New Verticillin and Glisoprenin Analogues from *Gliocladium catenulatum*, a Mycoparasite of *Aspergillus flavus* Sclerotia

Biren K. Joshi,[†] James B. Gloer,*[,†] and Donald T. Wicklow[‡]

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, and Bioactive Agents Research Unit, Agricultural Research Service, National Center for Agricultural Utilization Research, United States Department of Agriculture, Peoria, Illinois 61604

Received November 23, 1998

Four new compounds (**1**−**4**) have been isolated from solid−substrate fermentation cultures of the sclerotial mycoparasite *Gliocladium catenulatum*. The structures of verticillins D (**1**), E (**2**), and F (**3**) were assigned on the basis of MS and NMR data and chemical derivatization. The structure of glisoprenin F (**4**) was assigned by analysis of MS and NMR results, and comparison of its spectral data to those of the known compound glisoprenin D (**6**).

Mycoparasites are fungi that colonize and parasitize other fungi, often causing damage or cell death in the host.[1] In some instances, the damage caused to the fungal host is due, at least in part, to the production of antibiotic secondary metabolites by the mycoparasite. During a study of sclerotium survival in soil,[2] *Gliocladium catenulatum* Gilman & Abbott (an asexual ascomycete, probably Nectriaceae) was observed as a colonist of *Aspergillus flavus* Link:Fr. sclerotia that were buried for two years in a Georgia cornfield. *G. catenulatum* (and other species) were observed to colonize the sclerotia and sometimes render them inviable.[2] A representative strain of *G. catenulatum* (NRRL 22970) was isolated from an individual *A. flavus* sclerotium. An organic extract from cultures of this isolate showed activity against *A. flavus*, as well as antibacterial effects, and was therefore subjected to chemical investigation. This investigation led to the isolation of four new compounds, which we named verticillins D (**1**), E (**2**), and F (**3**), and glisoprenin F (**4**). The names given to **1**−**4** are proposed by virtue of their structural similarities with previously reported compounds. Verticillins D, E, and F are new antimicrobial analogues of verticillins A−C (e.g., verticillin A; **5**),[3,4] while glisoprenin F is a new polyterpenoid closely related to glisoprenins A−E (e.g., glisoprenin E; **6**).[5−7] This report describes the isolation and structure elucidation of compounds **1**−**4**.

Results and Discussion

G. catenulatum (NRRL 22970) was isolated from an *A. flavus* sclerotium that had been buried in a Georgia cornfield for two years. Separation of the EtOAc extract of solid−substrate fermentation cultures of *G. catenulatum* by Si gel vacuum−liquid chromatography (VLC), Sephadex LH-20 column chromatography, and reversed-phase HPLC afforded compounds **1**−**4**.

The ^{1}H, ^{13}C, and DEPT NMR data (Table 1) for verticillin D (**1**) contained signals corresponding to a 1,2-disubstituted benzenoid unit, three methine carbons (two attached to heteroatoms), two carboxy carbonyls, three quaternary sp^3 carbons, two exchangeable protons, one aliphatic methyl group, and one *N*-methyl group. Although the ^{13}C NMR spectrum showed resonances for 16 carbons, analysis of HRESIMS data revealed the molecular formula to be $C_{32}H_{32}O_8N_6S_4$, indicating that verticillin D (**1**) is a symmetrical dimer. Furthermore, these data suggested that **1** is a member of the epidithiodioxopiperazine class of fungal antibiotics and that it is closely related to verticillins A−C, which were originally isolated from *Verticillium* spp.[3] Comparison of the data to those reported for verticillin A (**5**) supported this conclusion and suggested that the core dimeric structures of **1** and **5** are identical. Analysis of the spectral data for **1** indicated that the difference between the structures lies in the identities of the amino acid-derived *R* and *R*′ substituents. Although each corresponding unit of verticillins A−C is derived from alanine (R = CH_3) or serine (R = CH_2OH), the data for compound **1** clearly revealed its origin from two threonine units (R = CH_3CHOH).

