Chloriolide, a 12-Membered Macrolide from *Chloridium virescens* var. *chlamydosporum* (NRRL 37636)

Ping Jiao,[†] Dale C. Swenson,[†] James B. Gloer,*[,†] and Donald T. Wicklow[‡]

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, and Mycotoxin Research Unit, Agricultural Research Service, National Center for Agricultural Utilization Research, USDA, Peoria, Illinois 61604

Received November 29, 2005

A new 12-membered macrolide (chloriolide; **1**) was obtained from solid-substrate fermentation cultures of *Chloridium virescens* var. *chlamydosporum* that was originally isolated from decayed wood. The structure of **1** was determined by analysis of 1D NMR, 2D NMR, and MS data and confirmed by X-ray crystallographic analysis. The absolute configuration of **1** was assigned by application of the modified Mosher method using the (*R*)- and (*S*)-MTPA derivatives of a rearrangement product obtained under standard acylation conditions. The assignment made by analysis of the corresponding $\Delta\delta$ values was verified by X-ray crystallographic analysis of the (*S*)-MTPA derivative.

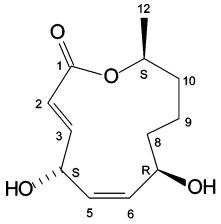
In the course of our ongoing studies of mycoparasitic and fungicolous fungi as sources of new bioactive natural products,[1−4] chemical investigation of solid-substrate fermentation cultures of *Chloridium virescens* var. *chlamydosporum* (J.F.H. Beyma) W. Gams & Holubova-Jechova (anamorph: Lasiosphaeriaceae) led to the isolation of a new 12-membered macrolide, chloriolide (**1**), along with two other known macrolides. Details of the isolation, structure elucidation, and stereochemical assignment of chloriolide (**1**) are presented here.

The isolate of *C. virescens* var. *chlamydosporum* employed in this work (MYC-1299 = NRRL 37636) was obtained from an area of conspicuous fungal colonization on the underside of a decaying hardwood branch collected in a Florida pine-oak forest and was considered to be a fungicolous isolate. The term "fungicolous" is sometimes used to refer specifically to fungi found as colonists of macromycetes, but can also be employed more widely to embrace a range of fungus−fungus relationships.[5] Hawksworth observed that saprophytic Hyphomycetes may be found growing intermixed with one another, and in some instances overgrowth or facultative parasitism may be exhibited, as with *C. botryoideum* (Corda) Hughes on various dematiaceous fungal species on wood.[5] *C. virescens* var. *chlamydosporum* is most commonly isolated from wood in advanced stages of decay and from forest soils rich in organic matter.[6,7] The fungus was one of five Hyphomycetes shown to increase in frequency with the decomposition of fallen logs of *Picea glauca* that were also colonized by Basidiomycetes.[8] There are no prior reports of chemistry from this species and only one report of chemistry from any member of the genus—an unrelated compound described from an unidentified *Chloridium* spp.[9]

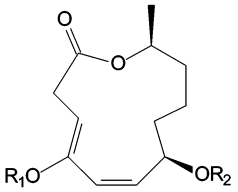
The EtOAc extract of solid-substrate fermentation cultures of *C. virescens* var. *chlamydosporum* exhibited activity against *Aspergillus flavus* and *Fusarium verticillioides* and was fractionated by Sephadex LH-20 column chromatography, followed by flash silica gel chromatography and HPLC, to afford a new 12-membered macrolide (**1**) and the known bioactive macrolides monorden (radicicol)[10,11] and pochonin B.[12] The known compounds were identified by comparison of their spectroscopic data with published values.[10−12]

Results and Discussion

Chloriolide (**1**) was obtained as a white powder that afforded NMR and EIMS data consistent with the molecular formula



