New Cyclic Peptide and Bisindolyl Benzenoid Metabolites from the Sclerotia of *Aspergillus sclerotiorum*

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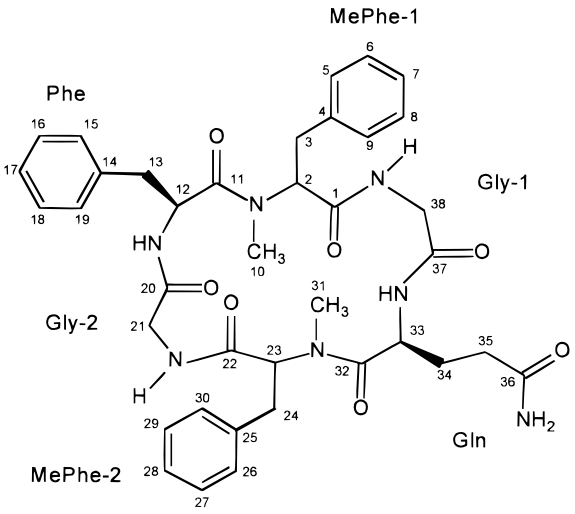
Scleramide (**1**), a new cyclic hexapeptide, and a new oxidized bisindolyl benzenoid derivative (2″-oxoasterriquinol D methyl ether, **2**) were isolated from extracts of the sclerotia of *Aspergillus sclerotiorum* (NRRL 5167). The structures of these compounds were determined by analysis of 1D and 2D NMR experiments.

Our interest in fungal sclerotia as sources of new antiinsectan metabolites prompted us to investigate the chemistry of the sclerotia of *Aspergillus sclerotiorum* Huber (NRRL 5167; Trichocomaceae). We have previously reported a variety of antiinsectan compounds from the sclerotia of *Aspergillus* spp.,[1,2] and initial studies of *A. sclerotiorum* afforded a potent new antiinsectan compound of the paraherquamide class called sclerotiamide.[3] Further studies of the sclerotial extracts of *A. sclerotiorum* were undertaken in an effort to isolate additional analogues that might show similar effects. Although additional metabolites with potent activity were not encountered, these studies did lead to the isolation of two additional new compounds; a cyclic hexapeptide (scleramide, **1**) and an oxidized bisindolyl benzenoid derivative (2″-oxoasterriquinol D methyl ether **2**). The known compound asterriquinol D methyl ether (**3**) was also obtained. Details of the isolation and structure determination of these metabolites are described here.



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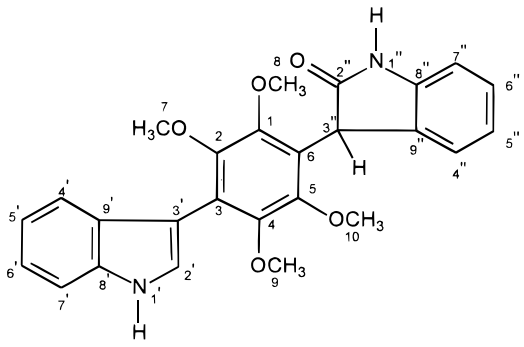
Chromatography of the $CHCl_3$ extract of the sclerotia on Sephadex LH-20, followed by reversed-phase HPLC, resulted in the isolation of scleramide (**1**). Scleramide has the molecular formula $C_{38}H_{45}N_7O_7$, as deduced from ^{13}C NMR (Table 1) and HRFABMS data [$(M + H)^+$ at *m/z* 712.3444, Δ 1.5 mmu]. The ^1H, ^{13}C, and DEPT NMR spectra of **1** contained resonances characteristic of a peptide, and revealed the presence of seven amide carbonyls, six amide NH protons, two *N*-methyl groups, and three phenyl groups. The presence of the phenyl groups was consistent with the observation of only 32 carbon signals in the ^{13}C NMR spectrum. These data accounted for 19 of the 20 unsaturations, and suggested that scleramide (**1**) is a cyclic peptide.



