Full Papers

Annularins A−H: New Polyketide Metabolites from the Freshwater Aquatic Fungus *Annulatascus triseptatus*

Chen Li, Meaghan V. Nitka, and James B. Gloer*

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242

Jinx Campbell and Carol A. Shearer

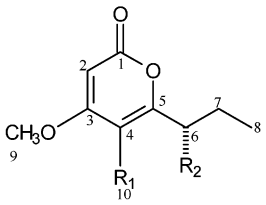
Department of Plant Biology, University of Illinois, Urbana, Illinois 61801

Received May 14, 2003

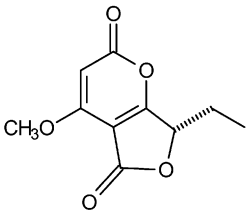
Eight new polyketide metabolites, annularins A−H (**1**−**8**), along with the known compound (−)-(*S*)-*p*-hydroxyphenyllactic acid, were isolated from the organic extracts of the freshwater fungus *Annulatascus triseptatus*. Compounds **1**−**6** are 3,4,5-trisubstituted α-pyrones, and the fused bicyclic pyrone−furanone system in annularin F (**6**) has not been reported previously among natural products. Compounds **7** and **8** are 3,4-disubstituted α,*β*-unsaturated *γ*-lactones. Annularins A (**1**), B (**2**), C (**3**), and F (**6**) exhibited antibacterial activity.

Freshwater aquatic fungi have not been extensively investigated as potential sources of new bioactive secondary metabolites. As part of an ongoing research project focusing on chemical studies of freshwater fungal species, our group has reported a variety of new bioactive compounds.[1−4] This report describes the isolation, structure elucidation, and bioactivities of eight new compounds (annularins A−H, **1**−**8**) and the known compound (−)-(*S*)-*p*-hydroxyphenyllactic acid from the newly discovered freshwater fungus *Annulatascus triseptatus* (A-353-1B).

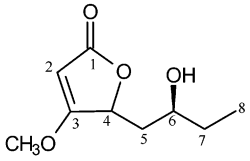
A. triseptatus is the first member of a new genus in the family Annulatascaceae (Sordariales). This family occurs commonly on submerged woody debris in lotic habitats throughout North America and also in Costa Rica, Scotland, and Venezuela. These fungi typically form soft rot cavities on balsa wood in peptone−yeast extract−glucose (PYG) culture. An EtOAc extract of *A. triseptatus* solid-substrate fermentation cultures showed antifungal and antibacterial activity. Chemical investigation of this extract led to the isolation of eight new compounds, which we have named annularins A−H (**1**−**8**).



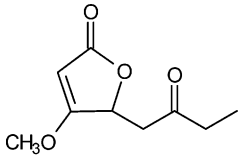
1	$R_1 = CH_3$	$R_2 = OH$
2	$R_1 = CH_2OH$	$R_2 = H$
3	$R_1 = CH_2OH$	$R_2 = OH$
4	$R_1 = CH_3$	$R_2 = H$
5	$R_1 = H$	$R_2 = H$



6



7



8

Results and Discussion

The molecular formula for annularin A (**1**) was determined to be $C_{10}H_{14}O_4$ (four degrees of unsaturation) on the basis of NMR and HRESIMS data. Analysis of the ^1H and ^{13}C NMR data for **1** (Tables 1 and 2) revealed the presence of a 1-hydroxypropyl group, a methoxy group, an olefinic methine unit, and four nonprotonated sp^2 carbons. The structure of annularin A (**1**) was solved through analysis of HMBC data. The methoxy signal (H_3-9) showed a strong correlation with C-3, enabling its direct connection with C-3. The only olefinic proton (H-2; δ_H 5.46) showed correlations with C-1, C-3, and C-4. These data, together with the extreme chemical shifts of C-2 and C-3 (δ_C 88.6 and 170.6), suggested the presence of the *β*-oxygenated α,*β*-unsaturated ester unit shown in **1**. H_3-10 showed HMBC correlations with C-3, C-4, and C-5, allowing the construction of the C1−C5/C9/C10 subunit. The 1-hydroxypropyl side chain was attached to C-5 on the basis of HMBC correlations between H-6 and C-4 and between H_2-7 and

* To whom correspondence should be addressed. Tel: 319-335-1361. Fax: 319-335-1270. E-mail: james-gloer@uiowa.edu.