The structure of verticillin D (**1**) was verified by detailed analysis of HMBC and HMQC data for its acetylation product (**7**). Interestingly, treatment of symmetrical compound **1** with acetic anhydride afforded an asymmetrical triacetate (**7**), as evidenced by HRFABMS data, the presence of three acetyl methyl singlets in the ^{1}H NMR spectrum, and the generation of a doubled set of signals for the remaining elements of the molecule. Although both

Table 1. NMR Data for Verticillins D (**1**) and E (**2**) in Pyridine-d_5

	1		**2**	
position	δ_H (mult., J_{HH})	δ_C	δ_H (mult., J_{HH})	δ_C
1, 1′		167.9		166.5
3, 3′		82.4		87.8
4, 4′		162.6		160.7
5a, 5a′	5.88 (s)	84.0	5.85 (s)	83.7
6a, 6a′		151.3		150.7
7, 7′	6.66 (br d, 7.8)	110.6	6.74 (br d, 7.7)	110.4
8, 8′	7.07 (dd, 7.8, 7.8)	130.5	7.13 (dd, 7.7, 7.7)	130.4
9, 9′	6.72 (dd, 7.8, 7.8)	119.9	6.75 (dd, 7.7, 7.7)	119.6
10, 10′	8.32 (br d, 7.8)	129.1	8.33 (br d, 7.7)	128.7
10a, 10a′		131.3		130.3
10b, 10b′		67.7		67.9
11, 11′	6.38 (br s)	83.3	6.41 (d, 3.9)	82.3
11a, 11a′		79.0		78.8
12, 12′	3.45 (s)	30.0	2.92 (s)	27.5
13, 13′	5.00 (m)	68.0		197.0
14, 14′	1.51 (d, 5.8)	20.5	2.28 (s)	30.3
11, 11′-OH[a]	8.29 (br s)		8.78 (d, 3.8)	
6, 6′-NH	7.45 (s)		7.74 (s)	

[a] Signals for the 13 and 13′-OH protons were not observed but may be buried under the solvent peaks.

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

10.1021/np980530x CCC: $18.00
Published on Web 05/28/1999

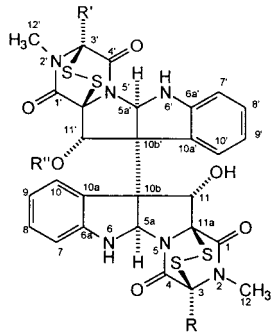
Table 2. NMR Data for Verticillin D Triacetate (**7**) in $CDCl_3$

position	δ_H (mult., J_{HH})	δ_C	HMBC correlations (H → C #)	position	δ_H (mult., J_{HH})	δ_C	HMBC correlations (H → C #)
1		166.4		10b		65.8	
1′		163.8		10b′		64.9	
3		79.2[a]		11	5.01 (d, 1.2)	81.8	5a, 10a, 10b
3′		79.8[a]		11′	6.95 (s)	80.0	5a′, 10a′, 10b′, 17′
4		160.3[b]		11a		76.2	
4′		160.2[b]		11a′		74.8	
5a	5.16 (s)	83.2	10a, 11a, 10b′	12	3.11 (s)	29.9	1, 3
5a′	5.15 (s)	82.6	6a′, 10a′, 11a′, 10b	12′	3.05 (s)	30.5	1′, 3′
6a		148.9		13	5.82 (q, 6.6)	68.0	4, 14, 15
6a′		148.4		13′	5.82 (q, 6.6)	67.9	3′, 4′, 14′, 15′
7	6.66 (d, 7.8)	111.2	9, 10a, 10b[e]	14	1.57 (d, 6.6)	17.1	3
7′	6.63 (d, 7.8)	110.7	9′, 10a′	14′	1.56 (d, 6.6)	17.1	3′
8	7.18 (dd, 7.8, 7.8)	130.6	6a, 10	15		169.4[c]	
8′	7.13 (dd, 7.8, 7.8)	130.4	6a′, 10′	15′		169.3[c]	
9	6.87 (dd, 7.8, 7.8)	120.5	7, 10, 10b[e]	16	2.10 (s)	20.9[d]	15
9′	6.84 (dd, 7.8, 7.8)	120.7	7′, 10a′	16′	2.09 (s)	20.9[d]	15′
10	7.62 (d, 7.8)	125.8	6a, 8, 10b	17′		168.3	
10′	7.89 (d, 7.8)	128.3	6a′, 8′, 10b′	18′	2.50 (s)	22.0	17′
10a		128.3		11-OH	5.38 (d, 1.2)		10b, 11
10a′		128.7					

