JOURNAL OF NATURAL PRODUCTS

Subscriber access provided by ISTANBUL TEKNIK UNIV

Isokotanins A-C: New Bicoumarins from the Sclerotia of Aspergillus alliceus

Jodi A. Laakso, Elizabeth D. Narske, James B. Gloer, Donald T. Wicklow, and Patrick F. Dowd

J. Nat. Prod., **1994**, 57 (1), 128-133• DOI: 10.1021/np50103a018 • Publication Date (Web): 01 July 2004

Downloaded from http://pubs.acs.org on April 4, 2009

More About This Article

The permalink http://dx.doi.org/10.1021/np50103a018 provides access to:

- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

ACS Publications
High quality. High impact.

Journal of Natural Products is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Journal of Natural Products
Vol. 57, No. 1, pp. 128-133, January 1994

ISOKOTANINS A–C: NEW BICOUMARINS FROM THE SCLEROTIA OF *ASPERGILLUS ALLIACEUS*

JODI A. LAAKSO, ELIZABETH D. NARSKE, JAMES B. GLOER,*

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

DONALD T. WICKLOW, and PATRICK F. DOWD

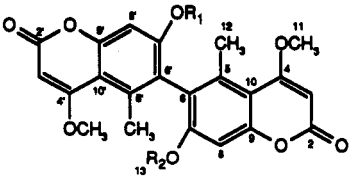
Agricultural Research Service, National Center for Agricultural Utilization Research, USDA, Peoria, Illinois 61604

ABSTRACT.—Three new bicoumarin metabolites, isokotanins A [**1**], B [**2**], and C [**3**], were isolated from the sclerotia of *Aspergillus alliaceus*. Isokotanin A is a regioisomer of the known bicoumarin kotanin [**4**]. The structures and spectral assignments for **1–3** were determined on the basis of selective INEPT, HMQC, HMBC, and NOESY nmr data, as well as by chemical interconversions. Isokotanins B and C show activity against the corn earworm *Helicoverpa zea* and the dried fruit beetle *Carpophilus hemipterus*. The known compounds kotanin [**4**], desmethylkotanin [**5**], nominine, and paspaline were also isolated from extracts of *A. alliaceus* sclerotia.

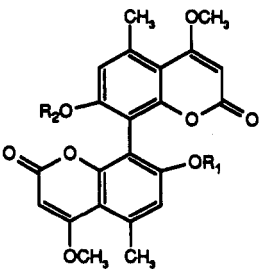
Many fungi produce durable physiological structures called sclerotia as a mechanism for the long-term survival and propagation of the species (1–3). Some sclerotia contain secondary metabolites that appear to play a role in protecting them from potential predators (e.g., fungivorous insects) (2,3). In our efforts to discover and identify bioactive sclerotial metabolites from *Aspergillus* spp., we have isolated a variety of compounds with antiinsectan activity (4). Chemical studies of the sclerotia of *Aspergillus alliaceus* Thom and Church (Aspergillaceae) (NRRL 315) have now yielded three new bicoumarin metabolites, which we have named isokotanins A [**1**], B [**2**], and C [**3**]. The isokotanins are related to the known fungal metabolites kotanin [**4**] and desmethylkotanin [**5**] (5), orlandin [**6**] (6), aflavarin (7), and desertorins A–C (8). In addition to the isokotanins, the known metabolites **4** and **5**, nominine (9), and paspaline (10) were isolated from the sclerotia of *A. alliaceus* (NRRL 315 and 317). Details of these studies are reported here.

RESULTS AND DISCUSSION

Sclerotia from *A. alliaceus* NRRL 315 were produced by solid substrate fermentation on corn kernels (2). The ground sclerotia were extracted first with pentane, then with CH_2Cl_2 using a Soxhlet apparatus. The CH_2Cl_2 extract exhibited antiinsectan activity against the economically important crop pest *Helicoverpa zea* and the ecologically relevant fungivorous beetle *Carpophilus hemipterus*. Therefore, this extract was further fractionated using Si gel cc and reversed-phase hplc, affording **1–5**. The identities of **4** and **5** were



1 $R_1=R_2=CH_3$
2 $R_1=H$, $R_2=CH_3$
3 $R_1=R_2=H$



4 $R_1=R_2=CH_3$
5 $R_1=H$, $R_2=CH_3$
6 $R_1=R_2=H$

determined by comparing their spectral properties to the data published for these compounds (5,11). Since no ^{13}C-nmr data have been previously published for **5**, its structure was confirmed by analysis of HMQC, HMBC, and NOESY nmr data.