10.1021/np030225y CCC: $25.00
Published on Web 10/02/2003

Table 1. ^{1}H NMR Data δ_H (mult., J_H) for Annularins A−D (**1**−**4**) and F (**6**) in $CDCl_3$

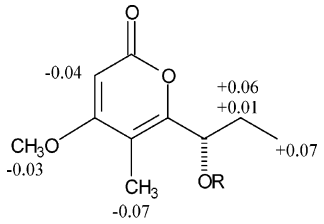
no.	annularin A (**1**)[a]	annularin B (**2**)[b]	annularin C (**3**)[b]	annularin D (**4**)[b]	annularin F (**6**)[b]
2	5.46 (s)	5.46 (s)	5.50 (s)	5.42 (s)	5.45 (s)
6	4.53 (br q; 8)	2.56 (t; 8)	4.62 (br t; 7)	2.46 (t; 7)	5.08 (dd; 4, 7)
7a	1.76 (m)	1.70 (sextet; 8)	1.85 (m)	1.66 (m)	2.10 (m)
7b	1.83 (m)				1.84 (m)
8	0.91 (t; 7.8)	0.95 (t; 7.2)	0.96 (t; 7.8)	0.93 (t; 7.5)	1.01 (t; 7.8)
9	3.81 (s)	3.85 (s)	3.87 (s)	3.80 (s)	3.95 (s)
10	1.90 (s)	4.44 (s)	4.52 (br s)	1.86 (s)	
OH	2.40 (6-OH, br d; 8)	1.83 (10-OH, br s)	not obsd		

[a] Recorded at 600 MHz. [b] Recorded at 400 MHz.

Table 2. ^{13}C NMR Data (δ_C) for Annularins A−D (**1**−**4**) and F (**6**) in $CDCl_3$

no.	annularin A (**1**)[a]	annularin B (**2**)[a]	annularin C (**3**)[b]	annularin D (**4**)[b]	annularin F (**6**)[b]
1	163.8	163.9	163.2	164.8	161.6
2	88.6	88.2	89.1	87.6	87.5
3	170.6	170.1	169.8	170.9	166.1
4	107.4	110.7	111.3	106.8	101.0
5	158.8	164.4	163.0	161.0	179.3
6	69.8	32.6	70.7	32.7	78.4
7	28.7	21.2	28.5	20.6	24.8
8	9.7	13.6	9.9	13.6	8.3
9	56.2	56.3	56.5	56.0	56.8
10	8.7	55.4	53.9	9.3	164.0

[a] Recorded at 90 MHz. [b] Recorded at 100 MHz.



1a: R=(*S*)-MTPA
1b: R=(*R*)-MTPA

Figure 1. $\Delta\delta$ values ($\delta_S - \delta_R$) for the MTPA esters **1a** and **1b** in ppm.

C-5. Carbonyl carbon C-1 and oxygenated sp^2 carbon C-5 must be linked through the remaining oxygen atom to form an α-pyrone ring, thereby completing the structural assignment of annularin A (**1**).

The absolute stereochemistry of **1** was determined by application of the modified Mosher NMR method.[5] Treatment of **1** with (*S*)-MTPACl or (*R*)-MTPACl afforded the (*R*)-MTPA ester (**1a**) or the (*S*)-MTPA ester (**1b**), respectively. Formation of the esters was confirmed by a significant downfield shift of the signal for H-6 and the appearance of the expected new aromatic and methoxy signals in the ^{1}H NMR spectra. Comparison of the ^{1}H NMR chemical shifts for **1a** and **1b** ($\Delta\delta$ values shown in Figure 1) led to assignment of the *S*-configuration at C-6.