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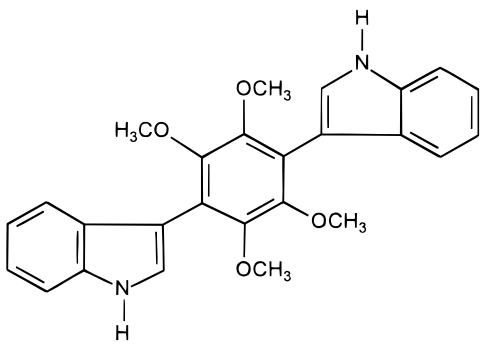
The structures of the constituent amino acids of **1** were determined from HMQC, HMBC (Table 1), and COSY data. For example, the presence of two MePhe residues was deduced on the basis of HMBC correlations of the *N*-methyl protons with the corresponding α-carbon signals, COSY correlations observed between the α- and *β*-protons, and HMBC correlations from the α- and *β*-protons to the neighboring aromatic carbons. Two Gly units, one Phe unit, and one Glx residue were also detected.

Two- and three-bond HMBC correlations from α- and *β*-protons of most of the amino acid residues to the



3

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

position	δ_H (mult., J_{HH})[b]	δ_C[c]	HMBC[d] (δ_C)	position	δ_H (mult., J_{HH})[b]	δ_C[c]	HMBC[d] (δ_C)
MePhe-1				MePhe-2			
N−CH_3	2.66 (s)	40.1	69.0, 172.8	NCH_3	2.82 (s)	40.2	68.6, 173.6
C=O		170.7		C=O		170.3	
α-CH	3.73 (dd, 11, 3.0)	69.0	34.7, 140.1, 170.7, 172.8	α-CH	3.91 (dd, 10, 4.2)	68.6	34.6, 40.2, 139.9, 170.3
β-CH_2	2.95 (dd, 14, 3.0)	34.7	69.0, 130.3, 140.1, 170.7	β-CH_2	3.33 (dd, 14, 10)	34.6	68.6, 130.3, 139.9, 170.3
	3.28 (m)				3.45 (dd, 14, 4.2)		
Ar-1		140.1		Ar-1		139.9	
2/6	7.31 (m)	130.3	34.7, 127.0, 129.3, 140.1	2/6	6.71 (m)	130.1	34.6, 127.3, 129.2, 139.9
3/5	7.42 (m)	129.3	127.0, 129.3, 140.1	3/5	7.16 (m)	129.2	127.3, 130.1, 139.9
4	7.15 (m)	127.0	129.3	4	7.25 (m)	127.3	130.1, 139.9
Gly-1				Gly-2			
NH	7.58 (br s)			NH	7.63 (d, 9.6)		170.3
C=O		169.8		C=O		169.4	
α-CH_2	3.34 (d, 10)	43.6	169.8, 170.7	α-CH_2	3.41 (dd, 13)	43.6	169.4, 170.3
	4.08 (dd, 10, 7.8)				4.11 (dd, 13, 9.6)		
Gln				Phe			
NH	7.60 (d)		169.8	NH	7.80 (d, 9.6)		169.4
C=O		173.6		C=O		172.8	
α-CH	5.05 (m)	48.4	28.5, 31.7, 173.6	α-CH	5.15 (m)	50.6	39.0, 138.5, 169.4, 172.8
β-CH_2	1.44 (m)	28.5	31.7, 48.4	β-CH_2	2.96 (dd, 13, 7.8)	39.0	50.6, 131.0, 138.5, 172.8
	2.20 (m)				3.40 (m)		
γ-CH_2	1.25 (m)	31.7	28.5, 48.4, 175.0	Ar-1		138.5	
	2.24 (m)			2/6	7.43 (m)	131.0	39.0, 127.5, 129.4, 138.5
NH_2	6.22 (br s)			3/5	7.36 (m)	129.4	127.5, 138.5
	6.89 (br s)			4	7.30 (m)	127.5	131.0
γ-C=O		175.0					

[a] Chemical shifts were referenced to residual solvent signals (7.24 or 77.0 ppm). [b] Recorded at 300 MHz. [c] Recorded at 75 MHz. [d] Recorded at 600 MHz (proton dimension).