[a−d]Assignments denoted by identical superscripts may be interchanged. [e] Weak, but detectable four-bond HMBC correlations.

side-chain hydroxy groups react smoothly, acetylation of one of the ring hydroxy groups apparently introduces a conformational bias that hinders the acetylation of the second ring hydroxy group to give asymmetrical triacetate **7**. Similarly, it has been reported that treatment of verticillin A (**5**) with acetic anhydride leads to formation of an asymmetrical monoacetate.[3] Analysis of the spectral data for **7** (Table 2) confirmed its pseudodimeric structure and enabled independent structure elucidation. The downfield shifts of H-13, H-13′, and H-11′ relative to their positions in **1** and relative to the position of H-11 in **7** were consistent with the formation of a triacetate retaining a free OH group at position 11. For example, HMBC correlations of the new acetyl methyl proton signals (H_3-16, -16′, and -18′) with the corresponding carboxyl carbons (C-15, -15′, and -17′, respectively) enabled identification of the acetate carbonyl signals. Correlations of the overlapping methine quartets H-13 and H-13′ with two new acetyl carbonyls (C-15 and -15′), the corresponding acetyl methyl carbons (C-16 and -16′), the quaternary sp^3 carbons of the epidithiodioxopiperazine rings (C-3 and -3′), and the corresponding amide carbonyls C-4 and C-4′ supported the side-chain assignments. A correlation of H-11′ with the third acetate carbonyl located the site of the third acylation and further correlations enabled assignment of the signals for both the diacylated and monoacylated monomeric units. These two units were connected via the C-10b−C-10b′ bond by virtue of HMBC correlations of the methine protons H-5a and H-5a′ with the quaternary carbons C-10b′ and C-10b, respectively. All other NMR data and HMBC correlations (Table 2) were fully consistent with the structure assignment for **7**.

The structures of verticillins E (**2**) and F (**3**) were elucidated by comparison of their spectral data to those for **1**. The ^1H NMR spectra of **1** and **2** are very similar. However, the ^1H NMR spectrum of **2** shows a methyl ketone singlet at δ 2.30 in place of the methyl doublet at δ 1.51 (H_3-14, -14′), as well as the absence of a signal corresponding to the oxygenated methine proton (H-13, -13′). In addition, the ^{13}C NMR spectrum of **2** shows a signal for a ketone carbonyl at δ 197.0 (C-13, -13′) in place of the oxygenated methine carbon signal found in the spectrum of **1**. Hence, it was clear that the secondary alcohol group in each side-chain in **1** ($R = R' = CH_3CHOH$) is oxidized to a keto group in **2** ($R = R' = CH_3CO$) and that the structures are otherwise identical.