1



2 R_1 = (*R*)-MTPA R_2 = H
3 R_1 = R_2 = (*R*)-MTPA
4 R_1 = R_2 = (*S*)-MTPA
5 R_1 = R_2 = (*R*)-MPA

$C_{12}H_{18}O_4$ (four unsaturations). The NMR data (Table 1) indicated the presence of two 1,2-disubstituted olefin moieties and one carboxy carbon, requiring chloriolide to be monocyclic. The ^{1}H−^{1}H COSY spectra, together with chemical shift data, revealed a single spin-system corresponding to the C2−C12 unit in **1**. The geometries of the C2−C3 and C5−C6 double bonds were assigned as *E* and *Z*, respectively, on the basis of ^{1}H−^{1}H coupling constants (J_{H2-H3} = 16 Hz, J_{H5-H6} = 12 Hz). HMBC correlations observed from olefinic proton H-2 and deshielded oxymethine proton H-11 to carboxy carbon C-1 established the location of the ester linkage, leading to the gross structure of **1** as shown.

The geometry of the double bonds in **1** was confirmed, and the relative configurations at C-4, C-7, and C-11 were assigned by X-ray crystallographic analysis. Colorless prisms of **1** were obtained from CH_2Cl_2/CH_3OH. X-ray diffraction analysis of **1** revealed the relative configuration as shown in Figure 1. Intermolecular hydrogen bonding was observed between the two hydroxyl groups in the crystal structure.

Table 1. NMR Data for Chloriolide (**1**) in $CDCl_3$[a]

position	δ_H	δ_C	HMBC (H → C#)
1		166.7	
2	6.15 (dd, 16, 2.2)	119.8	1, 3, 4
3	7.20 (dd, 16, 2.5)	152.7	1, 2, 4
4	4.80 (dt, 7.3, 2.5)	68.2	2, 3, 5, 6
5	5.75 (dd, 12, 7.3)	126.8	3, 4, 6
6	5.55 (dd, 12, 8.2)	140.5	4, 5
7	4.60 (br t, 8.2)	69.9	5, 8, 9
8	1.69 (m), 1.58 (m)	36.8	7, 9, 10
9	1.83 (m), 1.22 (m)	21.5	7, 8, 10, 11
10	1.78 (m), 1.45 (dt, 13, 8.4)	34.4	8, 9, 11
11	5.20 (m)	74.1	1, 10
12	1.35 (d, 6.4)	19.8	10, 11

[a] Recorded at 300 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, and 600 MHz for HMBC.

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

10.1021/np0504964 CCC: $33.50
Published on Web 02/14/2006

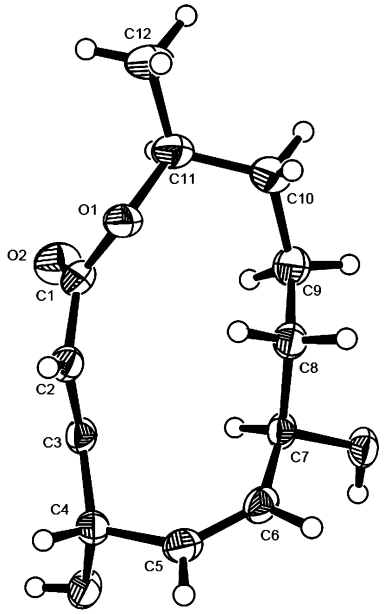
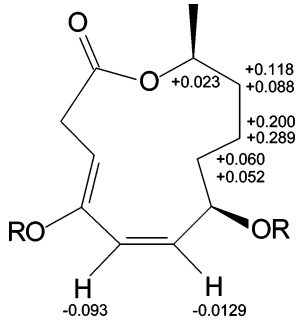


Figure 1. X-ray crystal structure of chloriolide (**1**).

Efforts to determine the absolute configuration of compound **1** using the modified Mosher's method[13] were undertaken. Although two secondary hydroxyl groups are present in **1**, 1 equiv of (*S*)-MTPACl [α-methoxy-α-(trifluoromethyl)phenylacetyl chloride] was added in an effort to prepare a monoacyl derivative. Two major products were obtained. The EIMS molecular ion peak at *m*/*z* 442 for one product (**2**) was consistent with the result expected for a monoacylated product. However, the ^{1}H NMR spectrum showed that the core structure of **1** had changed during the reaction. The two olefinic protons of the C2−C3 *E*-double bond were replaced by a methine triplet at δ_H 5.30 and a methylene doublet at δ_H 2.95, which were mutually coupled, as indicated by homonuclear decoupling experiments. The C5−C12 portion of the molecule remained the same as in compound **1**, but this spin-system was insulated from the new unit. Analysis of these data suggested that the product had undergone a double-bond rearrangement accompanied by a monoacylation to give structure **2**.