corresponding carbonyl carbon signals enabled assignment of the carbonyl signals of individual amino acyl units. For example, HMBC correlations observed from the Phe α- and β-protons to the carbonyl carbon resonating at δ 172.8, and correlation of the α-proton with the carbonyl carbon signal at δ 169.4, indicated that the carbonyl carbon of the Phe residue resonates at δ 172.8 and that the signal at δ 169.4 must be that of the neighboring residue that acylates the nitrogen atom of the Phe unit. In cases where assignments for the α-carbonyl and the acylating carbonyl could not be clearly differentiated, results for other amino acyl units were used to make the distinction. For example, the Gly-2 α-protons showed HMBC correlations with the carbonyl carbon signals at δ 169.4 and 170.3. Because the carbon resonating at δ 169.4 must be the carbonyl that acylates Phe, then the carbonyl carbon signal at δ 169.4 must be the α-carbonyl carbon of Gly-2, and the carbon resonating at δ 170.3 must be that of the adjacent acylating unit.

The presence of Gln rather than Glu in scleramide (**1**) was deduced on the basis of the HRFABMS data, and on the presence of two broad singlets in the ^{1}H NMR spectrum (6.22 and 6.89 ppm) corresponding to the NH_2 protons of the γ-amide group. The peptide linkage of the Gln residue was determined on the basis of HMBC information. An HMBC correlation from the Gln α-proton signal to the Gln α-carbonyl carbon resonance at δ 173.6 was observed, together with a correlation to the same carbonyl from the neighboring MePhe-2 methyl proton signal. These data, in conjunction with an HMBC correlation observed from the Gln γ-protons to the Gln γ-carbonyl carbon signal, indicated an α-peptide linkage for this residue.

HMBC correlations from the *N*-methyl and α-protons were especially useful in determining the amino acid sequence of **1**. HMBC correlations from the MePhe-1 methyl signal to the Phe carbonyl carbon, and from the Gly-1 α-proton signals to the MePhe-1 carbonyl carbon, permitted the establishment of the partial sequence Phe→MePhe-1→Gly-1. Correlations from the Phe amide NH signal to the carbonyl signal of Gly-2 and from the Gly-2 α-proton signals to the MePhe-2 carbonyl resonance allowed assignment of the partial sequence MePhe-2→Gly-2→Phe. Finally, correlations from the Gln α-amide NH signal to the Gly-1 carbonyl resonance and from the MePhe-2 methyl group to the carbonyl resonance of Gln provided evidence for a Gly 1→Gln→MePhe-2 sequence. Based on these data, the gross structure of scleramide was assigned as shown in **1**.

Chiral capillary GC analysis of the *N*-(pentafluoropropionyl)isopropyl (PFP−IPA) ester derivatives[4] of the amino acid residues present in the total acid hydrolyzate revealed that the Phe and Gln residues in **1** have the L-configuration. However, the PFP−IPA derivatives of the D- and L-MePhe standards did not resolve under the GC conditions employed. Attempts to resolve the Marfey derivatives[5] of D- and L-MePhe standards were also unsuccessful. Ultimately, the stereochemistry of the MePhe units was determined by direct chiral HPLC analysis of the hydrolyzate,[6] which led to assignment of the L-configuration.

A known bisindolyl benzenoid, asterriquinol D methyl ether (**3**), was also isolated from the chloroform extract. Compound **3** has previously been isolated from *Aspergillus terreus*,[7] along with several other compounds of the same structural type. Many substituted bisindolyl benzenoids and quinones are known as fungal metabolites.[8−10] The sample of **3** isolated from *A. sclerotiorum* was identified based on comparison of NMR and MS data with those obtained for a sample obtained from *A. terreus* NRRL 2399. As expected, the ^{1}H and ^{13}C NMR spectra show signals corresponding to only half the molecule due to the symmetry of **3**.