Structure elucidation of annularins B−E (**2**−**5**) was straightforward because of their close structural relationships to annularin A (**1**). Annularin B (**2**) possesses the same molecular formula as **1**, as deduced from its NMR and MS data. An isolated *n*-propyl unit was identified from ^{1}H and ^{13}C NMR analysis (Tables 1 and 2), while the methyl group (H_3-10) in **1** was replaced by a new oxygenated methylene group (δ_H 4.44, s). This information led to identification of the structure of **2** as shown, which differs from **1** only in the position of the hydroxy group.

The molecular formula of annularin C (**3**) contained an additional oxygen atom on the basis of its HRMS and NMR data. Comparison of its ^{1}H and ^{13}C NMR data (Tables 1 and 2) with those of **1** and **2** revealed the existence of hydroxy groups at both C-6 and C-10, thus leading to assignment of the structure of annularin C as shown in **3**. Annularin C is assumed to possess the *S*-configuration at C-6, by analogy with that determined for **1**.

The molecular formula of annularin D (**4**) was determined to be $C_{10}H_{14}O_3$ by analysis of its EIMS and 1D NMR data. Straightforward NMR comparisons with compounds **1**−**3** (Tables 1 and 2) revealed that compound **4** differs from **1** in the absence of the hydroxy group on the side chain at C-6.

Annularin E (**5**) was obtained in very limited quantity (ca. 0.3 mg), but its ^{1}H NMR data (see Experimental Section) clearly showed the absence of the signal for H_3-10 and revealed its replacement by a new olefinic proton that showed a weak coupling to H-2 (H-4; δ_H 5.75, d; J = 2 Hz). The EI mass spectrum of **5** showed a molecular ion 14 mass units lower than **4**. These data were consistent with the replacement of the C-10 methyl group with a proton. The propyl group present in **2** and **4** was also clearly evident from the NMR data for **5**, leading to assignment of the structure of annularin E (**5**) as shown.

The NMR data for annularin F (**6**; Tables 1 and 2) revealed that it contained a methoxy/propyloxy-substituted pyrone unit similar to that of the other members of this group discussed above. However, there were some distinctive structural differences. Its molecular formula ($C_{10}H_{10}O_5$), as deduced from HRESI data, required two more unsaturations than for annularins A−E (**1**−**5**). In addition, the ^{1}H and ^{13}C NMR signals for the C-10 methyl group were replaced by a carboxyl carbon (δ_C 164.0) in the data for **6**, while the C-5 signal shifted downfield dramatically from δ_C 158.8 to δ_C 179.3. Because no additional olefinic or carbonyl carbons were present, compound **6** must contain an additional ring. The signals for the oxygenated methine C-6 (δ_H 5.08; δ_C 78.4) were shifted significantly downfield compared to the corresponding C-6 signals in **1** and **3**, implying acylation at this position. This conclusion was supported by the IR spectrum, which showed an additional ester/lactone absorption, but no OH absorption band. Key HMBC correlations of H_2-7 with C-5 and of H-6 with C-4, C-5, and carbonyl carbon C-10 permitted assignment of the structure of annularin F as shown in **6**. The significant

Table 3. NMR Data for Annularins G (**7**) and H (**8**) in $CDCl_3$[a]

	annularin G (**7**)			annularin H (**8**)	
no.	^{1}H δ_H (mult.; J_H)	^{13}C δ_C	HMBC (H→C)	^{1}H δ_H (mult.; J_H)	^{13}C δ_C
1		172.4			171.8
2	5.05 (d; 1)	88.4	1, 4	5.07 (d; 1)	88.9
3		183.0			181.8
4	5.04 (ddd; 1, 3, 10)	76.2	2, 3, 5, 6	5.24 (ddd; 1, 4, 8)	74.6
5a	1.92 (ddd; 3, 10, 14)	39.4	3, 6, 7	2.69 (dd; 8, 16)	43.8
5b	1.57 (ddd; 4, 10, 14)		3, 4, 7	2.86 (dd; 4, 16)	
6	3.82 (br m)	69.3	4, 8		206.0
7	1.50 (m)	30.8	5, 6, 8	2.48 (q; 7)	37.0
8	0.94 (t; 7)	9.7	6, 7	1.06 (t; 7)	7.4
9	3.87 (s)	59.4	3	3.88 (s)	59.6
OH	1.66 (br d; 4)				

[a] ^{1}H NMR data were recorded at 600 MHz; ^{13}C NMR data were recorded at 100 MHz.

downfield shift of the C-5 signal is consistent with NMR data for analogous β-oxygenated α,β-unsaturated γ-lactone rings.[1,6,7] Considering the clear structural relationships between annularins F (**6**) and A (**1**), compound **6** is again proposed to have the analogous *S*-configuration.