Many spectral similarities between isokotanin A [**1**] and **4** were evident. The ^{1}H-nmr spectrum of each showed signals for two vinylic/aromatic protons, two MeO groups, and one aryl Me group (Table 1), although the chemical shifts of the aryl Me protons

TABLE 1. ^{1}H-nmr Data for Isokotanins A–C [**1–3**].[a]

H #	Isokotanin A	Isokotanin B	Isokotanin C
3	5.57 (s)	5.60 (s)	5.62 (s)
8	6.75 (s)	6.87 (s)	6.70 (s)
11	3.92 (s)	4.00 (s)	3.97 (s)
12	2.21 (s)	2.31 (s)	2.29 (s)
13	3.70 (s)	3.78 (s)	
3′		5.56 (s)	
8′		6.73 (s)	
11′		4.01 (s)	
12′		2.25 (s)	

[a]Data were recorded at 300 MHz in $CDCl_3$, Me_2CO-d_6, and CD_3OD for isokotanins A, B, and C, respectively.

differ significantly (2.70 ppm for **4**, 2.21 ppm for **1**). The ^{13}C-nmr data for **1** contained signals for one carboxyl, two MeO, one Me, and eight aromatic/vinylic carbons (Table 2). Although these data suggested the formula $C_{12}H_{11}O_4$, the molecular weight of **1** was established as 438 by eims ($C_{24}H_{22}O_8$). The nmr, ir, and uv spectra were all consistent with the presence of a substituted coumarin unit. Therefore, **1** was recognized as a symmetrical coumarin dimer wherein each subunit is substituted with two MeO groups and one Me group.

An HMQC nmr experiment was conducted to establish single-bond carbon-proton correlations, while selective INEPT data provided long-range correlations. The upfield shifts of H-8 and C-8 indicated a disposition ortho or para to two oxygen substituents, and the aryl methyl showed selective INEPT correlations to three non-oxygenated, non-protonated aromatic carbons (C-5, -6, and -10). One MeO group was located at the 7-position on the basis of a selective INEPT correlation with C-7. The second MeO group was located at C-4 on the basis of a selective INEPT correlation with C-4, as well as chemical shift considerations (C-4 at 170.0 ppm; C-3 at 87.9 ppm). The ^{13}C-nmr shifts reported for siderin (the monomeric subunit of **1**) (12) are all within 1.5 ppm of the values obtained for **1** except for that of C-6, which is the proposed attachment point for the two monomer units. These results could only be accounted for by the substitution pattern shown in **1**. Selective INEPT correlations observed for H-3 and H-8 (see Experimental) were fully consistent with the assignment. Further confirmation for this proposed structure was obtained by analysis of NOESY data. Intense cross-peaks correlating each aromatic/vinylic proton singlet with the appropriate ortho MeO group were observed in the NOESY spectrum, and a NOESY correlation was also observed between H_3-11 and H_3-12.

The ^{1}H-nmr spectrum of isokotanin B [**2**] (Table 1) indicated that it is closely related to **1**, but has an asymmetric structure. Only three MeO signals were present in the ^{1}H-nmr spectrum of **2**, and hreims data indicated a molecular formula of $C_{23}H_{20}O_8$. Therefore, isokotanin B was proposed to be a desmethyl analog of **1**. The location of the phenolic OH group was assigned on the basis of HMQC and HMBC nmr data (Table 3).

TABLE 2. ^{13}C-nmr Data for Isokotanins A–C [**1–3**].[a]

C #	Isokotanin A	Isokotanin B	Isokotanin C
2	163.0	162.4	166.0
3	87.9	88.1	87.5
4	170.0	170.5	172.6
5	137.2	138.3	139.3
6	123.4	123.9	124.4
7	160.1	161.4	160.5
8	97.4	97.8	101.6
9	156.2	157.4	157.3
10	108.1	108.8	108.3
11	56.2[b]	56.6	56.9
12	18.7	19.0	19.2
13	56.0[b]	56.5	
2′		162.4	
3′		87.7	
4′		170.5	
5′		138.3	
6′		123.2	
7′		159.1	
8′		101.0	
9′		156.8	
10′		108.2	
11′		56.7	
12′		19.1	

[a]Data were recorded at 75.6 MHz in $CDCl_3$, Me_2CO-d_6, and CD_3OD for isokotanins A, B, and C, respectively.

