays, compounds **1**−**4** showed modest activity against *Pseudomonas aeruginosa* (multiple antibiotic-resistant strain, ATCC 27853), and decaspirone G (**2**) also displayed activity against *Lactococcus lacti* (PCM 2379).

Several natural products containing the spiroketal moiety have been isolated from fungal sources, such as the pressomerins,[1−3] the palmarumycins,[4,5] diepoxin,[6,7] the cladospirone bisepoxides,[8,9] sch49209,[10] the spiroxins,[11] and the recently reported spiromamakone A.[12] During our ongoing investigations of rarely studied fungal species as sources of new bioactive natural products, a subculture of an isolate of *Helicoma viridis* (2203), obtained from a split of decaying branches of an unidentified tree on Wuling Mountain, Hebei Province, People's Republic of China, was grown in liquid culture, and the organic extract displayed antibacterial activity against *Pseudomonas aeruginosa* (multiple antibiotic-resistant strain, ATCC 27853). Bioassay-guided fractionation of this extract led to the isolation of three new spirobisnaphthalene derivatives and one new non-spirobisnaphthalene, which have been named decaspirones F−I (**1**−**4**), along with the known compound diepoxin η (**5**). Details of the isolation, structure elucidation, and antimicrobial activity of these compounds are reported here.



1 R = H
1a R = CO-*P*-C_6H_4Br

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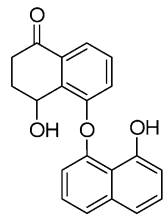

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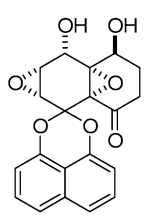

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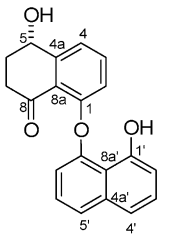

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Results and Discussion

The molecular formula of decaspirone F (**1**) was determined to be $C_{20}H_{18}O_5$ (12 degrees of unsaturation) by HRESIMS analysis [*m/z* 361.1033 $(M + Na)^+$; Δ +1.3 mmu], and this conclusion was supported by the ^1H and ^{13}C NMR data (Table 1). Detailed analysis of ^1H, ^{13}C, and HMQC NMR data for decaspirone F (**1**) revealed the presence of five methine carbons (three of which are oxygenated), four protonated olefinic carbons, 10 aromatic carbons (six of which are protonated), and one doubly oxygenated quaternary carbon (δ_C 101.3). These data accounted for all but three exchangeable protons and required decaspirone F (**1**) to be pentacyclic. Analysis of the COSY NMR data led to the identification of three isolated proton spin-systems corresponding to the C-2−C-8 (including C-4a−C-8a), C-2′−C-4′, and C-5′−C-7′ subunits of structure **1**. HMBC correlations of H-2, H-3, H-8, and H-8a with C-1 led to the completion of a decalin system. Correlations of H-2′ with C-1′ and C-8a′, H-7′ with C-8 and C-8a′, and H-4′ and H-5′ with C-4a′ and C-8a′ revealed that the C-1′−C-4′ and C-5′−C-8′ subunits were joined at C-4a′ and C-8a′, leading to the completion of a naphthalene moiety. The chemical shifts of C-1′ (δ_C 154.4) and C-8′ (δ_C 153.8) indicated that C-1 and C-8′ were oxygenated. Both the chemical shift of C-1 (δ_C 101.3) and the pentacyclic nature of decaspirone F suggested the presence of a ketal moiety at C-1 between the decalin and naphthalene systems. In addition, the molecular formula of **1** requires that C-4, C-5, and C-8 bear free hydroxyl groups. On the basis of these data, the planar structure of decaspirone F was established as depicted in **1**.