The final two new metabolites, annularins G (**7**) and H (**8**), are structurally related to each other, but showed some significant spectral differences from annularins A−F (**1**−**6**). EIMS data for **7** revealed a molecular formula of $C_9H_{14}O_4$ (three unsaturations), and the NMR data (Table 3) revealed the presence of a carbonyl carbon and an oxygenated, trisubstituted olefin unit, indicating that annularin G is monocyclic. A 2-hydroxybutyl side chain attached to an oxygenated sp^3 methine unit (C-4) was identified through examination of the ^{1}H NMR data. Analysis of HMBC data led to the proposal of an α,β-unsaturated γ-lactone structure. Olefinic proton H-2 (δ_H 5.05 d, J = 1 Hz) was shifted upfield by 0.4 ppm compared to the olefinic proton signal of annularins A−E and showed HMBC correlations with ester carbon C-1, oxygenated olefinic carbon C-3, and oxygenated sp^3 carbon C-4, while oxygenated methine proton H-4 showed correlations with carbons 2, 3, 5, and 6. The methoxy protons (H_3-9) again correlated with C-3 (δ_C 183.0). Because no direct evidence for an ester linkage was obtained from HMBC data, and no OH signal was observed in the ^{1}H NMR spectrum, an acetylation reaction was performed to confirm the proposed structure. Treatment of compound **7** with acetic anhydride in pyridine resulted in formation of a monoacetate wherein the signal for H-6 was shifted significantly downfield (from δ_H 3.82 to 5.01), indicating the presence of one free hydroxy group at C-6. Carbons 1 and 4 must therefore be connected through an ester linkage to complete the structure of **7**. The stereochemistry at C-6 is proposed to be the same as in annularins A (**1**), C (**3**), and F (**6**) due to their structural similarities, but the stereochemistry at C-4 was not determined.

Upon solving structure **7**, the identification of its close analogue, annularin H (**8**), was readily achieved. Compound **8** has two fewer hydrogens than **7**. Comparison of the ^{1}H and ^{13}C NMR data of compounds **7** and **8** clearly indicated that the hydroxy group at C-6 in **7** was oxidized to a ketone carbonyl (C-6, δ_C 206.0) in **8**. Chemical shift and coupling constant differences at positions 5 and 7 (Table 3) were fully consistent with this conclusion.

The known compound (−)-(*S*)-*p*-hydroxyphenyllactic acid was also obtained from this extract and was identified by comparison of its NMR, MS, and optical rotation data with literature values.[8] Both enantiomers of *p*-hydroxyphenyllactic acid have been previously reported from various natural sources, including fungi.[8−11]

Annularins A (**1**), B (**2**), C (**3**), and F (**6**) exhibited activity against *Bacillus subtilis* (ATCC 6051) in standard disk assays, each causing zones of inhibition of 8−10 mm at 200 μg/disk. Only annularin C (**3**) displayed activity against *Staphylococcus aureus* (ATCC 29213), affording a 14 mm zone of inhibition at 200 μg/disk. None of these compounds exhibited activity against *Candida albicans* (ATCC 90029) at this level. All annularins except for annularins D (**4**) and E (**5**) (due to sample limitations) were screened for antifungal activity against *Aspergillus flavus* (NRRL 6541), but none showed activity in this assay. Thus, although the compounds described here include the most abundant metabolites evident in the crude extract based on NMR analysis, the components responsible for the original antifungal activity of the extract have not been identified.