[b]These carbon assignments may be interchanged.

Each MeO proton signal showed a single HMBC correlation to the adjoining aromatic carbon. Each aromatic/vinylic proton signal showed HMBC correlation(s) to the corresponding ortho oxygenated carbon(s). H-8′ was correlated with two oxygenated aromatic carbons, but was the only aromatic/vinylic proton signal not showing an HMBC correlation to a methoxy-bearing carbon, so it was possible to locate the phenolic OH group at C-7′ as shown in **2**. The MeO groups attached to C-4 and C-4′ were differentiated based on their NOESY correlations to neighboring ortho protons (Table 3). As expected from the HMBC results, H-8′ was the only aromatic/vinylic proton

TABLE 3. HMBC and NOESY Data for Isokotanin B [**2**].[a]

H #	HMBC correlations (C#)	NOESY correlations (H#)
3	2, 4, 10	11
8	4[b], 6, 7, 9, 10	13
11	4	3, 12
12	5, 6, 10	11
13	7	8
3′	2′, 4′, 10′	11′
8′	4′[b], 6′, 7′, 9′, 10′	
11′	4′	3′, 12′
12′	5′, 6′, 10′	11′

[a]Data were recorded at 600 MHz (^{1}H dimension) in Me_2CO-d_6.

[b]These correlations represent 4-bond couplings. All other HMBC correlations represent 2- or 3-bond couplings.

signal to lack a NOESY correlation with a MeO signal. Further support for the structure assignment was obtained by methylation of **2** with diazomethane to yield **1**.

The ^{1}H-nmr spectrum of isokotanin C [**3**] exhibited two vinylic/aromatic proton signals, one aryl Me signal, and only one MeO resonance (Table 1). The hrei mass spectrum indicated the molecular formula $C_{22}H_{18}O_8$. These data suggested that **3** is a symmetrical desmethyl analog of **2**. Analysis of selective INEPT data (see Experimental section), and comparison with the selective INEPT and HMBC results for compounds **1** and **2**, allowed assignment of the structure of isokotanin C as **3**. Treatment of **3** with diazomethane again yielded compound **1**, thereby supporting the structure assignment.

Isokotanins A–C [**1–3**] differ from kotanin, desmethylkotanin, and orlandin, respectively, in the connection of the two coumarin subunits. The isokotanins are linked between carbons 6 and 6′, whereas the kotanins and orlandin [**4–6**] are linked in an 8,8′-fashion. Desertorins A–C are all asymmetrical compounds linked in a 6,8′-manner. Isokotanins A–C are chiral due to restricted rotation about the single bond connecting the two coumaryl units. Each compound showed optical activity, but no stereochemical assignments were made.

Interestingly, the chemical conversion of isokotanin C [**3**] to isokotanin A [**1**] yielded a product with an optical rotation opposite in sign to that of the naturally occurring **1**. The analogous reaction converting isokotanin B [**2**] to **1** yielded a product identical in all respects (including optical rotation) to the naturally occurring compound. One possible explanation for this result assumes racemic **3** as a biosynthetic precursor of **1** and **2**. Siderin and kotanin [**4**] are reportedly formed via the polyketide pathway (11,13). If enzymatic methylation of **3** to give **2** proceeded with some substrate selectivity for one enantiomer (atropisomer) of **3**, the unreacted **3** would be enriched in the other enantiomer. Therefore, chemical methylation of the residual, naturally occurring **3** would yield a product enriched in the opposite enantiomer of **1**. Although this would rationalize the results obtained, further investigation would be required to unambiguously determine the origin of these stereochemical differences.

Paspaline, a known indole-derived metabolite (10), was also isolated from the pentane extract of the sclerotia of *A. alliaceus* (NRRL 315). A second isolate of *A. alliaceus* (NRRL 317) showed a sclerotial metabolite profile nearly identical to that found for the NRRL 315 strain. However, the CH_2Cl_2 extract from the sclerotia of *A. alliaceus* NRRL 317 also yielded the known antiinsectan metabolite nominine. Nominine was originally isolated from *Aspergillus nomius* (9), a member of the *Aspergillus flavus* taxonomic group, and it has also been isolated from members of the *Aspergillus niger* group (14). To our knowledge, this is the first report of the isolation of this metabolite from a member of the *Aspergillus ochraceus* taxonomic group. Another strain of *A. alliaceus* is known as the source organism for asperlicin, a potent non-peptidal cholecystokinin antagonist (15).

