Nominine: A New Insecticidal Indole Diterpene from the Sclerotia of *Aspergillus nomius*

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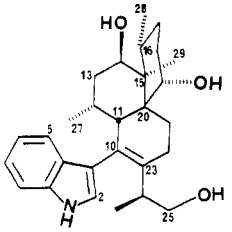
Received January 3, 1989

Nominine (**2**), a new indole diterpenoid biogenetically related to the aflavinines, has been isolated as the major organic-soluble component of the sclerotia of the fungus *Aspergillus nomius* (NRRL 13137). *A. nomius* is closely related to the common fungi *A. flavus* and *A. parasiticus*, both of which selectively allocate antiinsectan aflavinine derivatives to their sclerotia. This compound was identified primarily through NMR decoupling studies and selective INEPT experiments and by spectral comparison with aflavinine derivatives isolated from *A. flavus*. Nominine exhibits potent activity against the widespread crop pest *Heliothis zea*, causing 40% mortality and a 97% reduction in weight relative to controls when incorporated into a standard test diet at 100 ppm dry weight.

Many fungi produce specially adapted morphological structures called sclerotia that are critical to the long-term survival and propagation of the species.[1-3] The factors that permit the long-term survival of sclerotia in soil are not fully understood. Many vascular plants are known to selectively allocate metabolites to important physiological structures as chemical defenses against herbivory.[4] By analogy, it has been suggested that fungal sclerotia may have evolved chemical defenses against predation by fungivorous insects that commonly encounter sclerotia in soil.[3,5] However, aside from the sclerotia (ergot) of *Claviceps* spp. (which produce the ergot alkaloids), sclerotia have not been commonly explored for the production of unique, biologically active secondary metabolites. We have previously reported the isolation of four antiinsectan aflavinine derivatives (e.g., 14,25-dihydroxyaflavinine, **1**) that are selectively allocated to the sclerotia of *Aspergillus flavus* in concentrations effective against insects that encounter sclerotia under natural conditions.[2,3] Chemical studies of the sclerotia produced by a recently identified member of the *A. flavus* taxonomic group, *A. nomius*, have led to the isolation of a new, related indole diterpenoid (**2**), which we have named nominine. Nominine exhibits potent activity against the widespread crop pest *Heliothis zea* in controlled feeding experiments. Details of the isolation, structure elucidation, and biological activity of this compound are presented here.



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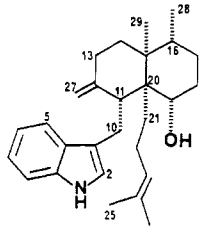

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Results and Discussion

Sclerotia of *Aspergillus nomius* Kurtzman, Horn, and Hesseltine (NRRL 13137)[6] were produced by solid substrate fermentation on corn kernels. Examination of the hexane extracts of the sclerotia indicated the presence of a major antiinsectan metabolite not detected earlier during bioassay-directed studies of sclerotial metabolites from other *Aspergillus* spp. Reversed-phase HPLC of this extract afforded an indole metabolite with a molecular formula of $C_{28}H_{39}NO$, as determined by HREIMS and ^{13}C NMR data. This result matched the formula of aflavinine derivatives that had been isolated earlier, and NMR data also indicated a close similarity, but the new compound

Table I. Proton and Carbon NMR Data for Nominine (2)[a]

position	^1H	^{13}C
1	7.88 (br s)	
2	6.93 (br s)	121.2 (d)
3		117.1 (s)
4		127.7 (s)
5	7.59 (br d, 7.8)	118.5 (d)
6	7.11 (dd, 7.1, 7.8)	119.1 (d)
7	7.17 (dd, 7.1, 8.1)	121.8 (d)
8	7.32 (br d, 8.1)	111.0 (d)
9		136.0 (s)
10	3.04 (br d, 15.4)	21.4 (t)
	3.10 (dd, 9.0, 15.4)	
11	3.22 (br d, 9.0)	45.9 (d)
12		148.9 (s)
13	2.11 (ddd, 3.2, 3.5, 12.7)	33.5 (t)
	2.21 (m)	
14	1.51 (m)	34.4 (t)
	1.68 (m)	
15		40.9 (s)
16	2.45 (m)	31.1 (d)
17	1.33 (m)	25.4 (t)
	1.70 (m)	
18	1.64 (m)	28.8 (t)
	1.92 (m)	
19	4.52 (br s)	70.0 (d)
20		47.9 (s)
21	1.36 (m)	29.2 (t)
	1.75 (m)	
22	2.21 (m)	23.9 (t)
23	5.17 (br t, 7.1)	125.8 (d)
24		131.2 (s)
25	1.67 (br s)	17.8 (q)
26	1.71 (br s)	25.8 (q)
27	4.81 (br s)	107.7 (t)
	4.92 (br s)	
28	0.82 (d, 6.6)	16.6 (q)
29	1.01 (s)	18.7 (q)