The relative configuration of decaspirone F (**1**) was proposed as shown in Figure 1 by analysis of $^1H-^1H$ coupling constants and NOESY data. The large *trans*-diaxial-type coupling constant observed between H-4a and H-8a (14 Hz) in **1** indicated that H-4a and H-8a must be in pseudoaxial orientations with respect to the corresponding six-membered rings and *trans* to each other in the decalin system. The small coupling constants observed for H-4 (4.7 Hz) and H-8 (2.9 Hz) indicated that H-4 and H-8 must adopt pseudoequatorial orientations, and the observed coupling constant of 7.9 Hz for H-5 was used to position it in a pseudoaxial orientation. Key NOESY correlations between H-4 and H-5, H-4 and H-8a, H-4a and H-8, and H-5 and H-8a further supported the above assignments (Figure 1).

Ultimately, the structure of decaspirone F (**1**) was confirmed by single-crystal X-ray diffraction analysis of its 5-bromobenzoate derivative (**1a**), and a perspective ORTEP plot is shown in Figure 2. The X-ray data also allowed determination of the absolute configuration of all chiral centers in **1** (4*S*, 5*S*, 4a*S*, 8a*S*, and 8*R*).

Decaspirone G (**2**) was assigned the same molecular formula $C_{20}H_{18}O_5$ as decaspirone F (**1**) on the basis of HRESIMS analysis [*m/z* 361.1044 $(M + Na)^+$; Δ + 0.2 mmu] and NMR data (Table 2). Analysis of ^1H and ^{13}C NMR data of **2** revealed the presence

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10.1021/np0603830 CCC: $33.50
Published on Web 10/14/2006

Table 1. NMR Spectroscopic Data for Decaspirone F (**1**) in CD_3OD

position	δ_H[a] (*J* in Hz)	δ_C,[b] mult.	HMBC (H → C#)	NOESY
1		101.3, qC		
2	5.82, d (10)	126.1, CH	3, 8a, 4	4
3	6.05, dd (10, 4.7)	134.7, CH	4a, 4, 1, 2	
4	4.41, t (4.7)	62.6, CH	4a, 8a, 2, 3	2, 8a
4a	2.60, ddd (14, 7.9, 4.7)	39.7, CH	8a, 5, 8, 1	8
5	4.65, dd (7.9, 2.8)	63.3, CH		4, 8a, 7
6	5.93, dd (9.9, 2.8)	134.8, CH	4a, 5, 8, 7	8
7	6.00, dd (9.9, 5.1)	129.7, CH	8a, 5, 8	5
8	4.74, dd (5.1, 2.9)	67.5, CH	1, 7, 6, 8a	4a, 6
8a	2.49, dd (14, 2.9)	43.1, CH	4a, 4, 5, 8, 1′, 8′, 1′	4a, 6
1′		148.3, qC		
2′	6.91, d (7.6)	110.6, CH	8a′, 4′, 4a′, 1′	4′
3′, 6′	7.42, m	128.4/128.6, CH	2′, 7′, 8a′, 4a′, 1′	
4′, 5′	7.50, d (8.1)	121.9/121.7, CH	2′, 7′, 8a′, 4′, 5′, 4a′, 1′, 8′	2′, 7′
4a′		135.7, qC		
7′	6.93, d (7.6)	110.7, qC	8a′, 5′, 4a′, 8′	5′
8′		148.7, qC		
8a′		115.0, qC		

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

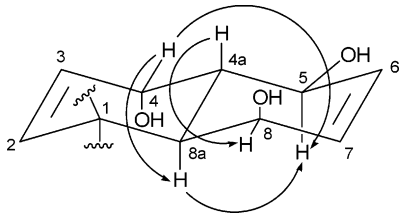

Figure 1. Key NOESY correlations for decaspirone F (**1**).