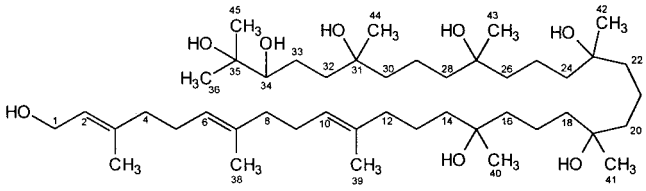


	R	R′	R″
1	$CH(OH)CH_3$	$CH(OH)CH_3$	H
2	$COCH_3$	$COCH_3$	H
3	$COCH_3$	$CH(OH)CH_3$	H
5	CH_3	CH_3	H
7	$CH(OAc)CH_3$	$CH(OAc)CH_3$	OAc

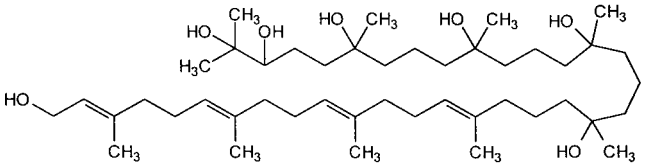
The ^1H and ^{13}C NMR spectra of verticillin F (**3**) showed two slightly different sets of signals, indicating that it has an asymmetrical structure. The ^1H NMR spectrum of **3** showed a quartet for an oxygenated methine proton at δ 4.63 (H-13′), which was coupled to a methyl doublet at δ 1.54 (H_3-14′), as in verticillin D (**1**). However, the ^1H NMR spectrum of **3** also showed a methyl ketone singlet at δ 2.50 (H-14), as in verticillin E (**2**). Thus, one monomeric unit each from **1** and **2** are joined together in verticillin F (**3**). This conclusion was fully consistent with all of the NMR and MS data for **3**. The stereochemistry shown for **1**−**3** is proposed by analogy to other known compounds in this class[3,4,8,9] based on close agreement of relevant ^1H and ^{13}C NMR chemical shifts.

The fourth new metabolite, glisoprenin F (**4**), is unrelated to **1**−**3**. The molecular formula of **4** was determined to be $C_{45}H_{86}O_8$ on the basis of ^{13}C NMR, DEPT, and HRFABMS data. All three unsaturations were accounted for by six olefinic carbon signals, indicating an acyclic structure. The ^1H, ^{13}C, and DEPT NMR data, in combination with the

molecular formula, indirectly indicated the presence of eight exchangeable protons, requiring all of the oxygen atoms in the molecule to be present as OH groups. The structure was elucidated by analysis of 1D NMR and HMBC data, and by comparison of its spectral data to those of the previously known glisoprenins,[5−7] especially glisoprenin D (**6**).[7]



4



6

The NMR data for **4** indicated that one oxygenated quaternary carbon and an additional methylene unit replace two olefinic carbons present in **6**. In addition, one of the vinylic CH_3 groups was replaced by a CH_3 singlet at a position indicating its connection to a quaternary oxygen-bearing sp^3 carbon. These data suggested that compound **4** differs from **6** by hydration of one of the internal olefinic units found in **6**. Although there was considerable overlap in the NMR spectra, the signals were well resolved enough to enable confirmation of the presence of key structural units analogous to those of **6** by analysis of HMBC data recorded at 600 MHz. For example, the presence of the C-34−C-35 terminal vicinal diol unit was confirmed by observation of HMBC correlations of two of the nonvinylic methyl singlets (H_3-36 and H_3-45) to the same pair of oxygenated carbons (methine C-34 and quaternary carbon C-35), as well as to C-45 and C-36, respectively. HMBC correlations of the vinylic protons H-2 and H-6 with the methylene carbon C-4, and of the vinylic proton H-10 and the methyl signal H_3-38 with the methylene carbon C-8, indicated that the C-1−C-11 triene system found in **6** was intact in **4**. These conclusions were also supported by close agreement in the NMR chemical shifts for these portions of the two structures.[7] The HMBC correlations shown by the methyl singlets for H_3-40, -41, -42, -43, and -44 overlapped, so it was not possible to assign individual HMBC correlations for these methyl groups. However, the remaining NMR data were fully consistent with the presence of five internal, repeating hydroxylated prenyl units, as depicted in **4**, rather than four such units, as found in **6**. These require that the C-14−C-15 double bond in **6** must be hydrated in **4**, thereby leading to assignment of the structure of **4** as shown.

