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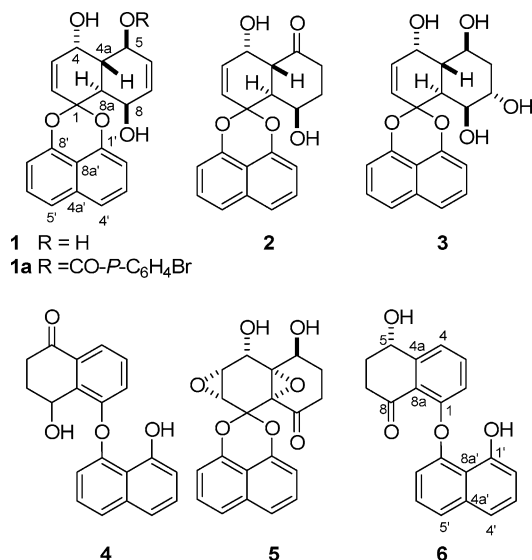
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Decaspirones F–H (**1**–**3**), three new spirobisnaphthalene derivatives, and one new non-spirobisnaphthalene, decaspirone I (**4**), have been isolated from cultures of an isolate of the saprophytic fungus *Helicoma viridis*. The structures of these 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 spiro-mamakone 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 **5** (**5**). Details of the isolation, structure elucidation, and antimicrobial activity of these compounds are reported here.



Results and Discussion

The molecular formula of decaspirone F (**1**) was determined to be C₂₀H₁₈O₅ (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 ¹³C NMR data (Table 1). Detailed analysis of ¹H, ¹³C, 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** (4S, 5S, 4aS, 8aS, and 8R).

Decaspirone G (**2**) was assigned the same molecular formula C₂₀H₁₈O₅ 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 ¹³C NMR data of **2** revealed the presence

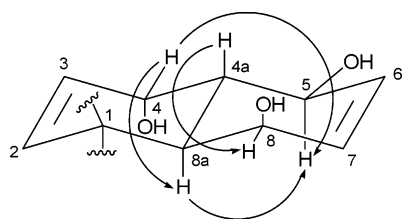
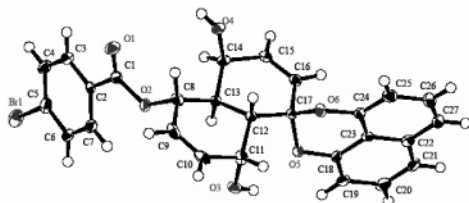
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Table 1. NMR Spectroscopic Data for Decaspirone F (**1**) in CD₃OD

position	δ_{H}^a (J in Hz)	δ_{C}^b mult.	HMBC (H \rightarrow 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, H₂-6, and H₂-7 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 4S, 5S, 4aR, 8aS, 8R, by analogy with decaspirone F (**1**).

The elemental composition of decaspirone H (**3**) was established as C₂₀H₂₀O₆ by analysis of its HRESIMS [*m/z* 379.1166 (M + Na)⁺; Δ –1.4 mmu] and NMR data. Detailed analysis of the ¹H and ¹³C 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 C₂₀H₁₆O₄ (13 degrees of unsaturation) by HRESIMS [*m/z* 343.0950, (M + Na)⁺] and its NMR data. The ¹H and ¹³C 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 ¹³C 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 H₂-7 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 $\mu\text{g}/\text{disk}$ (ciprofloxacin: 22 mm zone of inhibition at 100 $\mu\text{g}/\text{disk}$). Decaspirone G (**2**) also exhibited activity against *Lactococcus lactis* (PCM 2379), causing a zone of inhibition of 12 mm at 100 $\mu\text{g}/\text{disk}$. 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 $\mu\text{g}/\text{disk}$.

In all probability, the biosynthesis of decaspirones F–H (**1**–**3**) proceeds in a manner similar to that of other spirobisanaphthalenes, 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 CR₁.¹⁴

Table 2. ¹H and ¹³C NMR Spectroscopic Data for Decaspirones G (2) and H (3) in CD₃OD

position	decaspirones G (2)		decaspirones H (3)	
	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 CD₃OD

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. ¹H and ¹³C NMR data were acquired with a Bruker Avance-400 spectrometer using solvent signals (CD₃OD; 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% CaCO₃; 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 C₁₈ 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 H₂O over 10 min, 40–70% over 30 min), the known compound diepoxin **7** (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 H₂O over 10 min, 40–60% over 30 min), decaspirone H (3; 3.7 mg; *t_R* 20 min; 40% MeOH in H₂O over 10 min, 40–60% over 30 min), and decaspirone I (4; 5.3 mg; *t_R* 35 min; 60% MeOH in H₂O over 10 min, 60–80% over 40 min).