The ^{1}H NMR and MS data for the second product (**3**) clearly revealed that two MTPA units had been added and that the same double-bond rearrangement had occurred. The diastereotopic proton resonances of the new methylene group appeared at slightly different positions in this case. In addition, the signal for H-7 was shifted significantly downfield relative to that of **1**, indicating that the hydroxyl group at C-7 was acylated in **3**. HMBC and HMQC data were obtained for **3** in order to verify the proposed structure. These experiments allowed assignment of three new carbon signals around δ_C 114.7, 144.5, and 32.0. The HMBC correlations observed from H-3 to C-4, from H-5 to C-3, and from H-6 to C-4 indicated that the double bond formed involving carbons at δ_C 114.7 and 144.5 (C-3 and C-4) was conjugated with the *Z*-double bond of the C5−C12 unit. Correlations between the methylene protons and the ester carbonyl confirmed the occurrence of the double-bond rearrangement.

Upon treatment of **1** with 2 equiv of (*R*)-MTPACl, bis-(*S*)-MTPA ester **4** was obtained as the major product. ^{1}H−^{1}H COSY data enabled assignment of the proton signals for **4**, and comparison of these results with the 1D and 2D NMR data for **3** allowed assignment of the corresponding signals for **3**. Analysis of the $\Delta\delta_{S-R}$ values for the two diastereomeric esters **3** and **4** (Figure 3)[13] enabled assignment of the absolute configuration at C-7 as *R*. 4*S* and 11*S* configurations could then be assigned to **1** on the basis of the knowledge of the relative configuration from the X-ray structure. However, the geometry of the new C3−C4 double bond formed in **2**−**4** proved to be difficult to assign unambiguously by NMR.



3 R = (*R*)-MTPA
4 R = (*S*)-MTPA

Figure 2. $\Delta\delta$ values (=$\delta_S - \delta_R$; in ppm) obtained for (*S*)- and (*R*)-MTPA esters **4** and **3**.

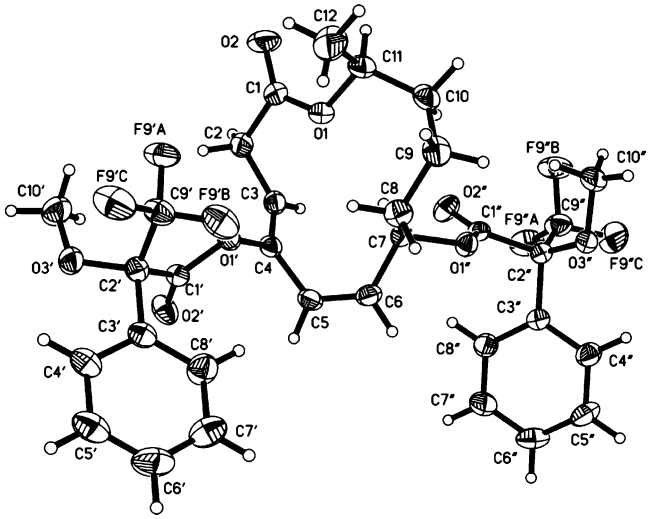


Figure 3. X-ray crystal structure of (*S*)-MTPA ester **4**.

Fortunately, a crystal of **4** was obtained from EtOAc/hexanes. X-ray diffraction analysis revealed that the new enol ester double bond has the *Z* geometry and also provided independent confirmation of the absolute configuration assignment for **4** arrived at by the modified Mosher protocol, and therefore for **1**−**3** as well.