A strain of *G. catenulatum* has been registered in the U. S. as "Primastop Biofungicide" (Kemira Agro Company), primarily for garden use.[10] However, to our knowledge, the only prior report of chemistry from this species describes the isolation of a tetracycline antibiotic unrelated to **1**−**4**.[11] Neither verticillins D−F (**1**−**3**) nor glisoprenin F (**4**) were responsible for the activity of the *G. catenulatum* extract against *A. flavus*. The *A. flavus*-active agents are structurally unrelated to **1**−**4** and are still under investigation. However, all three verticillin analogues did show antibacterial activity at 100 μg/disk in standard Petri-plate assays, affording inhibitory zone sizes of 23 to 26 mm against *Bacillus subtilis* (ATCC 6051) and of 11 to 14 mm against *Staphylococcus aureus* (ATCC 14053). A gentamicin sulfate standard (Sigma Chemical Co.) showed ca. 25-mm zones of inhibition in both assays at 50 μg/disk.

The simplest analogue of **1**−**3** (verticillin A; **5**) is known to exhibit antitumor activity.[3] Several other analogues of this structural type are known. Two such compounds (Sch 52900 and 52901), containing an ethyl or 1-hydroxyethyl group in place of one of the side-chain methyl groups in **5**, were reported together with verticillin A as inhibitors of *c-fos* proto-oncogene induction from an unidentified *Gliocladium* species.[4] Another series of compounds called leptosins, isolated from a *Leptosphaeria* sp., possesses hydroxymethyl or isopropyl side-chains and varying numbers of sulfur atoms in the bridges across the dioxopiperazine rings.[8,9] Some of these compounds show in vivo antitumor effects.[9] Glisoprenins C−E have been reported to inhibit appressorium formation in the plant pathogenic fungus *Magnaporthe grisea*.[7] The other known glisoprenins (A and B) are inhibitors of cholesterol acyl transferase.[5,6]

Experimental Section

General Experimental Procedures. The ^{1}H NMR spectra for **1** and **2**, and all ^{13}C NMR spectra, were recorded on a Bruker AC-300 spectrometer at 300 and 75 MHz, respectively. ^{1}H NMR spectra for **3**, **4**, and **7**, and all 2D NMR data, were acquired using a Bruker AMX-600 spectrometer at 600 MHz (^{1}H dimension). HMQC and HMBC experiments were optimized for $^1J_{CH}$ = 152 Hz and $^nJ_{CH}$ = 8 Hz, respectively. Chemical shifts are reported in parts per million downfield from TMS using the solvent signals as references. Solvents employed were pyridine-d_5 (δ_H 7.22, 7.58, 8.74; δ_C 123.9, 135.9, 150.4), Me_2CO-d_6 (δ_H 2.04, δ_C 29.8), and $CDCl_3$ (δ_H 7.24, δ_C 77.0). All ^{13}C NMR assignments are consistent with DEPT data. HRFABMS data were recorded on a VG ZAB-HF mass spectrometer, and HRESIMS data were recorded on a Fisons Autospec instrument. IR spectra were acquired using a Mattson Cygnus 25 FT spectrophotometer. UV spectra were recorded using a Hewlett-Packard 8452A diode array spectrophotometer. Optical rotations were determined on a JASCO DIP-1000 polarimeter. Melting points (uncorrected) were measured using a Fisher−Johns micro melting point apparatus.

Fungal Material. *G. catenulatum* (NRRL 22970) was isolated from a sclerotium of *A. flavus* that was buried for two years in a field continuously planted to corn near Tifton, Georgia. Fermentation was carried out in two 2.8-L Fernbach flasks each containing 200 g of rice. The contents of the flask were soaked overnight in distilled H_2O, autoclaved, cooled to room temperature, inoculated with spore suspension, and incubated for 45 days at 25 °C. After incubation, the fermented rice was mechanically fragmented and extracted repeatedly with EtOAc (3 × 500 mL).