Annularins A−F (**1**−**6**) are new α-pyrones, members of a general class that is commonly found among fungal sources. Biogenetically, they are presumably derived from the polyketide (tetraketide) pathway. The closest known fungal metabolites include cladobotrins, pestalopyrones, and pestalotins,[12−17] which differ from annularins in the length and/or functionality of the side chain, as well oxidation and methylation patterns. Biosynthetic studies support a polyketide origin for members of this class.[17] Annularins G (**7**) and H (**8**) are envisioned to arise via an analogous biogenetic route, but with a different ring closure step. α,β-Unsaturated γ-lactones are also precedented among fungal metabolites, with examples including the well-known compound penicillic acid.[6] To our knowledge, the fused α-pyrone−furanone system in annularin F (**6**) has not been encountered previously among natural products, although some compounds with similar ring systems have been synthesized.[18,19]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. ^{1}H NMR data were obtained at 300 (Bruker AC-300), 400 (Bruker DRX-400), or 600 MHz (Bruker AMX-600), and ^{13}C NMR data were obtained at 90 MHz (Bruker WM-360) or 100 MHz (Bruker DRX-400). HMQC and HMBC data were recorded at 600 MHz (^{1}H-dimension; Bruker AMX-600). NMR data were recorded in $CDCl_3$, and the chemical shifts were referenced to the residual solvent signals (δ_H 7.24/δ_C 77.0). Other general procedures and instrumentation have been described previously.[20]

Isolation, Cultivation, and Fermentation of Fungal Material. The culture employed in this work (A353-1B) was identified as a member of a new genus and species, *Annulatascus triseptatus*.[21] Voucher specimens are deposited at the University of Illinois herbarium collection (ILLS), and cultures are deposited in the University of Illinois Culture Collection. Submerged woody debris was collected from Shaker Pond, Alfred, Maine, and transported to the laboratory in plastic bags containing paper towels. The woody debris was incubated in plastic refrigerator boxes containing moistened paper towels under ambient light (fluorescent light, 12 h light/12 h dark) and temperature conditions (~25 °C) and examined periodically for the appearance of ascomata. Cultures were obtained from selected ascomata using procedures that have been described previously.[22,23]

A culture of *A. triseptatus* was used to inoculate five Pyrex storage dishes, each containing 50 g of Botan rice sterilized in 50 mL of deionized water. The dish cultures were sealed with Parafilm and incubated at 25 °C on a 12 h light/12 h dark cycle for 30 days.

Extraction and Isolation. The fermented rice was extracted with EtOAc, and the solvent was then evaporated to afford 380 mg of extract. The EtOAc extract of *A. triseptatus* was then partitioned between CH_3CN and hexane. The

CH_3CN-soluble portion (236 mg) was subjected to Sephadex LH-20 column chromatography with a step-gradient elution sequence of hexane−CH_2Cl_2 (1:4), CH_2Cl_2−acetone (4:1), and CH_2Cl_2−acetone (2:3) and yielded nine fractions. The first fraction (25 mg) eluted with hexane−CH_2Cl_2 (1:4) was further separated on reversed-phase HPLC (Alltech HS Hyperprep 100 BDS C_{18} column, 5 μm particles, 1.0 × 25 cm; flow rate 2.0 mL/min; 40% to 60% CH_3CN in H_2O over 20 min) to yield annularin D (**4**, 0.4 mg, t_R 19.6 min), annularin E (**5**, 0.3 mg, t_R 16.8 min), annularin F (**6**, 12 mg, t_R 12.9 min), and annularin H (**8**, 0.7 mg, t_R 10.5 min). The third fraction (32 mg) that was also eluted with hexane−CH_2Cl_2 (1:4) from the LH-20 column was separated on reversed-phase HPLC (same column as above; flow rate 1.5 mL/min; 30% CH_3CN in H_2O) to yield annularin A (**1**, 12 mg, t_R 17.5 min) and another sharp peak (9 mg, t_R 15.2 min). This sharp peak actually represented a mixture of related compounds which was eventually separated on the same HPLC column by employing a MeOH−H_2O solvent system (flow rate 2.0 mL/min; 50% MeOH in H_2O) to give annularin B (**2**, 6 mg, t_R 13.6 min) and annularin G (**7**, 1 mg, t_R 10.8 min). The seventh fraction (75 mg), which was eluted with CH_2Cl_2−acetone (4:1) from the LH-20 column, was further separated on a silica gel column using a step gradient EtOAc−acetone solvent system. A subfraction (9 mg) that eluted with EtOAc−acetone (1:1) was further separated on reversed-phase HPLC (same column as above; flow rate 2.0 mL/min; 40% to 50% CH_3CN in H_2O over 10 min) to yield annularin C (**3**, 3 mg, t_R 6.0 min). The eighth fraction (30 mg) eluted with hexane−CH_2Cl_2 (1:4) from the LH-20 column was separated by reversed-phase HPLC (same column as above; flow rate 2.0 mL/min; 30% CH_3CN in H_2O) to yield (−)-(*S*)-*p*-hydroxyphenyllactic acid (11 mg, t_R 4.0 min).