In an effort to prevent the occurrence of the double-bond rearrangement, a different acylation reaction of **1** under somewhat milder conditions using *R*-MPAOH (α-methoxyphenylacetic acid) with EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide][14] as the coupling reagent was conducted. However, only products displaying the same double-bond rearrangement were observed, as exemplified by diacylation product **5**, which was isolated and characterized. At the same time, a blank control experiment carried out exposing **1** only to DMAP resulted in little if any reaction, with only a possible trace of the keto form of the nonacylated version of **2**−**4** being observed. Treatment of **1** with dilute HCl resulted in no rearrangement product, but instead afforded NMR signals consistent with hydrolytic cleavage of the lactone ring. The reason for the prevalence of this rearrangement under acylation reaction conditions is not clear, although limited molecular modeling calculations (CambridgeSoft Chem 3D Pro version 9.0.1) indicate that the products arising from the double-bond rearrangement are somewhat more stable than their unrearranged counterparts.

Chloriolide is a member of a family of polyketide-derived fungal macrolactones. The closest known analogues are patulolides A−C from *Penicllium urticae*[15,16] and cladospolides A−D produced by *Cladosporium* spp.[17−19] These precedents also possess 12-membered lactone rings, but with different arrangements in functionality. Interestingly, the 11*S* configuration assigned to **1** is different from

that reported for the analogous centers in monorden,[11] pochonin B,[12] patulolides A−C,[15,16] and cladospolides A−D,[17−19] although it does match the configuration recently assigned at the corresponding center in *iso*-cladospolide B and pandangolides 1 and 1a.[20]

Although the related cladospolides and patulolides have been reported to show antifungal and antibacterial activity,[15−17] chloriolide (**1**) was inactive against *Aspergillus flavus* (NRRL 6541) and *Fusarium verticillioides* (NRRL 25457) in standard disk assays[1] at 200 μg/disk. Chloriolide (**1**) was also inactive in antibacterial disk assays against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Bacillus subtilis* (ATCC 6051). The antifungal activity of the original extract in this case was attributed to the presence of the well-known antifungal metabolite monorden. Monorden (also known as radicicol) is produced by *Monicillium nordinii*, a mycoparasite isolated from fungi that attack forest trees, as well as a variety of other fungi.[1,21]

Experimental Section

General Experimental Procedures. The optical rotation was determined with a Rudolph automatic polarimeter, model AP III 589, and UV data were recorded with a Varian Cary III UV−visible spectrophotometer. ^{1}H and ^{13}C NMR spectra were acquired on Bruker DPX-300 and DRX-400 spectrometers, respectively. HMQC and HMBC data were obtained on a Bruker Avance-600. HPLC was carried out using a Beckman system Gold instrument with a model 166 UV detector. Other general procedures and instrumentation have been described previously.[22]

Fungal Material. A culture of *C. virescens* var. *chlamydosporum* (MYC-1299) was isolated from a visibly decayed portion of a dead hardwood branch collected from a long-leaf pine-oak forest at Wakulla Springs State Park near Crawfordsville, Florida, in May 2002. A subculture has been deposited in the Agricultural Research Service (ARS) collection at the NCAUR with the accession number NRRL 37636. General fermentation procedures used have been published elsewhere.[2,22] The culture was incubated on rice (3 × 50 g) at 25 °C for 30 days and extracted with EtOAc (3 × 500 mL). The combined, filtered EtOAc solution was evaporated to dryness, yielding 389 mg of crude extract.

Extraction and Isolation. The crude extract (389 mg) was partitioned between hexane and MeCN. The MeCN fraction (242 mg) was chromatographed using a Sephadex LH-20 column to yield 20 fractions. Fraction 12 consisted of essentially pure monorden (27 mg). Fraction 14 (26 mg) was further purified by flash silica column chromatography by gradient elution with hexanes/EtOAc. Subfractions 7, 8, 9, and 10 from fraction 14 were combined to afford chloriolide (**1**; 15 mg). Further purification of fraction 15 (20 mg) by HPLC (MeCN/H_2O, 40−50% over 15 min, 50−100% over 10 min) on an Alltech HS BDS 8-μm C_{18} column (10 × 250 mm) at a flow rate of 2 mL/min with UV detection at 215 nm gave pochonin B (2.7 mg).