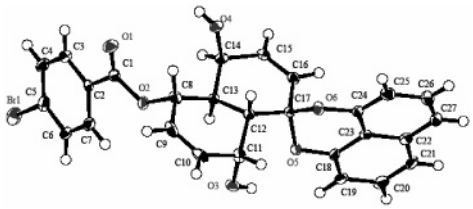

Figure 2. Thermal ellipsoid representation of 5-bromobenzoyldecaspirone F (**1a**).

of structural features similar to those found in **1**, except that the oxygenated methine carbon (C-5) and one olefinic unit (C-6−C-7) in **1** were replaced by a new ketone carbon (δ_C 211.8) and two aliphatic methylene units (δ_C 30.9, and 36.1, respectively). COSY NMR data for **2** indicated that these two methylene units are adjacent to each other and one of them (δ_C 30.9) is joined to C-8. The molecular formula for **2** required that the ketone carbon be connected to C-4a and C-6, leading to assignment of structure **2** for decaspirone G. HMBC correlations of H-4, H-4a, H_2-6, and H_2-7 with the ketone carbon at δ_C 211.8 further confirmed this assignment. The relative configuration of decaspirone G (**2**) was established by analysis of $^1H-^1H$ NMR coupling constants and NOESY data and by comparison of its ^1H NMR data with those of decaspirone F (**1**). The small coupling constant (1.4 Hz) observed between H-8 and H-8a indicated that H-8 must adopt a pseudoequatorial orientation. The absolute configuration was proposed as 4*S*, 5*S*, 4a*R*, 8a*S*, 8*R*, by analogy with decaspirone F (**1**).

The elemental composition of decaspirone H (**3**) was established as $C_{20}H_{20}O_6$ by analysis of its HRESIMS [*m/z* 379.1166 $(M + Na)^+$; Δ −1.4 mmu] and NMR data. Detailed analysis of the ^1H and ^{13}C NMR data for **3** (Table 2) indicated that signals for the olefinic unit (C-6−C-7) in **1** were replaced by one oxygenated methine unit (δ_H 3.94; δ_C 69.2) and one methylene unit (δ_H 2.00, 2.12; δ_C 35.9) in the NMR spectra of **3**. On the basis of these observations, the structure of decaspirone H was proposed as shown in **3** and confirmed by analysis of its COSY NMR data. The small coupling constant observed between H-6 and H-7 (2.4 Hz) indicated that H-7 adopts a pseudoequatorial orientation. The stereochemistry of decaspirone H was deduced as shown in **3** by comparison of its NMR data with those of **1**.

The molecular formula of the final compound decaspirone I (**4**) was determined to be $C_{20}H_{16}O_4$ (13 degrees of unsaturation) by HRESIMS [*m/z* 343.0950, $(M + Na)^+$] and its NMR data. The ^1H and ^{13}C NMR data (Table 3) for **4** differed significantly from those of **1**−**3**, suggesting a substantial structural change. Upon extensive analysis of COSY and HMBC correlations, two structural subunits corresponding to the C-1−C-8 (including C-4a and C-8a) and C-1′−C-8′ (including C-4a′ and C-8a′) moieties were established. The chemical shifts of C-1 (δ_C 156.7), C-1′ (δ_C 154.4), C-8 (δ_C 61.8), and C-8′ (δ_C 153.8) in the ^{13}C NMR spectrum of **4** indicated that these carbons were oxygenated. Because no exchangeable protons were observed in the ^1H NMR spectrum of **4** when it was recorded in methanol-d_4, no HMBC correlations could be used to connect the two structural subunits. When acetone-d_6 was used as solvent, two additional signals for exchangeable protons at δ_H 4.70 and 9.02 were observed in the ^1H NMR spectrum of **4**. Key HMBC correlations of one exchangeable proton (δ_H 4.70) with C-7 and C-8a and the other exchangeable proton (δ_H 9.02) with C-1′, C-2′, and C-8a′ were observed, indicating that C-8 and C-1′ bear free hydroxyl groups. These results required C-1 and C-8′ to be connected to the same oxygen atom to form an ether linkage. On the basis of these data, the structure of decaspirone I was established as shown in **4**. The small $^1H-^1H$ coupling constant observed between H_2-7 and H-8 indicated that H-8 must adopt a pseudoequatorial orientation in the six-membered ring.