Annularin A (1): white solid; mp 102−105 °C; $[\alpha]_D$ +33° (*c* 0.4, CH_2Cl_2); UV (MeOH) λ_{max} 206 (ϵ 4700), 278 (ϵ 1100); IR (CH_2Cl_2) ν_{max} 3383, 2969, 1705, 1646, 1565, 1458, 1407 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; HMBC data ($CDCl_3$) H-2 → C-1, 3, 4; H-6 → C-4, 7, 8; H_2-7 → C-5, 6, 8; H-8 → C-6, 7; H_3-9 → C-3; H_3-10 → C-3, 4, 5; 6-OH → C-5, 6, 7; EIMS (70 eV) *m/z* 198 (M^+; rel int 41), 180 (2), 169 (34), 154 (2), 139 (83), 111 (16), 83 (100); HRESIMS *m/z* 199.0980 (calcd for $C_{10}H_{14}O_4$ + H, 199.0970).

***(R)* and *(S)*-MTPA Esters of Annularin A (1).** A solution of **1** (0.8 mg, 4 μmoL) in pyridine (200 μL) was treated with (*S*)-2-methoxy-2-trifluoromethylphenylacetyl chloride [(*S*)-MTPACl, 10 μL, 50 μmoL], and the mixture was stirred at 25 °C for 50 h. Aqueous saturated $NaHCO_3$ (1 mL) was added, and the solution was extracted with CH_2Cl_2 (3 × 1.5 mL). The combined organic extracts were evaporated to give a colorless oil identified as (*R*)-MTPA ester **1a**, along with some residual MTPA acid. Analogous treatment of **1** (0.8 mg) using (*R*)-MTPACl afforded (*S*)-MTPA ester **1b**, again with residual MTPA acid. Further purification was not pursued, because all of the relevant proton signals for products **1a** and **1b** (i.e., except for the phenyl group signals) were well resolved from those of the MTPA acids.

^1H NMR data for (*R*)-MTPA ester 1a: (300 MHz, $CDCl_3$; other than phenyl signals) δ 5.67 (1H, t, 7, H-6), 5.44 (1H, br s, H-2), 3.76 (3H, br s, H_3-9), 1.974 (1H, m, H-7a), 1.89 (1H, m, H-7b), 1.92 (3H, br s, H_3-10), 0.88 (3H, t, 7, H_3-8).

^1H NMR data for (*S*)-MTPA ester 1b: (300 MHz, $CDCl_3$; other than phenyl signals) δ 5.65 (1H, t, 7, H-6), 5.48 (1H, br s, H-2), 3.79 (3H, br s, H_3-9), 1.969 (1H, m, H-7a), 1.83 (1H, m, H-7b), 1.99 (3H, br s, H_3-10), 0.81 (3H, t, 7, H_3-8).

Annularin B (2): white solid; mp 92−95°C; UV (MeOH) λ_{max} 213 (ϵ 5200), 263 (ϵ 2300); IR (CH_2Cl_2) ν_{max} 3331, 2979, 1720, 1645, 1566, 1460, 1412 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 198 (M^+; rel int 66), 180 (23), 170 (35), 155 (25), 152 (100); HREIMS *m/z* 198.0899 (calcd for $C_{10}H_{14}O_4$, 198.0892).