Isokotanin B [**2**] causes a 21% reduction in feeding by *C. hemipterus* larvae relative to controls when incorporated into a standard test diet at 100 ppm (wet weight). Isokotanin C induces a 19% reduction in *C. hemipterus* adult feeding at 100 ppm. Isokotanin A, the kotanins, and paspaline are inactive in these assays at this dietary level. Isokotanin A and kotanin were also tested at levels that more closely approximated their sclerotial concentrations (500 and 1000 ppm, respectively). In each case, some feeding reduction was observed in assays against *C. hemipterus* adults (17% and 23%, respectively). Isokotanin C [**3**] possesses mild activity against the corn earworm *H. zea*, causing a reduction in weight gain of 11% at the 100 ppm dietary level after one week. Although nominine is much more active against *H. zea* (9), neither this metabolite nor **1–5** are present in levels sufficient to account for the level of *H. zea* activity displayed by the CH_2Cl_2 extracts. Thus, further studies of these *A. alliaceus* isolates are in progress.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr data were obtained at 300 and 75.0 MHz, respectively. HMQC experiments were recorded at 300 or 600 MHz (^{1}H dimension), while HMBC experiments were carried out at 600 MHz. Spectra were recorded in $CDCl_3$, Me_2CO-d_6, or CD_3OD, and chemical shifts were referenced using the corresponding solvent signals: 7.24 ppm/77.0 ppm, 2.04 ppm/29.8 ppm, 3.30 ppm/49.0 ppm, respectively. Multiplicities of carbon signals were verified through DEPT experiments. Selective INEPT experiments were optimized for $^nJ_{CH}$ values of 4, 7, or 10 Hz, while HMBC and HMQC experiments were optimized for $^nJ_{CH}$=8.3 Hz and $^1J_{CH}$=150.2 Hz, respectively. Hplc separations employed a Beckman Ultrasphere ODS column (5-μm particles, 250×10 mm) at a flow rate of 2.0 ml/min with uv monitoring at 215 nm. Details of bioassays and other experimental procedures have been described elsewhere (2,3,16).

CULTIVATION OF THE ORGANISM.—Cultures of *A. alliaceus* (NRRL 315 and 317) were obtained from the Agricultural Research Service (ARS) collection of the USDA National Center for Agricultural Utilization Research in Peoria, IL. The NRRL 315 strain was originally isolated from a dead blister beetle, while NRRL 317 was isolated from a garlic bulb (17). The sclerotia of *A. alliaceus* may eventually ripen to form ascospores (18). This teleomorph state of the fungus is properly named *Petromyces alliaceus* Malloch and Cain. Sclerotia were produced by solid substrate fermentation on autoclaved corn kernels using general procedures described previously (2). The harvested sclerotia were then ground to a powder and stored at 4° until extraction.

ISOLATION AND CHARACTERIZATION OF **1–5**.—Ground sclerotia of *A. alliaceus* NRRL 315 (150.0 g) were sequentially extracted with pentane and CH_2Cl_2 using a Soxhlet apparatus. A portion (280 mg) of the total CH_2Cl_2 extract (973 mg) was fractionated by Si gel cc. A stepwise gradient from 0–10% (v/v) MeOH in $CHCl_3$ was employed, resulting in the elution of several fractions. Fractions eluting between 3 and 8% MeOH were separated further by reversed-phase hplc (MeOH-H_2O 75:25 or 70:30) to give isokotanin A (**1**, 2.1 mg), isokotanin B (**2**, 14.4 mg), isokotanin C (**3**, 1.7 mg), kotanin (**4**, 1.2 mg), and desmethylkotanin (**5**, 3.7 mg).

Isokotanin A [**1**].—Compound **1** was isolated as a yellow solid with the following properties: mp 223–226° (dec); $[\alpha]_D$ +21.4° (*c*=0.22 g/dl, $CHCl_3$); hplc R_t 11.5 min at MeOH-H_2O (75:25); uv (MeOH) 214 (ε 23200), 238 (15600), 291 (9800), 313 (13400), 321 nm (12700); ir (neat) 3432, 2927, 2852, 1722, 1607, 1595, 1457, 1366, 1254, 1171, 977, 809 cm^{-1}; ^{1}H- and ^{13}C-nmr data, Tables 1 and 2, respectively; selective INEPT nmr data: H-3→C-4, 10; H-8→C-6, 7, 9, 10; H_3-11→C-4; H_3-12→C-5, 6, 10; H_3-13→C-7; eims (70 eV) *m/z* 438 $[M]^+$ (100), 423 (3.7), 410 (13), 392 (11), 364 (6.8), 219 (9.7), 191 (19), 69 (29), 55 (27), 44 (80); hreims obsd 438.1294, calcd for $C_{24}H_{22}O_8$ 438.1318.