A new analogue of **3**, 2″-oxoasterriquinol D methyl ether (**2**), was also isolated from this sclerotial extract. Compound **2** differs from the known members of this class of compounds because it is oxidized at the 2″ position. 2″-Oxoasterriquinol D methyl ether (**2**) has the molecular formula $C_{26}H_{24}N_2O_5$ (16 unsaturations), as deduced from HRFABMS data, and the formula differs from that of **3** by the addition of one oxygen atom. The NMR data clearly showed that the symmetry evident in the spectra of **3** was no longer present in **2**. The ^{13}C, ^{1}H, and DEPT NMR data

Table 2. NMR Data for 2″-Oxoasterriquinol D Methyl Ether (**2**) in $CDCl_3$

position	δ_H (mult., J_{HH})[a]	δ_C[a]	HMBC[b] correlations (C#)	position	δ_H (mult., J_{HH})[a]	δ_C[a]	HMBC[b] correlations (C#)
1		148.6		5′	7.11 (dd, 9, 6)	119.7	7′, 9′
2		147.4		6′	7.19 (m)	121.9	4′, 8′
3		123.7		7′	7.41 (d, 9)	110.9	5′, 9′
4		147.5		8′		135.8	
5		147.8		9′		127.1	
6		123.5		1″	7.81 (br s)		9″
7	3.50 (s)	60.5	2	2″		179.3	
8	4.00 (s)	62.0	1	3″	5.15 (s)	44.0	1, 5, 6, 2″, 8″, 9″
9	3.30 (s)	59.9	4	4″	7.07 (d, 6)	124.0	3″, 6″, 8″
10	3.30 (s)	60.1	5	5″	6.98 (dd, 9, 6)	122.3	7″, 9″
1′	8.37 (br s)			6″	7.22 (m)	127.7	4″, 8″
2′	7.29 (d, 3)	124.3	3′, 8′, 9′	7″	6.93 (d, 9)	109.2	5″, 9″
3′		108.4		8″		141.5	
4′	7.54 (d, 6)	121.1	3′, 6′, 8′	9″		130.9	

[a] Spectra recorded at 300 MHz (^1H) and 75 MHz (^{13}C). [b] HMBC data were recorded at 600 MHz (^1H dimension).

for **2** (Table 2) suggested the presence of nine olefinic or aromatic protons, four methoxy groups, two NH protons, a carboxyl or amide carbon, and one isolated sp^3-methine proton. Signals corresponding to 20 olefinic or aromatic carbons and a carbonyl group were observed in the ^{13}C NMR spectrum, accounting for 11 unsaturations. The five remaining unsaturations must be attributed to rings. The IR spectrum contained an absorption at 1670 cm^{-1} consistent with the presence of an amide group.

COSY data and ^1H NMR coupling values revealed that each partial structure contained a 1,2-disubstituted benzenoid ring. HMBC data (Table 2) enabled extension of these two partial structures to 3-indolyl and 3-(2-oxo)-indolyl groups. Correlations of amide NH-1″ with C-9″; of H-4″ with C-3″, C-6″, and C-8″; and of the methine proton H-3″ with amide carbonyl C-2″, C-8″, and C-9″ were instrumental in construction of the 3-(2-oxo)indolyl subunit. These units accounted for all but six nonprotonated aromatic carbons and four methoxy groups, requiring the presence of a fully substituted benzenoid unit bearing four methoxy groups and the two indolyl subunits as substituents. Each methoxy signal showed an HMBC correlation to its corresponding oxygenated aromatic carbon. Additional correlations of H-3″ with C-1, C-5, and C-6 indicated that the 3-(2-oxo)indolyl group is directly connected to the benzene ring at C-6, and that it is flanked by two methoxy-bearing carbons. The indole proton signal for H-2′ of the indole unit did not show HMBC or selective INEPT correlations with any of the carbon resonances of the fully substituted benzenoid ring. However, the indole units were placed *para* to each other on the benzenoid ring because ^{13}C NMR chemical shift considerations require this assignment and because of the structural analogy to **3**. These data afforded the structure shown in **2**.

The presence of identical substituents at C-1, C-2, C-4, and C-5 in **2** initially suggested that identical ^1H NMR chemical shifts should be expected for H_3-7 and H_3-9 and for H_3-8 and H_3-10. This reasoning would also suggest that the ^{13}C NMR chemical shifts for C-1 and C-5 should be identical, as should those for C-2 and C-4. However, very slight differences were observed between the corresponding resonances (Table 2). Presumably, the combination of a significant barrier to rotation and the presence of the chiral center (C-3″) results in different environments for the methoxy groups and the benzenoid carbons to which they are attached. 2″-Oxoasterriquinol D methyl ether (**2**) is optically active, but the stereochemistry was not assigned. A variety of bisindolyl benzenoids have been reported in the literature,[7−10] but, to our knowledge, compound **2** is the first oxindole analogue of this family to be described. Members of this general structural class have been reported to display antitumor properties,[11] as well as other interesting activities,[12] and have also been the subject of recent synthetic investigations.[13] The asterriquinones have been proposed to originate biosynthetically from tryptophan and mevalonic acid.[8−9]