Annularin C (3): colorless oil; $[\alpha]_D$ +10° (*c* 0.2, CH_2Cl_2); UV (MeOH) λ_{max} 210 (ϵ 5500), 273 (ϵ 2000); IR (CH_2Cl_2) ν_{max} 3355, 2970, 1700, 1562, 1460, 1411 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 214 (M^+; rel int 41), 196 (7), 185 (7), 167 (52), 155 (100); HREIMS *m/z* 214.0838 (calcd for $C_{10}H_{14}O_5$, 214.0841).

Annularin D (4): colorless oil; UV (MeOH) λ_{max} 225 (ϵ 4900); ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 182 (M^+; rel int 89), 167 (3), 154 (63), 139 (38), 125 (100), 111 (14), 83 (62).

Annularin E (5): colorless oil; UV (MeOH) λ_{max} 206 (ϵ 3800); ^1H NMR (300 MHz, $CDCl_3$) δ 5.75 (1H, d, 2, H-4), 5.39 (1H, d, 2, H-2), 3.77 (s, H_3-9), 2.40 (2H, dd, 8, 8, H_2-6), 1.66 (qt, 7, 7, H_2-7), 0.96 (t, 7.5, H_3-8); EIMS (70 eV) *m/z* 168 (M^+; rel int 100), 153 (84), 139 (31), 125 (55), 124 (37), 111 (17), 93 (13), 83 (16), 69 (37).

Annularin F (6): white solid; mp 143−145°C; $[\alpha]_D$ −64° (*c* 0.5, CH_2Cl_2); UV (MeOH) λ_{max} 210 (ϵ 5100), 239 (ϵ 2100), 274 (ϵ 1000); IR (CH_2Cl_2) ν_{max} 1773, 1750, 1661, 1570, 1486, 1458 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; HMBC data ($CDCl_3$) H-2 → C-1, 3, 4; H-6 → C-4, 5, 7, 8, 10; H_2-7 → C-5, 6, 8; H_3-8 → C-6, 7; H_3-9 → C-3; EIMS (70 eV) *m/z* 210 (M^+; rel int 48), 195 (16), 182 (100), 167 (12), 152 (35), 140 (3), 124 (77); HRESIMS *m/z* 211.0623 (calcd for $C_{10}H_{10}O_5$ + H, 211.0606).

Annularin G (7): white solid; mp 89−94 °C; $[\alpha]_D$ +35° (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} 223 (ϵ 4700); ^1H NMR, ^{13}C NMR, and HMBC data, see Table 3; EIMS (70 eV) *m/z* 186 (M^+; rel int 3), 168 (13), 157 (49), 139 (14), 126 (77), 113 (100), 85 (59), 69 (58).

Monoacetate of 7. A solution of annularin G (0.4 mg), pyridine (0.1 mL), and acetic anhydride (0.5 mL) was stirred at RT for 48 h. H_2O (1 mL) was added, and the solution was extracted with $CHCl_3$ (3 × 1 mL). The organic layer was then evaporated under N_2 to afford the monoacetate of compound **7** (0.5 mg): ^1H NMR (300 MHz, $CDCl_3$) δ 5.05 (1H, d, 1, H-2), 5.01 (1H, m, H-6), 4.79 (1H, ddd, 1, 3, 10, H-4), 3.87 (3H, s, H_3-9), 2.14 (1H, ddd, 3, 10, 14, H-5a), 2.04 (3H, s, acetyl CH_3), 1.70 (1H, ddd, 4, 10, 14, H-5b), 1.63 (2H, br pentet, 7, H_2-7), 0.88 (3H, t, 7, H_3-8); ESIMS, *m/z* 251 (M + Na; rel int 100); HRESIMS *m/z* 251.0895 (calcd for $C_{11}H_{16}O_5$ + Na, 251.0895).