Isolation of Compounds 1−4. The EtOAc extract (9.4 g) was partitioned between hexane and 95:5 MeOH−H_2O. The MeOH−H_2O fraction (1.7 g) was subjected to Si gel VLC (5 × 5 cm column) and eluted with step gradients of 50, 20, and 0% hexane in CH_2Cl_2 (500 mL each) followed by step gradients of 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 17, and 20% MeOH in CH_2Cl_2 (500 mL each). The fraction eluting with 3% MeOH in CH_2Cl_2 (207 mg) was triturated with CH_2Cl_2 to afford 39 mg of insoluble residue. This residue was purified by reversed-phase HPLC (Rainin Dynamax C_{18} column; 10 × 250 mm; 5-μm particle size, 60 Å pore size, 60% CH_3CN in 0.1% HCOOH−H_2O at 3 mL/min) to afford verticillin D (**1**, 8.0 mg, t_R 12.0 min), verticillin E (**2**, 3.5 mg, t_R 19.2 min), and verticillin F (**3**, 3.5 mg, t_R 15.2 min). Interestingly, the symmetrical metabolites **1** and **2** were nearly insoluble in most solvents, requiring

the use of pyridine-d_5 for NMR experiments, while verticillin F (**3**) was freely soluble in Me_2CO-d_6.

The fractions eluting from the VLC column with 17 and 20% MeOH were combined (125 mg) and further purified on a Sephadex LH-20 column (27 × 1.5 cm), eluting successively with 4:1 CH_2Cl_2−hexane (350 mL), 4:1 CH_2Cl_2−Me_2CO (250 mL), 3:2 CH_2Cl_2−Me_2CO (300 mL), and 1:4 CH_2Cl_2−Me_2CO (300 mL). The fraction eluting with 3:2 CH_2Cl_2−Me_2CO (82 mg) was purified by reversed-phase HPLC (Hamilton PRP-1 column; 10 × 250 mm; 10-μm particle-size, 40 to 55% CH_3CN in 0.1% HCOOH−H_2O in 30 min at 3 mL/min) to give glisoprenin F (**4**, 4.4 mg, t_R 17.3 min).

Verticillin D (1): white powder; mp 244−247 °C; $[\alpha]_D$ +220° (*c* 0.1 mg/mL, MeOH); UV (MeOH) λ_{max} 214 (ϵ 19 000), 304 (1900); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS $[M + H]^+$ at *m/z* 757.1261 (calcd for $C_{32}H_{32}N_6O_8S_4$ + H, 757.1243).

Verticillin E (2): yellow powder; mp 211−214 °C; $[\alpha]_D$ +440° (*c* 0.1 mg/mL, MeOH); UV (MeOH) λ_{max} 218 (ϵ 26 000), 304 (4400); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS $[M + H]^+$ at *m/z* 753.0926 (calcd for $C_{32}H_{28}N_6O_8S_4$ + H, 753.0930).