[a] Data recorded in $CHCl_3$ at 360 and 90.7 MHz, respectively.

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(3) Wicklow, D. T.; Dowd, P. F.; TePaske, M. R.; Gloer, J. B. *Trans. Br. Mycol. Soc.* **1988**, *91*, 433.
(4) *Herbivores: Their Interaction with Secondary Plant Metabolites*; Rosenthal, G. A., Janzen, D. H., Eds.; Academic: New York, 1979.
(5) Wicklow, D. T.; Cole, R. J. *Can. J. Bot.* **1982**, *60*, 525.
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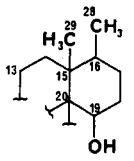
Table II. Selective INEPT Two- and Three-Bond Correlations for Nominine (2)

proton signal irradiated	carbon signals observed	proton signal irradiated	carbon signals observed
H-2	3, 4, 9	H-23	21, 22, 25, 26
H-5	3	H-26	23, 24
H-10	2, 12	H-27	11, 13
H-11	12, 20, 21, 27	H-28	15, 16, 17
H-16	15, 28, 29	H-29	14, 15, 16, 20
H-19	15		

clearly contained one more double bond than the aflavinines, suggesting the presence of one less ring. Proton spin systems were established by a series of homonuclear decoupling experiments conducted in two different solvents. Carbon assignments were made on the basis of a heteronuclear C–H shift correlation experiment. Proton and carbon NMR assignments are provided in Table I. These data alone did not permit a conclusive structural assignment, so a series of selective INEPT experiments[7] was performed to afford long-range C–H correlations (Table II).

The presence of partial structure a was established by the results of proton NMR decoupling experiments and by comparison of the NMR data to those obtained for the aflavinines.[2,8] Confirmation of this assignment was ob-



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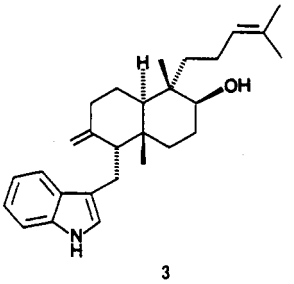
tained by long-range correlation of H_3-28 with C-15, 16, and 17, and correlation of H_3-29 with C-14, 15, 16, and 20. The presence of a 3-substituted indole moiety, a 4-methyl-3-pentenyl group, a downfield-shifted, isolated $CHCH_2$ unit, and an exo methylene group was also clearly indicated by proton NMR decoupling experiments and carbon NMR data. Three key observations resulting from selective INEPT experiments were instrumental in establishing the connectivity of these units. The geminal protons of the exo methylene unit were correlated with the CH carbon of the $CHCH_2$ unit and the terminal CH_2 carbon of partial structure a (C-11 and C-13, respectively). In addition, one of the H-10 protons was correlated with C-2 of the indole unit and the disubstituted carbon of the exo methylene group (C-12), suggesting the gross structure **2** for nominine. Finally, the proposed location of the 4-methyl-3-pentenyl group was confirmed by correlation of H-11 with carbons 12, 20, 21, and 27.

The relative stereochemistry for compound **2** was assigned as shown based on its close biogenetic relationship to the aflavinines. The relative stereochemistry for the aflavinines has been assigned as shown in structure **1** primarily on the basis of two X-ray crystallographic studies.[9,10] Support for the stereochemical analogy between these structural types was provided by comparison of parallel NMR data for **2** with those of the aflavinines and by a series of NOE experiments. Irradiation of H-16 in a difference NOE experiment enhanced the signal for H-11 (14%), and irradiation of H-11 enhanced the signal for H-16 (16%). Examination of molecular models suggests that these strong correlations are consistent only with a cis ring fusion and the relative stereochemistry shown for H-11 and H-16, with both protons assuming axial positions in a chair–chair conformation. NOESY data indicated that H-5, H-19, and one of the protons on C-27 are all spatially close to H_2-10. Since H-19 must be equatorial (no trans-diaxial coupling with neighboring protons), C-10 would also have to be equatorial in order to rationalize the NOESY correlation. This result also supports the placement of H-11 in an axial position. Only the relative stereochemistry shown for **2** (with the adoption of a chair–chair conformation) is fully consistent with all of these observations. Additional NOESY correlations were also consistent with the proposed stereochemistry.