Compounds **1**−**3** showed no significant activity in dietary assays against the corn earworm *Helicoverpa zea* at 100 ppm, though a sample of **1** did cause a modest (27%) reduction in feeding rate when tested against the fall armyworm *Spodoptera frugiperda* at 50 μg/cotton leaf disk. We have previously reported a cyclic peptide from the ascostromata of a *Eupenicillium* species,[14] but scleramide (**1**) is the first novel peptide encountered in our studies of *Aspergillus* sclerotial metabolites.

Experimental Section

General Experimental Procedures. Chiral GC analysis was performed on a Hewlett-Packard 5890 gas chromatograph, and chromatograms were recorded on a Shimadzu model CR3A integrator. General procedures and other instrumentation employed in this work (e.g., NMR, MS) have been described previously.[3]

Fungal Material. The culture of *A. sclerotiorum* (NRRL 5167) was obtained from the ARS Collection at the National Center for Agricultural Utilization Research in Peoria, IL. Production of sclerotia was accomplished by solid-substrate fermentation on autoclaved corn kernels and harvested using procedures previously described.[15]

Extraction and Isolation of 1−3. A sample of ground sclerotia (138 g) was extracted with 2 × 1 L of $CHCl_3$ to afford 1.6 g of extract, which was subjected to Sephadex LH-20 column chromatography eluting with CH_2Cl_2−MeOH (1:1). The first-eluting fraction (1.2 g) was subjected to a second Sephadex LH-20 column eluting with hexane−toluene−MeOH (3:1:1). The resulting fractions were pooled based on TLC results. Fraction 5 (211 mg) was subjected to preparative reversed-phase HPLC (Rainin Dynamax-60 Å 8-μm C_{18}, 2.14 × 25 cm; 10 mL/min) using CH_3CN−H_2O (50:50) to yield five fractions. Fraction 2 (36.4 mg) was further purified by semipreparative reversed-phase HPLC (Beckman Ultrasphere 5-μm C_{18}, 1 × 25 cm; 2 mL/min) using a gradient of 20 to 60% CH_3CN−H_2O over 15 min, then holding at 60%, to afford scleramide (**1**; 3.8 mg), 2″-oxoasterriquinol D dimethyl ether (**2**, 10.3 mg), and asterriquinol D dimethyl ether (**3**, 10.2 mg).

Scleramide (1): white solid; mp 167−169 °C (dec); UV (MeOH) 218 (ϵ 10 300), 260 (ϵ 3300); IR 3410, 2927, 2854, 1699, 1684, 1653 cm^{-1}; ^1H, ^{13}C NMR, and HMBC data, see Table 1; HRFABMS (3-nitrobenzyl alcohol) obsd *m/z* 712.3444 $[M + H]^+$, calcd for $C_{38}H_{45}N_7O_7$ + H, 712.3458.

Chiral GC Analysis of the PFP−IPA Derivatives Prepared from the Acid Hydrolyzate of Scleramide (1). A 0.8-mg sample of **1** was combined with 1 mL of 6 N HCl and

heated at 110 °C in a sealed vacuum hydrolysis tube for 24 h. The tube was cooled to room temperature, and the reagent was evaporated under a stream of dry N_2. The resulting hydrolyzate was treated with an excess of acetyl chloride in 2-propanol in a 4-mL Reacti-vial. The vial was capped and heated to approximately 100 °C. After 45 min, the excess reagent was evaporated under an N_2 stream at 115 °C. The residue was then cooled in an ice bath, and 1 mL of pentafluoropropionic anhydride was added along with 3 mL CH_2Cl_2. The vial was capped and heated to 100 °C for 15 min, cooled to room temperature, and evaporated to dryness under an N_2 flow.