Verticillin F (3): yellow powder; mp 221−224 °C; $[\alpha]_D$ −80° (*c* 0.1 mg/mL, MeOH); UV (MeOH) λ_{max} 214 (ϵ 18 000), 302 (1400); IR (CH_3CN) ν_{max} 3522, 2939, 1685, 1429, 1376 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 600 MHz) δ 7.88 (dd, 7.6, 1.0, H-10), 7.85 (dd, 7.6, 1.0, H-10′), 7.11 (2H, ddd, 7.7, 7.6, 1.0, H-8, H-8′), 6.76 (ddd, 7.6, 7.6, 1.1, H-9′), 6.75 (ddd, 7.6, 7.6, 1.1, H-9), 6.70 (br d, 7.7, H-7), 6.68 (br d, 7.7, H-7′), 6.04 (br s, NH-6′), 6.00 (br s, NH-6), 5.96 (d, 1.8, OH-11), 5.92 (d, 2.8, OH-11′), 5.73 (d, 2.8, H-11′), 5.70 (d, 1.8, H-11), 5.11 (d, 0.8, H-5a′), 5.10 (d, 0.7, H-5a), 4.80 (br d, 7.7, OH-13′), 4.63 (dq, 7.7, 6.6, H-13′), 3.19 (s, H_3-12), 2.91 (s, H_3-12′), 2.51 (s, H_3-14), 1.54 (d, 6.6, H_3-14′); ^{13}C NMR (Me_2CO-d_6, 75 MHz) δ 197.0 (C-13), 167.7 (C-1), 166.9 (C-1′), 162.1 (C-4′), 160.9 (C-4), 150.7 (C-6a′), 150.5 (C-6a), 130.6 (C-8), 130.6 (C-8′), 130.1 (C-10a), 130.1 (C-10a′), 128.8 (C-10), 128.7 (C-10′), 120.0 (C-9), 119.8 (C-9′), 110.7 (C-7), 110.6 (C-7′), 87.9 (C-3′), 83.6 (C-5a′), 83.4 (C-5a), 82.9 (C-11), 82.6 (C-11′), 80.2 (C-3), 78.1 (C-11a′), 77.5 (C-11a), 67.9 (C-13′), 67.2 (C-10b′), 66.9 (C-10b), 30.5 (C-12′), 28.5 (C-12), 27.9 (C-14), 19.9 (C-14′); HRESIMS $[M + H]^+$ at *m/z* 755.1118 (calcd for $C_{32}H_{30}N_6O_8S_4$ + H, 755.1086).

Glisoprenin F (4): colorless oil; $[\alpha]_D$ +272° (*c* 0.1 mg/mL, MeOH); UV (MeOH) λ_{max} 210 (ϵ 6900); IR (CH_3CN) ν_{max} 3022, 1231, 657 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.39 (dt, 1.2, 6.6, H-2), 5.10 (2H, br m, H-6, 10), 4.12 (2H, d, 6.6, H_2-1), 3.30 (d, 9.6, H-34), 2.01−2.14 (4H, m, H_2-5, 9), 1.92−2.01 (6H, m, H_2-4, 8, 12), 1.66 (s, H_3-37), 1.58 (s, H_3-38), 1.57 (s, H_3-39), 1.30−1.50 (32H, m, H_2-13, 14, 16, 17, 18, 20, 21, 22, 24, 25, 26, 28, 29, 30, 32, 33), 1.20 (s, H_3-45), 1.18 (s, H_3-44), 1.16 (9H, overlapping singlets, H_3-41, 42, 43), 1.15 (s, H_3-40), 1.14 (s, H_3-36); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 139.4 (C-3), 134.8 (C-7, 11), 124.4 (C-10), 123.9 (C-6), 123.5 (C-2), 78.9 (C-34), 72.8 (C-15, 19, 23, 27, 31), 72.6 (C-35), 59.3 (C-1), 43.5 (C-20), 42.6 (C-33), 42.3 (C-32), 42.2 (C-18, 22, 26, 30), 42.1 (C-16, 24, 28), 41.7 (C-14), 40.0 (C-12), 39.6 (C-8), 39.4 (C-4), 27.2 (C-42, 43, 44), 26.8 (C-40, 41), 26.5 (C-45), 26.3 (C-9), 26.2 (C-5), 23.5 (C-36), 22.2 (C-13), 18.1 (C-17, 21, 25, 29), 16.3 (C-37), 16.0 (C-38), 15.9 (C-39); selected HMBC correlations (H# → C#) H-1 → C-2, -3; H-2 → C-4, -37; H-6 → C-4; H-10 → C-8, -9; H_3-36 → C-34; H_3-37 → C-2, -3, -4; H_3-38 → 6, -7, -8; H_3-39 → 10, -11, -12; H_3-45 → C-34; HRFABMS $[M + Na]^+$ at *m/z* 777.6238 (calcd for $C_{45}H_{86}O_8$ + Na, 778.6220).