Recently, two similar metabolites called emindoles DA (**3**) and SA (the C-11 epimer of **3**) have been reported by other workers from cultures of *Emericella desertorum* and *E. striatum*.[11] *Emericella* is the genus name applied to perfect states of conidial fungi from the *Aspergillus nidulans* group. The emindoles have been proposed as close biogenetic relatives of paxilline (**4**), a tremorgenic metabolite produced by *Claviceps paspali* and *Aspergillus* spp., because both compound types apparently arise from the same condensation product of tryptophan and geranylgeraniol.[11] Interestingly, there appears to be a parallel



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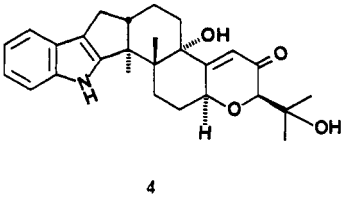

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biogenetic relationship between nominine and the aflavinines. The aflavinines are proposed to be derived from the same intermediate as paxilline by a different cyclization process,[9] and nominine appears to arise from the aflavinine pathway. Despite this structural relationship, nominine is not present in the sclerotia of *A. flavus* or *A. parasiticus*, and we have not detected any of the previously reported aflavinine derivatives in the sclerotia of *A. nomius*.

Nominine exhibits potent activity against the widespread crop pest *Heliothis zea*, causing 40% mortality when incorporated into a standard diet at 100 ppm (dry weight). Furthermore, the average weight of the insects surviving after 7 days on the test diet was only 2 mg, as opposed to 59 mg for controls. Nominine is substantially more effective than rotenone in this assay and exhibits activity comparable to that of permethrin. Nominine also elicits an antifeedant response in larvae of the fungivorous dried fruit beetle *Carpophilus hemipterus*.

Significantly, sclerotia are not formed in liquid cultures of *A. nomius*, and nominine is also not produced under such conditions. The restriction of nominine to the sclerotia of *A. nomius* parallels the selective allocation of aflavinines[2] and ergot alkaloids[12] to the sclerotia of *A. flavus*

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(8) The numbering system shown for the aflavinine ring system (e.g., **1**) has been revised here to better illustrate its biogenetic origin and to simplify structural comparison with nominine.
(9) Gallagher, R. T.; McCabe, T.; Hirotsu, K.; Clardy, J.; Nicholson, J.; Wilson, B. J. *Tetrahedron Lett.* **1980**, *21*, 243.
(10) Cole, R. J.; Dorner, J. W.; Springer, J. P.; Cox, R. H. *J. Agric. Food Chem.* **1981**, *29*, 293.
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and *Claviceps* spp., respectively. In addition, we have found that field-produced sclerotia formed by inoculation of corn ears in field test plots contain quantities of nominine similar to those found in laboratory-produced sclerotia. Taken together, these results suggest a possible ecological role for sclerotial metabolites and provide further evidence that fungal sclerotia are a unique and promising source of new bioactive natural products.

Experimental Section

General Procedures. Sclerotia from a strain of *A. nomius* (NRRL 13137) were obtained from the USDA Northern Regional Research Center in Peoria, IL. The sclerotia were prepared by solid substrate fermentation on autoclaved corn kernels using general procedures which have been previously described[3] and were stored at 4 °C until extraction. Proton and carbon NMR data were obtained in $CDCl_3$ on a Bruker WM-360 spectrometer, and chemical shifts were recorded using the signal for the residual protiated solvent (7.24 ppm) as a reference. Carbon multiplicities were established by a DEPT experiment. One-bond C–H correlations were obtained using an XHCORR pulse sequence optimized for 135 Hz. Proton signals studied with the selective INEPT technique were individually subjected to three separate experiments, optimizing for 7, 10, or 13 Hz. HREIMS data were obtained on a VG ZAB-HF instrument. Details of other experimental procedures and insect bioassays have been described elsewhere.[13,14]

