8.8 Hz, 1 H), 10.84 (s, 1 H); ^{13}C NMR -1.32 (q), 40.97 (t), 56.30 (q), 113.21 (d), 115.49 (s), 124.13 (d), 124.51 (d), 125.50 (d), 127.20 (s), 129.37 (d), 132.10 (s), 133.66 (d), 142.79 (d), 147.17 (s), 163.57 (s), 191.41 (d). Anal. Calcd for $C_{18}H_{22}O_2Si$: C, 72.44; H, 7.43. Found: C, 72.74; H, 7.46.

Acknowledgment. The authors are grateful to the National Institutes of Health for financial support of this program.

Registry No. 1, 93-04-9; **2**, 3401-47-6; **3**, 947-62-6; **4**, 51439-58-8; **5**, 94321-75-2; **6**, 94321-29-6; **7a**, 137869-07-9; **7b**, 137869-12-6; **7c**, 137869-13-7; **7d**, 137869-14-8; **7e**, 137869-15-9; **7g**, 137869-16-0; **7h**, 137869-17-1; **7i**, 137869-18-2; **8a**, 137869-08-0; **8b**, 137869-19-3; **9a**, 137869-09-1; **9b**, 137869-20-6; **10a**, 137869-10-4; **10b**, 137869-21-7; **11a**, 137869-11-5; **11b**, 137869-22-8; **11c**, 137869-23-9; BuLi, 109-72-8; *s*-BuLi, 598-30-1; *t*-BuLi, 594-19-4; (*Z*)-CH_3CH=$CHLi$, 6524-17-0; PhLi, 591-51-5; PhMgBr, 100-58-3; $LiMe_2N$, 3585-33-9; lithium diisopropylamine, 4111-54-0; lithium ephedrine methyl ether, 91525-92-7; allyltributylstannane, 24850-33-7; allyltrimethylsilane, 762-72-1; methyllithium, 917-54-4; 2-amino-2-methyl-1-propanol, 124-68-5.

Aspernomine: A Cytotoxic Antiinsectan Metabolite with a Novel Ring System from the Sclerotia of *Aspergillus Nomius*

Gail M. Staub,[†] James B. Gloer,*[,†,1] Donald T. Wicklow,[‡] and Patrick F. Dowd[‡]

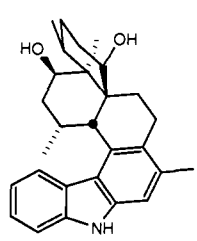
Contribution from the Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, and Agricultural Research Service, National Center for Agricultural Utilization Research, U. S. Department of Agriculture, Peoria, Illinois 61604. *Received August 30, 1991*

Abstract: Aspernomine (**3**), a new cytotoxic antiinsectan fungal metabolite with a previously undescribed ring system has been isolated from a pentane extract of the sclerotia of *Aspergillus nomius*. The structure of **3** was determined using NMR techniques, including COSY, NOESY, HMBC, and HMQC experiments conducted at 600 MHz.

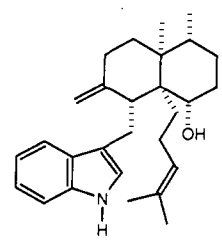
Studies of *Aspergillus* sclerotia as potential sources of new natural products with antiinsectan effects and other bioactivities have afforded a variety of new bioactive metabolites.[2a-f] Most of these compounds are indole diterpenoids (e.g., **1**), and several of them contain previously undescribed or rare ring systems. Our initial studies[2b] of sclerotial metabolites from *A. nomius* Kurtzman, Horn, and Hesseltine[3] led to the isolation of nominine (**2**), a compound exhibiting potent activity against the corn earworm *Helicoverpa zea* (formerly *Heliothis zea*).[4] Further studies of the pentane-soluble metabolites of an isolate of *A. nomius* have now afforded a unique new compound of similar biogenetic origin. This compound, which we have named aspernomine (**3**), possesses a new ring system and exhibits activity against *H. zea*, as well as significant cytotoxicity toward three human solid tumor cell lines. Details of the isolation, structure elucidation, and biological activity of aspernomine are presented here.