Chloriolide (1): white powder; $[\alpha]^{25}_D$ +107 (*c* 0.2, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 270 (4.76); ^{1}H and ^{13}C NMR data, see Table 1; COSY data, H-2 → H-3, 4; H-3 → H-2, 4; H-4 → H-3, 5, 6; H-5 → H-4, 6, 7; H-6 → H-5, 7; H-7→ H-5, 8; H-8 → H-9, 10; H-9 → H-8, 10; H-10 → H-9, 11; H-11 → H-10, 12; H-12 → H-10, 11; ESIMS *m*/*z* 475 $[2M + Na]^+$, 249 $[M + Na]^+$.

X-ray Crystallographic Analysis of Chloriolide (1).[23] A colorless crystal of **1** was obtained from CH_2Cl_2/MeOH (0.47 × 0.40 × 0.19 mm) that proved to be monoclinic (space group $P2_1$) with cell dimensions a = 5.0243(5) Å, b = 7.2167(7) Å, c = 16.7328(17) Å. Crystallographic data were collected on a Nonius Kappa CCD diffractometer (Mo Kα radiation, graphite monochromator) at 190(2) K (cold N_2 gas steam) using standard CCD techniques, yielding 10 090 data. Lorentz and polarization corrections were applied. A multiscan empirical absorption correction was also applied (T_{max} = 0.9826, T_{min} = 0.9578). The data were averaged, yielding 1488 independent data (1437 > 4σ(F_o), R(int) = 0.025). The computer programs from the HKL package were used for data reduction. The preliminary model of the structure was obtained using XS, a direct method program. Least-squares refining of the model versus the data was performed with the XL computer program. All of these programs are in the SHELXTL V5.1 package. Thermal ellipsoids shown in the illustration are at the 35% level. No other restraints or constraints were applied. The final refinement gave R_1 = 0.0273, wR_2 = 0.0692.

Table 2. NMR Data for Mosher Esters **2** and **3** in $CDCl_3$

	2	**3**	
position	δ_H[a]	δ_H[c]	δ_C[d]
1			169.3
2	2.95 (d, 8.3)	2.97 (ddd, 17, 8.3, 1.1) 2.89 (ddd, 17, 8.3, 1.1)	32.0
3	5.30 (t, 8.3)	5.47 (td, 8.3, 1.1)	114.5
4			144.5
5	6.00 (d, 10)	6.19 (br d, 11)	124.5
6	5.77 (dd, 10, 6.9)	5.80 (dd, 11, 6.5)	139.0
7	4.80 (m)	6.12 (m)	73.3
8	2.0−1.2[b]	2.02 (tdd, 13, 4.6, 3.0), 1.71 (m)	33.0
9	2.0−1.2[b]	1.55 (m), 1.16 (m)	32.0
10	2.0−1.2[b]	1.72 (m), 1.39 (ddd, 15, 10, 4.5)	30.5
11	5.10 (m)	5.05 (m)	72.5
12	1.22 (d, 6.5)	1.19 (d, 6.4)	17.4

[a] Recorded at 300 MHz. [b] Methylene signals H_2-8−H_2-10 in **2** were not specifically assigned due to extensive overlap. [c] Recorded at 600 MHz. [d] These ^{13}C NMR shifts were assigned on the basis of 600-MHz HMBC data.

Preparation of (*R*)-MTPA Esters 2 and 3. To a solution of **1** (1.5 mg, 0.067 mmol) in CH_2Cl_2 (1 mL) was added (*S*)-MTPACl (1.7 mg, 0.067 mmol) and DMAP (1 crystal). After stirring at ambient temperature for 14 h, saturated aqueous $NaHCO_3$ was added. The reaction mixture was extracted with CH_2Cl_2 (3 × 1 mL), and the organic layer was collected, evaporated to dryness, and subjected to RP-HPLC (Alltech HS BDS 8 μm C_{18} column, 250 × 10 mm; flow rate 2 mL/min, UV detection at 215 nm, eluent MeCN/H_2O, 30−100% over 30 min) to afford **2** (0.5 mg) and **3** (0.7 mg). Compound **2**: white solid; ^{1}H NMR data, see Table 2; EIMS *m*/*z* 442 (M^+; 7), 387 (14), 189 (100). Compound **3**: white solid; ^{1}H and ^{13}C NMR data, see Table 2; selected key HMBC data, H-2 → C-1, 3, 4; H-3 → C-1, 4, 5; H-5 → C-3, 7; H-6 → C-4; H-7 → C-5; ESIMS *m*/*z* 676 $[M + NH_4]^+$, 681 $[M + Na]^+$, 697 $[M + K]^+$.