Isokotanin B [**2**].—Compound **2** was isolated as a white solid; mp 213–216°; $[\alpha]_D$ +40.8° (*c*=0.6 g/dl, $CHCl_3$); hplc R_t 11.2 min at MeOH-H_2O (70:30); uv (MeOH) 213 (ε 19500), 216 (19700), 239 (12900), 292 (8900), 315 (13400), 323 nm (13200); ir (neat) 3247, 3013, 2944, 1705, 1593, 1558, 1456, 1370, 1089, 1063, 975, 808, 757 cm^{-1}; ^{1}H, ^{13}C, HMBC, and NOESY nmr data, Tables 1, 2, and 3; eims (70 eV) *m/z* 424 $[M]^+$ (100), 409 (7.1), 396 (19), 381 (5.6), 281 (6.9), 207 (9.4), 165 (10), 149 (16); hreims obsd, 424.1138, calcd for $C_{23}H_{20}O_8$ 424.1158.

Isokotanin C [**3**].—Compound **3** was isolated as a white solid; mp 219–222° (dec); $[\alpha]_D$ −29.1° (*c*=0.46 g/dl, MeOH); hplc R_t 9.5 min at MeOH-H_2O (70:30); uv (MeOH) 217 (ε 12600), 238 (7700), 293 (6200), 317 (10700), 324 nm (10800); ir (neat) 3245, 1685, 1592, 1559, 1457, 1378, 1247, 971, 807 cm^{-1}; ^{1}H- and ^{13}C-nmr data, Tables 1 and 2, respectively; selective INEPT nmr data, H-3→C-4, 10; H-8→C-4, 6, 7, 9, 10; H_3-11→C-4; H_3-12→C-5, 6, 10; eims (70 eV) *m/z* 410 $[M]^+$ (100), 395 (12), 382 (25), 281 (5.9), 265 (3.8), 237 (6.0), 207 (20), 206 (5.1), 205 (7.0), 177 (22); hrfabms obsd, 411.1062, calcd for $C_{22}H_{18}O_8$+H 411.1080.

Kotanin [**4**].—Compound **4** had hplc R_t 10.2 min at MeOH-H_2O (75:25); other properties of kotanin have been reported previously (5,12).

Desmethylkotanin [**5**].—Compound **5** had hplc R_t 7.6 min at MeOH-H_2O (75:25); ^{1}H nmr (Me_2CO-d_6) H-3, 5.52 (s); H-6, 6.74 (s); H-11, 3.93 (s); H-12, 2.70 (s); H-13, 3.81 (s); H-3′, 5.48 (s); H-6′, 6.70 (s); H-11′, 3.91 (s); H-12, 2.54 (s); ^{13}C nmr (Me_2CO-d_6) C-2, 163.9; C-3, 87.7; C-4, 170.2; C-5, 139.3; C-6, 111.7; C-7, 159.8; C-8, 106.0; C-9, 153.7*; C-10, 108.7; C-11, 56.1; C-12, 24.2; C-13, 5.2; C-2′, 163.2; C-3′, 87.1; C-4′, 170.2; C-5′, 138.2; C-6′, 116.0; C-7′, 157.4; C-8′, 105.0; C-9′, 153.9*; C-10′, 107.6; C-11′, 55.8; C-12′, 23.4 (*assignments for these carbon atoms may be interchanged); other properties of desmethylkotanin have been reported previously (5).

METHYLATION OF **2** AND **3** TO FORM **1**.—To 1.0 mg isokotanin B [**2**] or isokotanin C [**3**] in MeOH

(0.4 ml) was added an excess of freshly generated CH_2N_2 in Et_2O (3 ml). The solution was allowed to stand for 30 min at room temperature and then the solvent was evaporated. In each case, analysis by tlc indicated incomplete reaction, so the above procedure was repeated twice more, ultimately yielding 1.0 mg of material identical to **1** as judged by tlc, hplc, and ^{1}H-nmr analysis.