Decaspirones F−I (**1**−**4**) showed activity in standard disk assays against *Pseudomonas aeruginosa* (multiple antibiotic-resistant strain ATCC 27853), affording zones of inhibition of 8 to 10 mm at 100 μg/disk (ciprofloxacin: 22 mm zone of inhibition at 100 μg/disk). Decaspirone G (**2**) also exhibited activity against *Lactococcus lactis* (PCM 2379), causing a zone of inhibition of 12 mm at 100 μg/disk. However, none of these compounds displayed antimicrobial activity against *Staphylococcus aureus* (ATCC 6538), *Streptococcus mutans* (ATCC 25175), *Enterococcus faecalis* (ATCC 19433), and *Sarcina lutea* (CMCC B28001) or antifungal activity against *Geotrichum candidum* (AS2.498), *Candida albicans* (ATCC 10231), and *Aspergillus fumigatus* (ATCC 10894) at 100 μg/disk.

In all probability, the biosynthesis of decaspirones F−H (**1**−**3**) proceeds in a manner similar to that of other spirobisnaphthalenes, with both halves of the molecule being derived from dihydroxynaphthalene (DHN), as described in the literature.[13] Decaspirone I (**4**) could be a biosynthetic precursor for palmarumycin CP1, as suggested in a prior report.[4] The core structure of decaspirone F (**1**) is closely related to the known compound palmarumycin CR_1.[14]

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data for Decaspirones G (**2**) and H (**3**) in CD_3OD

	decaspirones G (**2**)		decaspirones H (**3**)	
position	δ_H[a] (*J* in Hz)	δ_C,[b] mult.	δ_H[a] (*J* in Hz)	δ_C,[b] mult.
1		101.7, qC		102.4, qC
2	5.79, d (10)	126.3, CH	5.80, d (10)	125.3, CH
3	5.97, dd (10, 4.2)	133.6, CH	6.04, dd (10, 4.9)	134.7, CH
4	4.70, t (4.2)	63.6, CH	4.40, dd (4.9, 4.6)	62.3, CH
4a	3.22, dd (13. 4.2)	47.8, CH	2.33, ddd (13, 12, 4.6)	41.0, CH
5		211.8, qC	4.33, ddd (13, 12, 5.2)	70.4, CH
6b	2.40, ddd (17, 6.5, 2.5)	36.1, CH_2	2.00, m	35.9, CH
6a	2.83, ddd (17, 12, 7.5)		2.12, dt (13, 2.4)	
7b	2.04, m	30.9, CH_2	3.94, m	69.2, CH
7a	2.16, m			
8	4.87, m	64.1, CH	4.44, dd (2.9, 1.6)	65.5, CH
8a	2.92, dd (13, 1.4)	45.0, CH	2.49, dd (13, 2.9)	39.4, CH
1′		148.2, qC		148.8, qC
2′	6.94, d (7.5)	110.7, CH	6.93, t (7.0)	110.9, CH
3′, 6′	7.45, m	128.5, CH	7.44, m	128.7, CH
		128.7, CH		128.5, CH
4′, 5′	7.54, dd (8.2, 2.0)	121.9, CH	7.53, d (8.2)	122.5, CH
		122.1, CH		121.7, CH
4a′		135.7, qC		135.7, qC
7′	6.97, d (7.4)	110.9, CH	6.93, t (7.0)	110.6, CH
8′		148.7, qC		148.0, qC
8a′		115.0, qC		115.0, qC