Preparation of (*S*)-MTPA Ester 4. A sample of **1** (1.7 mg, 0.0075 mmol) was treated with (*R*)-MTPACl (3.8 mg, 0.0015 mmol), and the reaction mixture was processed in the same fashion as described above to afford the (*S*)-MTPA ester **4** (2.4 mg). Compound **4**: white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.14 (m, H-7), 6.10 (d, J = 11 Hz, H-5), 5.67 (dd, J = 11, 6.3 Hz, H-6), 5.55 (t, J = 8.3 Hz, H-3), 5.07 (m, H-11), 3.07 (dd, J = 17, 8.3 Hz, H-2), 2.89 (dd, J = 17, 8.3 Hz, H-2), 2.08 (m, H-8), 1.82 (m, H-10), 1.76 (m, H-8), 1.74 (m, H-9), 1.51 (m, H-10), 1.45 (m, H-9), 1.19 (d, J = 6.5 Hz, H-12).

X-ray Crystallographic Analysis of (*S*)-MTPA Ester 4.[23] A colorless prism (0.24 × 0.24 × 0.20 mm; triclinic; space group P_1) obtained from 1:2 EtOAc/hexanes was selected having cell dimensions a = 8.9862(9) Å, b = 9.1107(9) Å, c = 10.8325(11) Å. Data were collected on a Nonius Kappa CCD diffractometer (Mo Kα radiation, graphite monochromator) at 190(2) K (cold N_2 gas stream) using standard CCD techniques, yielding 15 580 data. Lorentz and polarization corrections were applied. A correction for absorption using the multiscan techinique was also applied (T_{max} = 0.9757, T_{min} = 0.9710). Equivalent data were averaged, yielding 3531 unique data (R_{int} = 0.024, 2698 F > 4σ(F), Friedel pairs averaged). The computer program from the HKL package was used for data reduction. All other programs are in the SHELXTL v6.1 package, and the data were otherwise processed as described above for **1**. Due to the weak anomalous signal with Mo radiation and the fact that only determination of relative configuration was needed from the X-ray data for **1** and **4**, no absolute structure parameter (e.g., the Flack parameter) was refined in either case, and the Freidel pairs were averaged for the final cycles of refinement. The final refinement in this instance gave R_1 = 0.0391, wR_2 = 0.0940.

Preparation of (*R*)-MPA Ester 5. To a solution of **1** (1.6 mg, 0.071 mmol) with EDC (1.7 mg, 0.086 mmol) and DMAP (1 crystal) in CH_2Cl_2 (0.5 mL) was added (*R*)-MPAOH (1.4 mg, 0.084 mmol). After 1 h at ambient temperature, water was added. The resulting mixture was evaporated to dryness and directly subjected to reversed-phase HPLC (Alltech Apollo 5-μm C_{18} column, 250 × 10 mm; flow rate 2 mL/min, UV detection at 215 nm, eluting with MeCN/H_2O, 30−100%

over 30 min) to afford **5** (0.8 mg). Compound **5**: white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.86 (m, H-7), 5.80 (d, J = 11 Hz, H-5), 5.39 (dd, J = 11, 6.5 Hz, H-6), 5.33 (t, J = 7.8 Hz, H-3), 5.00 (m, H-11), 2.65 (d, J = 7.8 Hz, H-2), 2.10−1.20 (m, H8−H10), 1.18 (d, J = 6.5 Hz, H-12).

Acknowledgment. Financial support from the National Science Foundation (CHE-0315591) is gratefully acknowledged.