Results and Discussion

Pentane extracts were obtained by Soxhlet extraction of the sclerotia of an isolate of *A. nomius* (NRRL 6552)[5] produced by solid substrate fermentation on corn. Silica gel chromatography of the extracts using gradient elution from hexane to ethyl acetate yielded aspernomine, a metabolite with the molecular formula $C_{28}H_{39}NO_2$ as determined by ^{13}C NMR and HREIMS data. This formula differed from that of nominine (**2**) by the addition of one oxygen atom, and NMR data indicated some similarities between the two compounds. However, the appearance of the NH proton chemical shift at 4.35 ppm as compared to 7.88 ppm for nominine, along with other differences in the UV and ^{13}C NMR spectra, indicated the absence of an indole moiety. In addition, IR and ^{13}C NMR data revealed the presence of a ketone functionality (1698 cm^{-1} and 209.2 ppm, respectively). The presence of signals for only eight other sp^2-hybridized carbons indicated that the compound must be pentacyclic. Thus, it is clear that the structure of aspernomine is significantly different from that of **2**. Proton spin systems were determined by analysis of a series of decoupling experiments and a homonuclear proton COSY spectrum recorded at 600 MHz. Shift assignments for carbons bound to hydrogen atoms were established on the basis of HMQC[6] data. The remaining carbon NMR assignments and the connectivity of the spin systems were determined with the aid of long-range C-H correlations obtained through HMBC[7] and selective INEPT[8] experiments. All of these results are summarized in Table I.



1



2

The presence of a 1,2-disubstituted benzene ring and isolated $NHCHCH_2$, $CHCH_2$, and 4-methyl-3-pentenyl units were established from the COSY, decoupling, and HMQC data. Partial structure **a**, a structural subunit found in nominine, was also initially proposed by a comparison of NMR data with those obtained for nominine. HMBC correlation of H_3-28 with C-15, -16, and -17 and correlation of H_3-29 with C-14, -15, -16, and

[†] University of Iowa.
[‡] National Center for Agricultural Utilization Research.

(1) Alfred P. Sloan Fellow (1990-1992) and NIH Research Career Development Awardee (1990-1995; K04 CA01571).

(2) (a) Gloer, J. B.; TePaske, M. R.; Sima, J.; Wicklow, D. T.; Dowd, P. F. *J. Org. Chem.* **1988**, *53*, 5457. (b) Gloer, J. B.; Rinderknecht, B. L.; Wicklow, D. T.; Dowd, P. F. *J. Org. Chem.* **1989**, *54*, 2530. (c) TePaske, M. R.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Tetrahedron* **1989**, *45*, 4961. (d) TePaske, M. R.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *J. Org. Chem.* **1989**, *54*, 4743. (e) TePaske, M. R.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Tetrahedron Lett.* **1989**, *30*, 5965. (f) TePaske, M. R.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *J. Org. Chem.* **1990**, *55*, 5299.

(3) Kurtzman, C. P.; Horn, B. W.; Hesseltine, C. W. *Antonie van Leeuwenhoek* **1987**, *53*, 147.

(4) Stoetzel, M. B. *Common Names of Insects and Related Organisms*; Entomological Society of America: Lanham, MD, 1989; p 77.

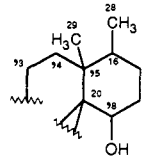
(5) The initial studies of *A. nomius* that led to the isolation of nominine were carried out with NRRL 13137. NRRL 6552 was employed in this study because it afforded a significantly higher yield of **3**.

(6) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565.

(7) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.

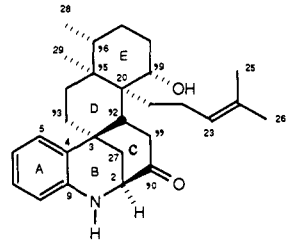
(8) Bax, A. J. *J. Mag. Reson.* **1984**, *57*, 314.

0002-7863/92/1514-1015$03.00/0



a

-20, along with COSY and other supporting data (Table I), confirmed the structure of **a**. These five spin systems accounted for all of the carbons except for the ketone carbon and one additional aliphatic quaternary carbon. The connectivity of these units was elucidated by analysis of long-range C–H correlations. The attachment of the 4-methyl-3-pentenyl group to C-20 was established by HMBC correlation of the downfield C-22 proton signal (2.18 ppm) with C-20. The upfield proton signal for H-22a was nearly superimposed on the signal for H-21b (1.98 vs 1.97 ppm), even when the solvent was varied. The resulting multiplet showed correlations to C-20 and C-15 consistent with the assigned location of the 4-methyl-3-pentenyl group. Correlation of the methine proton of the $NHCHCH_2$ unit (H-2) with C-9 of the 1,2-disubstituted aromatic ring, in conjunction with the downfield chemical shift of C-9 (142.7 ppm), linked the aromatic ring with the nitrogen atom of the $NHCHCH_2$ unit. An HMBC cross peak between the signal for the aromatic proton H-5 and the additional aliphatic quaternary carbon resonance (C-3) placed C-3 on the aromatic ring ortho to H-5 and to the nitrogen atom. The methine proton H-12 of the isolated $CHCH_2$ unit (comprised of C-11 and C-12) showed a variety of HMBC cross peaks that were especially useful in determining the structure of **3**. Correlations of the H-12