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

Table 3. NMR Spectroscopic Data for Decaspirone I (**4**) in CD_3OD

position	δ_H[a] (*J* in Hz)	δ_C,[b] mult.	HMBC (H → C#)	NOESY
1		156.7, qC		
2	7.00, d (8.2)	123.8, CH	4, 8a, 1	
3	7.40, m	130.6, CH	1, 4a	
4	7.80, d (7.7)	122.5, CH	2, 8a, 5	
4a		134.4, qC		
5		199.8, qC		
6a	2.60, dt (17, 3.6)	33.7, CH_2	7, 4a, 8, 5	
6b	3.11, dt (17, 9.4)		7, 8a, 5	8
7	2.34, dt (9.4, 3.6)	31.0, CH_2	6, 5, 8, 8a	
8	5.41, t (3.6)	61.8, CH	6, 1, 4a, 8a	6a
8a		135.7, qC		
1′		154.4, qC		
2′	6.84, d (8.0)	112.0, CH	8a′, 4′, 1′	
3′	7.35, m	128.6, CH	1′, 4a′	
4′	7.38, m	120.6, CH	2′, 8a′, 5′	
4a′		139.0, qC		
5′	7.65, d (8.2)	125.9, CH	7′, 8a′, 4′, 4a′	
6′	7.36, m	126.9, CH	8′, 4a′	
7′	6.90, d (7.4)	115.3, CH	8a′, 5′, 8′	
8′		153.8, qC		
8a′		118.2, qC		

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

However, decaspirones F−H (**1**−**4**) differ from most known palmarumycins by virtue of the presence of a C-2−C-3 olefinic unit and a 4-hydroxy methine unit rather than an epoxide moiety.[15,16] The structure of decaspirone I (**4**) is closely related to the hypothetical open-chain compound (**6**) proposed as a precursor to the palmarumycins,[17] but differs significantly in the identity of the substituents and substitution pattern on the top half of the compound.

To our knowledge, decaspirones F−I (**1**−**4**) are the first secondary metabolites to be reported from *H. viridis*.

Experimental Section

General Experimental Procedures. The optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^{1}H and ^{13}C NMR data were acquired with a Bruker Avance-400 spectrometer using solvent signals (CD_3OD; δ_H 3.30/δ_C 49.5) 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 data were obtained using a Bruker APEX III 7.0 T spectrometer.

Fungal Material. The isolate of *H. viridis* employed in this study was isolated by Mr. Guozhu Zhao from the split of decaying branches of an unidentified tree near the waterfall of Dragon Pond in Wuling Mountain, Hebei Province, on May 17, 2004. The isolate was identified by Mr. Zhao and assigned the accession number 2203 in the Professor X. Liu culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The isolate was subcultured on PDA slants at 25 °C for 15 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. Twenty 500 mL Erlenmeyer flasks, each containing 150 mL of liquid media (0.5% glucose, 3% soluble starch, 3% soybean flour, 0.5% corn steep powder, 0.5% yeast extract, and 0.3% $CaCO_3$; final pH 6.0), were individually inoculated with 15 mL of the seed culture and incubated at 25 °C on a rotary shaker at 150 rpm for 10 days.

Extraction and Isolation. The fermented material (3 L) was freeze-dried and extracted with MeOH (3 × 500 mL), and the organic solvent was evaporated to dryness under vacuum to afford 1.96 g of crude extract. The extract was partitioned between EtOAc and water three times, and the EtOAc portion (857 mg) was fractionated by silica gel VLC using petroleum ether−EtOAc gradient elution. The fractions that were eluted with 15% (200 mg), 20% (150 mg), and 100% (33 mg) EtOAc were further separated by semipreparative reversed-phase HPLC (Kramosil C_{18} column; 10 μm; 10 × 250 mm, 2 mL/min). Purification of these fractions using different gradients afforded decaspirone F (**1**; 100 mg, t_R 30 min; 40% MeOH in H_2O over 10 min, 40−70% over 30 min), the known compound diepoxin η (**5**; 8 mg, t_R 25 min; same gradient as in purification of **1**), decaspirone G (**2**; 3.9 mg; t_R 27 min; 40% MeOH in H_2O over 10 min, 40−60% over 30 min), decaspirone H (**3**; 3.7 mg; t_R 20 min; 40% MeOH in H_2O over 10 min, 40−60% over 30 min), and decaspirone I (**4**; 5.3 mg; t_R 35 min; 60% MeOH in H_2O over 10 min, 60−80% over 40 min).