Annularin H (8): colorless oil; $[\alpha]_D$ −38° (*c* 0.03, CH_2Cl_2); UV (MeOH) λ_{max} 225 (ϵ 5000); ^1H NMR and ^{13}C NMR data, see Table 3; EIMS (70 eV) *m/z* 184 (M^+; rel int 62), 155 (15), 127 (100), 113 (88), 99 (15), 85 (66), 69 (64); HREIMS *m/z* 184.0738 (calcd for $C_9H_{12}O_4$, 184.0736).

Acknowledgment. This research was supported by a grant from the National Institutes of Health (GM 60608).

Supporting Information Available: ^1H NMR spectra of annularins A−H (**1**−**8**) and ^{13}C NMR spectra of annularins A−D (**1**−**4**) and annularins F−H (**6**−**8**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Oh, H.; Swenson, D. C.; Gloer, J. B.; Shearer, C. A. *Tetrahedron Lett.* **2001**, *42*, 975−977.
(2) Oh, H.; Kwon, T. O.; Gloer, J. B.; Marvanová, L.; Shearer, C. A. *J. Nat. Prod.* **1999**, *62*, 580−583.
(3) Oh, H.; Gloer, J. B.; Shearer, C. A. *J. Nat. Prod.* **1999**, *62*, 497−501.
(4) Oh, H.; Swenson, D. C.; Gloer, J. B.; Shearer, C. A. *J. Nat. Prod.* **2003**, *66*, 73−79.
(5) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(6) Cole, R. J.; Cox, R. H. *Handbook of Toxic Fungal Metabolites*; Academic Press: New York, 1981; p 520.
(7) Suzuki, Y.; Kono, Y.; Inoue, T.; Sakurai, A. *Phytochemistry* **1998**, *47*, 997−1001.
(8) Valls, N.; Vallribera, M.; Lopez, M.; Bonjoch, J. *J. Org. Chem.* **2002**, *67*, 4945−4950.
(9) Arnone, A.; Assante, G.; Nasini, G.; Strada, S.; Vercesi, A. *J. Nat. Prod.* **2002**, *65*, 48−50.
(10) Ayer, W. A.; Browne, L. M.; Feng, M. C.; Orszanska, H.; Saeedi, H. *Can. J. Chem.* **1986**, *64*, 904−909.
(11) Maurya, R.; Ray, A. B.; Duah, F. K.; Slatkin, D. J.; Schiff, P. L. *J. Nat. Prod.* **1984**, *47*, 179−181.
(12) Strunz, G. M.; Heissner, C. J.; Kakushima, M.; Stillwell, M. A. *Can. J. Chem.* **1974**, *52*, 825−826.
(13) Kosemura, S.; Yamamura, S. *Tetrahedron Lett.* **1997**, *38*, 3025−3026.
(14) McGahren, W. J.; Ellestad, G. A.; Morton, G. O.; Kunstmann, M. P.; Martin, P.; Mullen, P. *J. Org. Chem.* **1973**, *38*, 3542−3544.
(15) Tezuka, Y.; Huang, Q.; Kikuchi, T.; Nishi, A.; Tubaki, K. *Chem. Pharm. Bull.* **1994**, *42*, 2612−17.
(16) Venkatasubbaiah, P.; Van Dyke, C. G.; Chilton, W. S. *Phytochemistry* **1991**, *30*, 1471−1474.
(17) Avent, A. G.; Hanson, J. R.; Truneh, A. *Phytochemistry* **1992**, *31*, 3447−3449.

(18) Kepe, V.; Kocevar, M.; Polanc, S. *Heterocycles* **1995**, *41*, 1299−1304.
(19) Stachel, H.; Schorp, M.; Maier, L.; Dandl, K. *Liebigs Ann. Chem*. **1994**, 1121−1127.
(20) Holler, U.; Gloer, J. B.; Wicklow, D. T. *J. Nat. Prod*. **2002**, *65*, 876−882.
(21) Campbell, J.; Shearer, C. A. *Mycologia* **2003**, in press.
(22) Fallah, P. M.; Shearer, C. A. *Mycologia* **2001**, *93*, 566−602.
(23) Shearer, C. A. *Nova Hedwigia* **1993**, *56*, 1−33.

NP030225Y