3

proton signal to both C-3 and C-4 indicated that C-12 is connected to C-3. Correlations of H-12 to C-19 and C-20 of subunit **a**, as well as to C-21 of the 4-methyl-3-pentenyl side chain, revealed the direct connection of C-12 to C-20. Thus, these results permitted the identification of all of the atoms directly linked to C-12. A further correlation of the H-12 proton to the methylene carbon of the isolated $NHCHCH_2$ unit (C-27) implied connection of C-27 to C-3 to form the six-membered B-ring, since C-27 cannot be directly attached to C-12, C-11, or the NH group. The ^{13}C NMR chemical shifts for the resulting tetrahydroquinoline subunit agree very well with literature values,[9] especially when the shift effects of the quaternary carbon (C-3) are considered.[10]

The remaining atom linked to the quaternary carbon C-3 was established as C-13 (of subunit **a**) on the basis of observation of correlations of the downfield-shifted H_{ax}-13 proton with C-3, C-4, and C-27. These results also confirmed the linkage of C-27 to C-3. A final correlation of H-12 with the ketone carbon (C-10) showed that C-10 must be connected to either C-3, C-11, C-12, or C-20. Since all of the connections to C-3, C-12, and C-20 are already accounted for, C-10 must be attached to C-11. Supporting evidence was provided by additional correlations of C-10 with H-2, H_a-11, and H_a-27. The only remaining positions available for connection are C-10 and C-2. Linkage of these two positions is supported by the HMBC correlations of H-2 and H_a-27 with C-10, along with a correlation of H_b-11 with C-2. On the basis of these

(9) Shamma, M.; Hindenlang, D. M. *Carbon-13 NMR Shift Assignments of Amines and Alkaloids*; Plenum Press: New York, 1979; p 99.

(10) Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Tables of Spectral Data for Structure Determination of Organic Compounds*; Springer-Verlag: New York, 1983; p C120.

Table I. Proton and Carbon-13 NMR Data for Aspernomine (**3**)[a] in $CDCl_3$

position	^1H	^{13}C	HMBC/selective INEPT	NOESY
1	4.35 (br s)			2, 8
2	3.75 (br s)	56.97 (d)	3,[b] 9,[b] 10[b]	1, 25, 27a, 27b
3		36.30 (s)		
4		130.70 (s)		
5	7.43 (br d; 7.2)	125.59 (d)	3, 7, 9	13ax
6	6.77 (ddd; 1.1, 7.2, 7.2)	118.37 (d)	4, 8	
7	7.04 (ddd; 1.1, 7, 8, 7.8)	127.58 (d)	5, 9	
8	6.51 (dd; 1.1, 7.8)	114.65 (d)	4, 6	1
9		142.70 (s)		
10		209.20 (s)		
11a	2.08 (dd; 9.3, 17.6)	37.33 (t)	10, 12,[c] 20[c]	12, 19
11b	2.49 (d; 17.6)		2, 3, 10, 20[c]	12, 19, 25
12	2.64 (br d; 9.3)	47.25 (d)	3, 4, 10, 11, 19, 20, 21,[c] 27	11a, 11b, 16, 18ax, 19
13eq	1.28 (m)	30.62 (t)		27a
13ax	2.58 (ddd; 4.1, 14.5, 14.5)		3, 4, 14, 27	5, 14 eq
14eq	1.39 (m)	28.95 (t)		13ax, 28, 29
14ax	1.68 (m)			
15		40.09 (s)		
16	2.40 (m)	31.33 (d)	15,[b] 28[b]	12, 28
17a	1.34 (m)	24.97 (t)		
17b	1.73 (m)			
18eq	1.53 (br dd; 3.1, 14.0)	29.92 (t)	16,[b] 19,[b] 20[b]	19
18ax	1.82 (m)			12
19	4.01 (br s)	69.84 (d)	15, 17	11a, 11b, 12, 18eq, 22b
20		46.38 (s)		
21a	1.63 (m)	30.20 (t)		27b
21b	1.97 (m)[d]			
22a	1.98 (m)[d]	23.88 (t)		
22b	2.18 (m)		20, 21, 23, 24	19, 23, 25
23	5.01 (br t)	125.59 (d)	21, 25, 26	26
24		131.72 (s)		
25	1.60 (s)	17.91 (q)	23, 24, 26	2, 22b, 28, 29
26	1.68 (s)	25.57 (q)	23, 24, 25	23, 28
27a	1.79 (br d; 14.0)	34.63 (t)	2, 3, 10, 12	2, 13 eq
27b	2.93 (br dd; 3.7, 14.0)		3, 4	2, 21a
28	0.93 (d; 7.7)	15.92 (q)	15, 16, 17	14eq, 16, 25, 26, 29
29	1.09 (s)	18.84 (q)	14, 15, 16, 20	14eq, 25, 28

[a] Data were collected at 600 and 90.7 MHz, respectively. [b] These correlations were observed in selective INEPT experiments, but not in the HMBC experiment. [c] These correlations were observed by HMBC, but selective INEPT results were used to verify the locations of the corresponding carbon signals. [d] NOESY and HMBC correlations for these two signals could not be unequivocally distinguished due to overlap; however, HMBC correlations of the H-21b/H-22a multiplet with C-12, -15, -20, -21, -23, and -24 are supportive of the structure assignment. NOESY correlations of this multiplet with H-2, -11b, -23, -25, and -29 can also be rationalized by structure **3**.