Isolation and Properties of Nominine (2). Sclerotia of *A. nomius* (500–750 μm diameter, 53 g) were ground with a mortar and pestle and triturated repeatedly with hexane (5 × 200 mL). The combined hexane extracts were filtered and evaporated to afford 117 mg of a light yellow oil. This residue was subjected to reversed-phase semipreparative HPLC (5μ C_{18} column; 250 × 10 mm; 90:10 MeOH–H_2O at 2.0 mL/min) to afford 23.7 mg of nominine (**2**) as an off-white powder. The retention time for **2** under these conditions was 22.2 min. Compound 1: mp 54–55 °C; $[\alpha]_D$ +23.6° (*c* 0.85, MeOH); ^1H NMR and ^{13}C NMR ($CDCl_3$), Table I; EIMS (70 eV) 405 (M^+; rel intensity 57), 387 (100), 318 (15), 304 (52), 302 (70), 288 (17), 248 (15), 232 (15), 196 (42), 180 (40), 168 (28), 156 (16); HREIMS obsd 405.3035, calcd for $C_{28}H_{39}NO$ 405.3031.

Liquid Culture of *A. nomius*. A sterilized medium suitable for production of sclerotia on agar in petri dishes (1.5% glucose and 0.5% yeast extract; 50 mL) was inoculated with *A. nomius* and aerated by agitation on an orbital shaker at 200 rpm for 28 days. Although the fungus produced substantial mycelial growth under these conditions, sclerotia were not formed, and no trace of nominine was detected in organic extracts of the mycelium or the culture filtrate by analytical HPLC.

Detection of Nominine in Sclerotia from Field-Inoculated Corn. Several silking corn ears on growing corn plants at the USDA Northern Regional Research Center field plot were toothpick-wound inoculated with a conidial suspension of *A. nomius*. When the ears reached full maturity, small quantities of sclerotia were harvested from the ears and manually separated from all other fungal and plant material. Extraction of these sclerotia with $CHCl_3$ and analysis of the extract by analytical HPLC under the conditions above indicated the presence of nominine at a level similar to that found in laboratory-produced sclerotia.

Acknowledgment. This work was conducted under Cooperative Research Agreement No. 58-5114-M-010 between the USDA Agricultural Research Service and the University of Iowa. We thank Dr. George Crull for helpful NMR discussions.

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The Structures of Pradimicins A, B, and C: A Novel Family of Antifungal Antibiotics

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Received December 8, 1988

The structures of the novel antifungal antibiotics pradimicins A, B, and C, elaborated by a new strain of *Actinomadura hibisca*, have been determined on the basis of chemical degradations and spectral analysis. Acid hydrolysis cleaved pradimicin A to yield D-xylose, 4,6-dideoxy-4-(methylamino)-D-galactose, an aromatic chromophore fragment, and D-alanine. Extensive homo- and heteronuclear 2D NMR experiments assisted by the degradation results allowed us to assign *N*-[[(5*S*,6*S*)-5-*O*-[4,6-dideoxy-4-(methylamino)-3-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyl]-5,6,8,13-tetrahydro-1,6,9,14-tetrahydroxy-11-methoxy-3-methyl-8,13-dioxobenzo[*a*]-naphthacen-2-yl]carbonyl]-D-alanine for the structure of pradimicin A. Pradimicins B and C are desxylosyl and des-*N*-methyl analogues of pradimicin A, respectively.

Introduction

Although enormous screening efforts have been made in the past 30 years, there are relatively few antifungal antibiotics with clinical efficacy, particularly against systemic fungal infections. In our efforts to discover microbial metabolites active against fungal infections, we have found that cultured broth of *Actinomadura hibisca* No. P157-2 (ATCC 53557), isolated from a soil sample from Fiji Island, contained red pigments that strongly protected mice from lethal infections caused by *Candida*, *Aspergillus*, and *Cryptococcus* strains.[1] The active principals were precipitated from the broth filtrate at pH 5.0 and purified by column chromatography to yield three components, pradimicins A (**1a**), B (**1b**), and C (**1c**).[2] In the in vitro assay,

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