The PFP−IPA derivatives of (D,L)- and (L)-Phe, *N*-MePhe, and Gln were prepared as described above and used as chromatographic standards. Chiral GC analyses were performed on a Chirasil-Val column (25 mm × 0.32 mm i.d.). Comparison of the GC retention times for the derivatives of the Phe and Gln residues established that these amino acids have the L-configuration. The PFP−IPA derivatives of the D- and L-*N*-MePhe standards did not resolve under the GC conditions employed.

Chiral HPLC Analysis of the Acid Hydrolyzate of Scleramide (1). A 0.3-mg sample of **1** was subjected to hydrolysis for 20 h using the conditions described above. The solvent was evaporated, and the residue was redissolved in 70 μL of H_2O. Chiral HPLC analysis of the resulting solution was performed using a Phenomenex Chirex column (50 × 4.6 mm) and eluting with 2 mM aqueous $CuSO_4$−CH_3CN (9:1) at 0.5 mL/min with UV detection at 254 nm.[6] The MePhe present in the hydrolyzate coeluted with L-MePhe at 15.40 min, and was clearly resolved from the D-isomer (17.40 min).

2″-Oxoasterriquinol D dimethyl ether (2): white solid; mp 145−147 °C dec; $[\alpha]_D$ −13.9° (*c* 0.001 g/mL, MeOH); UV (MeOH) 276 (ϵ 2600), 232 (ϵ 5500); IR 3463, 2932, 2857, 1670 cm^{-1}; ^1H, ^{13}C, and HMBC NMR data, see Table 2; HRFABMS (thioglycerol), obsd *m*/*z* 445.1705 $([M+H])^+$, calcd for $C_{26}H_{24}N_2O_5$ + H, 445.1763.

Asterriquinol D dimethyl ether (3): white solid; mp 187−189 °C dec; EIMS *m*/*z* 428 (M^+; 100), 385 (29), 355 (40), 299 (9); other spectral data have been previously reported.[8]

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References and Notes

(1) Gloer, J. B. *Acc. Chem. Res.* **1995**, *28*, 343−350.
(2) Gloer, J. B. In *The Mycota IV*; Wicklow, D. T., Söderström, B., Eds.; Springer-Verlag: Heidelberg, 1997; pp 249−268.
(3) Whyte, A. C.; Gloer, J. B.; Dowd, P. F.; Wicklow, D. T. *J. Nat. Prod.* **1996**, *59*, 1093−1095.
(4) Frank, H.; Nicholson, G. J.; Bayer, E. *J. Chromatogr.* **1978**, *167*, 187.
(5) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.
(6) Pettit, G. R.; Xu, J.-P.; Williams, M. D.; Hogan, F.; Schmidt, J. M.; Cerny, R. L. *Bioorg. Med. Chem.* **1997**, *7*, 827−832.
(7) Arai, K.; Shimizu, S.; Yamamoto, Y. *Chem. Pharm. Bull.* **1981**, *29*, 1005−1012.
(8) O'Leary, M. A.; Hanson, J. R.; Yeoh, B. L. *J. Chem. Soc., Perkin Trans. 1* **1984**, 567−570.
(9) Arai, K.; Yamamoto, Y. *Chem. Pharm. Bull.* **1990**, *38*, 2929−2932.
(10) Gloer, J. B.; DeGuzman, F. S.; Bruss, D. R.; Rippentrop, J. M.; Gloer, K. B. *J. Nat. Prod.* **1994**, *57*, 634−639.
(11) Kaji, A.; Saito, R.; Nomura, M.; Miyamoto, K.; Kiriyama, N. *Biol. Pharm. Bull.* **1998**, *21*, 945−949.
(12) Alvi, K. A.; Pu, H.; Luche, M.; Rice, A.; App, H.; McMahon, G.; Dare, H.; Margolis, B. *J. Antibiot.* **1999**, *52*, 215−223.
(13) Liu, K.; Wood, H. B.; Jones, A. B. *Tetrahedron Lett.* **1999**, *40*, 5119−5122.
(14) Belofsky, G. N.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Tetrahedron Lett.* **1998**, *39*, 5497−5500.
(15) TePaske, M. R.; Gloer, J. B.; Dowd, P. F.; Wicklow, D. T. *Trans. Br. Mycol. Soc.* **1988**, *91*, 433−438.

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