Verticillin D triacetate (7). A 3-mg sample of **1** was dissolved in 200 μL of pyridine and 1.5 mL of Ac_2O, and the solution was stirred for 42 h at room temperature. After addition of H_2O (2 mL), the resulting solution was extracted with 2 × 3 mL of $CHCl_3$. The $CHCl_3$ layers were combined, dried ($MgSO_4$), and evaporated to afford 3.2 mg (91% yield) of verticillin D triacetate (**7**) as a yellow powder: mp 197−200 °C; $[\alpha]_D$ +331° (*c* 1.3 mg/mL, MeOH); UV (MeOH) λ_{max} 214 (ϵ 21 000), 246 (7400), 306 (2900); IR ($CHCl_3$) ν_{max} 2958 (sh), 2927, 2855, 1750, 1695, 1685, 1231, 1197, 1080 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2. [The NMR data presented for **7** are reported in $CDCl_3$ solution rather than pyridine-d_5 because **7** is soluble in $CDCl_3$, and better signal dispersion was achieved in that solvent. However, the spectrum of **7** recorded in pyridine-d_5 solution showed significantly downfield-shifted δ_H values for some of the signals that were more in line with the corresponding δ values observed for pyridine-d_5 solutions of **1** and **2** (Table 1).] FABMS (3-nitrobenzyl alcohol) $[M + H]^+$ at *m/z* 883; HRFABMS (PEG 1000) $[M + H]^+$ at *m/z* 883.1574 (calcd for $C_{38}H_{39}N_6O_{11}S_4$, 883.1560).

Acknowledgment. We thank the National Science Foundation (CHE-9708316) and Biotechnology Research and Development Corporation (24-1-078) for financial support of this work.

References and Notes

(1) Jeffries, P. In *The Mycota, IV*; Wicklow, D. T., Söderström, B., Eds.; Springer-Verlag: Heidelberg, 1997; pp 149−164.
(2) Wicklow, D. T.; Joshi, B. K.; Gamble, W. R.; Gloer, J. B.; Dowd, P. F. *Appl. Environ. Microbiol.* **1998**, *64*, 4482−4484.
(3) Minato, H.; Matsumoto, M.; Katayama, T. *J. Chem. Soc., Perkin 1* **1973**, 1819−1825.
(4) Chu, M.; Truumees, I.; Rothofsky, M. L.; Patel, M. G.; Gentile, F.; Das, P. R.; Puar, M. S.; Lin, S. L. *J. Antibiot.* **1995**, *48*, 1440−1445.
(5) Nishida, H.; Huang, X.; Tomoda, H.; Omura, S. *J. Antibiot.* **1992**, *45*, 1202−1206.
(6) Nishida, H.; Huang, X.; Tomoda, H.; Omura, S. *J. Antibiot.* **1992**, *45*, 1669−1676.
(7) Sterner, O.; Thines, E.; Eilbert, F.; Anke, H. *J. Antibiot.* **1998**, *51*, 228−231.
(8) Takahashi, C.; Numata, A.; Ito, E.; Matsumura, E.; Araki, H.; Iwaki, H.; Kushida, K. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1859.
(9) Takahashi, C.; Minoura, K.; Yamada, T.; Numata, A.; Kushida, K.; Shingu, T.; Hagishita, S.; Nakai, H.; Sato, T.; Harada, H. *Tetrahedron* **1995**, *51*, 3483−3498.
(10) Fed. Regist. **1998**, *63*, (132), 37286−37289. EPA registration no. 64137-8; Primastop Biofungicide Powder, July 2, 1998.
(11) Wong, S. M.; Kullnig, R.; Dedinas, J.; Appell, K. C.; Kydd, G. C.; Gillum, A. M.; Cooper, R.; Moore, R. *J. Antibiot.* **1993**, *46*, 214−221.

NP980530X