Decaspirone F (1): colorless platelets, mp 235–238 °C; [α]_D +87 (c 1.15, CH₃OH); UV (CH₃OH) λ_{max} 225 (ε 45 000), 300 (ε 7600) nm; IR (CH₂Cl₂) ν_{max} 3513, 3441, 3330, 3057, 2919, 2893, 1924, 1754, 1632, 1612, 1436, 1416, 1397, 1383 cm⁻¹; ¹H NMR, ¹³C NMR, NOESY, and HMBC data, see Table 1; HRESIMS *m/z* 361.1033 [M + Na]⁺, calcd for C₂₀H₁₈O₅Na, 361.1046.

5-Bromobenzoate of Decaspirone F (1a). A sample of 1 (7 mg) was dissolved in CH₂Cl₂ (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, Kromasil C₁₈ column; 10 μ m; 10 \times 250 mm; 80–100% MeOH in H₂O over 30 min, 2 mL/min) to afford the 5-bromobenzoate derivative of decaspironone **1a** (4.6 mg; yield: 43%; t_R 16.5 min) as colorless platelets: ¹H NMR (CDCl₃, 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 \times 0.28 \times 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 K α 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.

Decaspironone G (2): colorless powder, mp 230–233 °C; [α]_D +116 (c 0.16, CH₃OH); UV (CH₃OH) λ_{max} 225 (ϵ 44 000), 300 (ϵ 8900) nm; IR (CH₂Cl₂) ν_{max} 3453 (br), 3059, 2926, 2854, 1694, 1608, 1413, 1381 cm^{–1}; ¹H and ¹³C NMR data, see Table 2; HMBC data (CD₃OD, 400 MHz) H-7b \rightarrow C-8a, 5; H-6a \rightarrow C-8, 5; H-6b \rightarrow C-5; H-4 \rightarrow C-8a, 2; H-2 \rightarrow C-8a, 4; H-3 \rightarrow C-4a; H-2' \rightarrow C-8a', 4'; H-7' \rightarrow C-8a', 5'; H-3', 6' \rightarrow C-4a', 1', 8'; H-4', 5' \rightarrow C-2', 7', 8a', 4', 5'; key NOESY correlations (CD₃OD, 400 MHz) H-7a \rightarrow H-8a; HRESIMS m/z 361.1044 [M + Na]⁺, calcd for C₂₀H₁₈O₅, 361.1046.

Decaspironone H (3): colorless powder, mp 148–150 °C; [α]_D +189 (c 0.31, CH₃OH); UV (CH₃OH) λ_{max} 225 (ϵ 22 800), 300 (ϵ 3900) nm; IR (CH₂CH₂) ν_{max} 3386, 3059, 2925, 2854, 1609, 1637, 1584, 1413, 1381 cm^{–1}; ¹H and ¹³C NMR data, see Table 2; HMBC data (CD₃OD, 400 MHz) H-6a \rightarrow C-4a, 8; H-6b \rightarrow C-4a, 8; H-4a \rightarrow C-1, 8; H-8a \rightarrow C-4, 7, 8, 8'; H-7 \rightarrow C-8a; H-3 \rightarrow C-4a; H-5 \rightarrow C-4; H-4 \rightarrow C-2, 5, 8a; H-2 \rightarrow C-4, 8a; H-3 \rightarrow C-1, 4a; H-2', 7' \rightarrow C-4', 5', 8a'; H-3', 6' \rightarrow C-1', 8a', 4a'; H-4', 5' \rightarrow C-2', 7', 8a', 4', 5', 1', 8'; key NOESY correlations (CD₃OD, 400 MHz) H-6b \rightarrow H-4a; H-8a \rightarrow H-5; HRESIMS m/z 379.1166 [M + Na]⁺, calcd for C₂₀H₂₀O₆, 379.1152.

Decaspironone I (4): colorless powder, mp 147–150 °C; [α]_D +104 (c 0.18, CH₃OH); UV (CH₃OH) 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}; ¹H NMR, ¹³C NMR, NOESY, and HMBC data in methanol-*d*₄, see Table 3; ¹H 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 \rightarrow C-7, C-8a; OH-1' \rightarrow C-1', C-2', and C-8a'; HRESIMS m/z 343.0950 [M + Na]⁺, calcd for C₂₀H₁₆O₄, 343.0941.

Diepoxin η (5): ¹H NMR, ¹³C 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 μ g/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.

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Supporting Information Available: ¹H and ¹³C NMR spectra of decaspirones **1**–**4**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

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- 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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- 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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