Supporting Information Available: X-ray data tables for **1** and **4**, ^{1}H NMR spectra for compounds **1**−**3**, and the ^{13}C NMR spectrum of compound **1**. This material is available free of charge on the Internet at http://pubs.acs.org.

References and Notes

(1) Wicklow, D. T.; Joshi, B. K.; Gamble, W. R.; Gloer, J. B.; Dowd, P. F. *Appl. Environ. Microbiol.* **1998**, *64*, 4482−4484.
(2) Soman, A. G.; Gloer, J. B.; Angawi, R. F.; Wicklow, D. T.; Dowd, P. F. *J. Nat. Prod.* **2001**, *64*, 189−192.
(3) Li, C.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Org. Lett.* **2002**, *4*, 3095−3098.
(4) Che, Y.; Gloer, J. B.; Wicklow, D. T. *J. Nat. Prod.* **2002**, *65*, 399−402.
(5) Hawksworth, D. L. In *Biology of Conidial Fungi*, Vol. 1; Cole, G. T., Kendrick, B., Eds.; Academic Press: New York, 1981; pp 171−244.
(6) Gams, W.; Holubova-Jechova, V. *Stud. Mycol.* **1976**, *13*, 1−99.
(7) Omvik, A. *Mycologia* **1970**, *62*, 209−226.
(8) Lumley, T. C.; Gignac, L. D.; Currah, R. S. *Can. J. Bot.* **2001**, *79*, 76−92.
(9) Kim, Y.; Nishida, H.; Pang, C.; Saito, T.; Toshiyuki, S. S.; Tonai-Kachi, H.; Yoshikawa, N.; VanVolkburg, M.; Parker, J. C.; Kojima, Y. *J. Antibiot.* **2002**, *55*, 121−127.
(10) Ayer, W.; Lee, S. P. *Can. J. Microbiol.* **1980**, *26*, 766−773.
(11) Cutler, H. G.; Arrendale, R. F.; Springer, J. P.; Cole, P. D.; Roberts, R. G.; Hanlin, R. T. *Agric. Biol. Chem.* **1987**, *51*, 3331−3338.
(12) Hellwig, V.; Mayer-Bartschmid, A.; Muller, H.; Greif, G.; Kleymann, G.; Zitamann, W.; Tichy, H.; Stadler, M. *J. Nat. Prod.* **2003**, *66*, 829−837.
(13) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(14) Neighbors, J. D.; Beutler, J. A.; Wiemer, D. F. *J. Org. Chem.* **2005**, *70*, 925−931.
(15) Sekiguchi, J.; Kuroda, H.; Yamada, Y.; Okada, H. *Tetrahedron Lett.* **1985**, *26*, 2341−2342.
(16) Rodphaya, D.; Sekiguchi, J.; Yamada, Y. *J. Antibiot.* **1986**, *39*, 629−635.
(17) Hirota, A.; Sakai, H.; Isogai, A. *Agric. Biol. Chem.* **1985**, *49*, 731−735.
(18) Fujii, Y.; Fukuda, A.; Hamasaki, T.; Ichimoto, I.; Nakajima, H. *Phytochemistry* **1995**, *5*, 1443−1446.
(19) Hirota, A.; Sakai, H.; Isogai, A.; Kitano, Y.; Ashida, Y.; Hirota, H.; Takahashi, T. *Agric. Biol. Chem.* **1985**, 49, 903−904.
(20) Gesner, S.; Cohen, N.; Ilan, M.; Yarden, O.; Carmeli, S. *J. Nat. Prod.* **2005**, *68*, 1350−1353.
(21) *Dictionary of Natural Products*, Web Version 2004(2); Chapman and Hall/CRC Press.
(22) Höller, U.; Gloer, J. B.; Wicklow, D. T. *J. Nat. Prod.* **2002**, *65*, 876−882.
(23) Crystallographic data for compounds **1** and **4** have been deposited with the Cambridge Crystallographic Data Centre (deposition numbers CCDC 284778 for **1** and 284777 for **4**). 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).

NP0504964