ISOLATION OF PASPALINE AND NOMININE.—Ground sclerotia of *A. alliaceus* NRRL 317 (121 g) were sequentially extracted with pentane and CH_2Cl_2, using a Soxhlet apparatus. A portion (250 mg) of the total CH_2Cl_2 extract (361 mg) was fractionated by Si gel cc. Elution with a stepwise gradient from 0–10% MeOH in $CHCl_3$ afforded several fractions. The first fraction (9.5 mg) to elute from this column was purified by reversed-phase hplc MeOH-H_2O (90:10) to give nominine (1.3 mg). The nmr data for this material were identical to those of an authentic standard of nominine isolated from *A. nomius* (9). Also isolated from this extract using conditions described above for NRRL 315 were **2** (1.9 mg), **3** (4.6 mg), **4** (28.6 mg), and **5** (9.2 mg).

A second sample of *A. alliaceus* NRRL 315 sclerotia (11.5 g) was ground with a mortar and pestle and then extracted with hexane. The hexane extract (20.3 mg) was separated using reversed-phase hplc MeOH-H_2O (90:10) to yield paspaline (2.0 mg). This material had spectral properties (nmr and ms) identical to those of an authentic standard of paspaline (10) isolated from *A. flavus* (7).

ACKNOWLEDGMENTS

This research was supported by grants from the National Science Foundation (CHE-9211252) and Biotechnology Research and Development Corporation. The University of Iowa nmr and ms Central Facilities are gratefully acknowledged. We also thank the Midwest Center for Mass Spectrometry for hrms analyses. Support for J.B.G. in the form of an Alfred P. Sloan Fellowship and an NIH Research Career Development Award (K04 CA01571) is gratefully acknowledged.

LITERATURE CITED

1. H.J. Willetts, *Biol. Rev. Cambridge Philos. Soc.*, **46**, 387 (1971).
2. D.T. Wicklow, P.F. Dowd, M.R. TePaske, and J.B. Gloer, *Trans. Br. Mycol. Soc.*, **91**, 433 (1988).
3. J.B. Gloer, M.R. TePaske, J. Sima, D.T. Wicklow, and P.F. Dowd, *J. Org. Chem.*, **53**, 5457 (1988).
4. J.A. Laakso, J.B. Gloer, D.T. Wicklow, and P.F. Dowd, *J. Org. Chem.*, **57**, 2066 (1992).
5. G. Buchi, D.H. Klaubert, R.C. Shank, S.M. Weinreb, and G.N. Wogan, *J. Org. Chem.*, **36**, 1143 (1971).
6. H.G. Cutler, F.G. Crumlet, R.H. Cox, O. Hernandez, R.J. Cole, and J.W. Dorner, *J. Agric. Food Chem.*, **27**, 592 (1979).
7. M.R. TePaske, J.B. Gloer, D.T. Wicklow, and P.F. Dowd, *J. Nat. Prod.*, **55**, 1080 (1992).
8. K. Nozawa, H. Seyea, S. Nakajima, S. Udagawa, and K. Kawai, *J. Chem. Soc., Perkin Trans. I*, 1735 (1987).
9. J.B. Gloer, B.L. Rinderknecht, D.T. Wicklow, and P.F. Dowd, *J. Org. Chem.*, **55**, 5555 (1989).
10. J.P. Springer and J. Clardy, *Tetrahedron Lett.*, **21**, 231 (1980).
11. J.B. Stothers and A. Stoessl, *Can. J. Chem.*, **66**, 2816 (1988).
12. R.D. Lapper, *Tetrahedron Lett.*, 4293 (1974).
13. K.K. Chexal, C. Fouwather, and J.S.E. Holker, *J. Chem. Soc., Perkin Trans. I*, 554 (1975).
14. M.R. TePaske, "Isolation and Structure Determination of Antiinsectan Metabolites from the Sclerotia of *Aspergillus* Spp." Ph.D. Thesis, University of Iowa, 1991.
15. R.S.L. Chang, V.J. Lotti, R.L. Monaghan, J. Birnbaum, E.O. Stapley, M.A. Goetz, G.A. Schonberg, A.A. Patchett, J.M. Liesch, O.D. Hensens, and J.P. Springer, *Science*, **230**, 177 (1985).
16. P.F. Dowd, *Entomol. Exp. Appl.*, **47**, 69 (1988).
17. K.B. Raper and D.I. Fennell, "The Genus *Aspergillus*." Williams and Wilkins, Baltimore, 1965.
18. D.I. Fennell and J.H. Warcup, *Mycologia*, **51**, 409 (1959).

Received 4 August 1993