Decaspirone F (1): colorless platelets, mp 235−238 °C; $[\alpha]_D$ +87 (*c* 1.15, CH_3OH); UV (CH_3OH) λ_{max} 225 (ϵ 45 000), 300 (ϵ 7600) nm; IR (CH_2Cl_2) ν_{max} 3513, 3441, 3330, 3057, 2919, 2893, 1924, 1754, 1632, 1612, 1436, 1416, 1397, 1383 cm^{-1}; ^{1}H NMR, ^{13}C NMR, NOESY, and HMBC data, see Table 1; HRESIMS *m*/*z* 361.1033 [M + Na]$^+$, calcd for $C_{20}H_{18}O_5Na$, 361.1046.

5-Bromobenzoate of Decaspirone F (1a). A sample of **1** (7 mg) was dissolved in CH_2Cl_2 (2 mL) in a 10 mL round-bottomed flask and combined with DMAP (4.5 mg) and 4-bromobenzoyl chloride (6.4 mg). The reaction vessel was sealed and the contents were stirred at room

temperature for 5 h. The reaction mixture was separated by reversed-phase HPLC (Agilent 1100, Kramosil C_{18} column; 10 μm; 10 × 250 mm; 80−100% MeOH in H_2O over 30 min, 2 mL/min) to afford the 5-bromobenzoate derivative of decaspirone F (**1a**; 4.6 mg; yield: 43%; t_R 16.5 min) as colorless platelets: ^{1}H NMR ($CDCl_3$, 400 Hz) 7.53 (1H, d, J = 8.4 Hz, H-4′ or H-5′), 7.52 (2H, d, 8.4 Hz, H-5′ or H-4′), 7.43 (2H, m, H-3′ and H-6′), 6.95 (1H, d, J = 7.4 Hz, H-2′ or H-7′), 6.90 (1H, d, J = 7.5 Hz, H-7′ or H-2′), 6.30 (1H, ddd, J = 8.4, 4.8, 1.8 Hz, H-7), 6.18 (1H, td, J = 8.4, 2.5 Hz, H-6), 6.06 (1H, dd, J = 4.4, 10 Hz, H-3), 5.95 (2H, m, H-2 and H-5), 4.94 (1H, dd, J = 4.8, 2.5 Hz, H-8), 4.30 (1H, t, J = 4.4 Hz, H-4), 3.97 (1H, br s, OH-8 or OH-4), 3.87 (1H, br s, OH-8 or OH-4), 3.02 (1H, ddd, J = 14.4, 8.4, 4.4 Hz, H-4a), 2.70 (1H, dd, J = 14, 2.5, 14 Hz, H-8a); ESIMS m/z 543 $[M + Na]^+$.

X-ray Crystallographic Analysis of 1a.[18] A colorless crystal of **1a** (0.32 × 0.28 × 0.08 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a Rigaku Saturn CCD area detector with graphite monochromator and Mo Kα radiation at −160(1) °C. Cell dimensions were determined to be a = 5.5563(13) Å, b = 11.793(3) Å, c = 16.708(4) Å. The 8021 measurements yielded 4847 independent reflections after equivalent data were averaged and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0262 and wR_2 = 0.0504.

Decaspirone G (2): colorless powder, mp 230−233 °C; $[\alpha]_D$ +116 (c 0.16, CH_3OH); UV (CH_3OH) λ_{max} 225 (ϵ 44 000), 300 (ϵ 8900) nm; IR (CH_2Cl_2) ν_{max} 3453 (br), 3059, 2926, 2854, 1694, 1608, 1413, 1381 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HMBC data (CD_3OD, 400 MHz) H-7b → C-8a, 5; H-6a → C-8, 5; H-6b → C-5; H-4 → C-8a, 2; H-2 → C-8a, 4; H-3 → C-4a; H-2′ → C-8a′, 4′; H-7′ → C-8a′, 5′; H-3′, 6′ → C-4a′, 1′, 8′; H-4′, 5′ → C-2′, 7′, 8a′, 4′, 5′; key NOESY correlations (CD_3OD, 400 MHz) H-7a → H-8a; HRESIMS m/z 361.1044 [M+ Na]$^+$, calcd for $C_{20}H_{18}O_5$, 361.1046.

Decaspirone H (3): colorless powder, mp 148−150 °C; $[\alpha]_D$ +189 (c 0.31, CH_3OH); UV (CH_3OH) λ_{max} 225 (ϵ 22 800), 300 (ϵ 3900) nm; IR (CH_2CH_2) ν_{max} 3386, 3059, 2925, 2854, 1609, 1637, 1584, 1413, 1381 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HMBC data (CD_3OD, 400 MHz) H-6a → C-4a, 8; H-6b → C-4a, 8; H-4a → C-1, 8; H-8a → C-4, 7, 8, 8′; H-7 → C-8a; H-3 → C-4a; H-5 → C-4; H-4 → C-2, 5, 8a; H-2 → C-4, 8a; H-3 → C− 1, 4a; H-2′, 7′ → C-4′, 5′, 8a′; H-3′, 6′ → C-1′, 8a′, 4a′; H-4′, 5′ → C-2′, 7’, 8a′, 4′, 5′, 1′, 8′; key NOESY correlations (CD_3OD, 400 MHz) H-6b → H-4a; H-8a → H-5; HRESIMS m/z 379.1166 [M + Na]$^+$, calcd for $C_{20}H_{20}O_6$, 379.1152.

Decaspirone I (4): colorless powder, mp 147−150 °C; $[\alpha]_D$ +104 (c 0.18, CH_3OH); UV (CH_3OH) λ_{max} 225 (ϵ 35 000), 300 (ϵ 2700) nm; IR (KBr) ν_{max} 3385, 3190, 3053, 2954, 2925, 2851, 1726, 1687, 1598, 1577 cm^{-1}; ^{1}H NMR, ^{13}C NMR, NOESY, and HMBC data in methanol-d_4, see Table 3; ^{1}H NMR (acetone-d_6, 400 MHz) δ 9.02 (1H, br, OH-1′), 7.79 (1H, dd, J = 7.7, 0.72 Hz, H-4), 7.67 (1H, dd, J = 8.3, 0.64 Hz, H-5′), 7.45 (4H, m, H-3, 3′, 4′, 6′), 7.24 (1H, dd, J = 8.1, 1.1 Hz, H-2), 6.91 (1H, dd, J = 7.3, 1.4 Hz, H-2′ or H-7′), 6.85 (1H, dd, J = 7.7., 0.84 Hz, H-7′ or H-2′), 5.38 (1H, dd, J = 6.9, 3.4 Hz, H-8), 4.70 (1H, d, J = 3.4 Hz, OH-8), 3.09 (1H, ddd, J = 17, 11, 7.4 Hz, H-6a), 2.53 (1H, td, J = 17, 3.5 Hz, H-6b), 2.34 (2H, m, H-7); key HMBC data (acetone-d_6, 400 MHz) OH-8 → C-7, C-8a; OH-1′ → C-1′, C-2′, and C-8a′; HRESIMS m/z 343.0950 [M + Na]$^+$, calcd for $C_{20}H_{16}O_4$, 343.0941.

Diepoxin η (5): ^{1}H NMR, ^{13}C NMR, and the ESIMS data were fully consistent with literature values.[7]

Bioassays. Antimicrobial and antifungal bioassays were conducted according to a literature procedure.[19] The bacterial strains were grown on Mueller-Hinton agar, 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 agar. The assay plates were incubated at 25 °C for 48 h (at 37 °C for 24 h for antimicrobial activity) and examined for the presence of a zone of inhibition.

Acknowledgment. We thank Professor J. B. Gloer of the University of Iowa for proofreading the manuscript and for valuable suggestions. Financial support from the Institute of Microbiology, Chinese Academy of Sciences (startup funding from the "100 Talents Program"), is gratefully acknowledged.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of decaspirones F−I (**1**−**4**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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(15) 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 five 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 *J. Nat. Prod.* (see ref 16).
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(18) Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 616787). 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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NP0603830