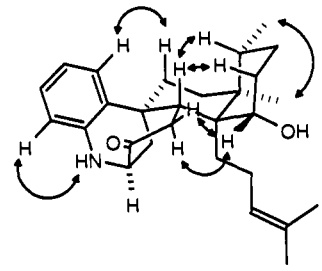


Figure 1. Selected NOESY correlations for aspernomine (**3**).

and other supporting data, the gross structure of aspernomine was assigned as **3**, which has a previously unreported ring system. The numbering system shown for **3** was chosen to facilitate spectral comparisons with nominine (**2**).[2b]

The relative stereochemistry of the E-ring and the D/E ring fusion of aspernomine (positions 15, 16, 19, and 20) is proposed to be analogous to that of nominine (**2**) and other related *Aspergillus* metabolites[2a-f,11-13] on the basis of biogenetic and NMR similarities. Confirmation of this hypothesis was obtained through analysis of NOESY data (Table I and Figure 1). A NOESY correlation was observed between H-12 and H-16. In order for these two protons to be spatially close, the relative stereochemistry at positions 15, 16, and 20 must be as shown. Furthermore, both protons must be axial (H-12 axial with respect to the D-ring), with the D- and E-rings most likely adopting a chair–chair conformation. Nominine and the aflavinines possess a similar cis D/E ring fusion. Additional supporting evidence was provided by NOESY correlations of H_3-28 with H_3-29, H_{ax}-18 with H-12, and H_{eq}-14 with both H_3-28 and H_3-29. H-19 must have an equatorial disposition (no trans-diaxial coupling with either neighboring proton). This observation, along with NOESY correlations of H-19 with both H-11 protons, plus a (very weak) correlation with H-12 establishes the relative stereochemistry at C-19 as shown. The remaining relative stereochemical assignments were proposed on the basis of other NOESY correlations and geometrical considerations. A strong correlation of the axial proton on C-13 with H-5 of the aromatic ring led to the assignment of the stereochemistry indicated at position 3. This assignment would also rationalize the substantial downfield shift of H_{ax}-13 (2.58 ppm) due to aromatic ring current effects. Geometrical constraints of the bridged B/C ring system require that H-2 must be cis to C-13 with respect to the B-ring. The NOESY correlations mentioned earlier of both C-11 protons with H-19, H-12 with H-16, and H-12 with H_{ax}-18 require the relative configuration shown for the remaining stereocenter (C-12). The C-ring would have to adopt a twisted conformation rather than the alternative chair-like form in order to account for the proximity of both C-11 protons to H-19. Thus, the relative stereochemistry is assigned as shown in structure **3**. However, the absolute configuration remains to be determined.

It is likely that compounds **1–3** arise biogenetically from a common digeranylindole precursor, with the pathway to **3** differing significantly from the pathway to the nominine/aflavinine class. The nontrivial skeletal differences between nominine (**2**) and compound **3** suggest that nominine is not a precursor to **3** and that the divergence may occur somewhat earlier in the biosynthetic process. This compound is the first quinoline-type alkaloid encountered in our studies of sclerotial metabolites.

Aspernomine (**3**) exhibits moderate activity against the corn earworm *H. zea*. Incorporation of this compound into a standard test diet at 100 ppm (dry weight) caused a 35% reduction in weight gain of the test insects relative to controls. This compound also exhibits cytotoxicity[14] toward three human solid tumor cell lines. ED_{50} values of 3.09, 4.93, and 3.08 μg/mL were observed in assays against the A-549 lung carcinoma, MCF-7 breast adenocarcinoma, and HT-29 colon adenocarcinoma cell lines, respectively.

Experimental Section

General Procedures. A strain of *A. nomius* (NRRL 6552) originally isolated from a pine sawfly (*Diprion similis*) was obtained from the ARS Culture Collection at the USDA National Center for Agricultural Utilization Research in Peoria, IL. Sclerotia were prepared by solid substrate fermentation on autoclaved corn kernels using general procedures which have been previously described.[15] Carbon multiplicities were determined by a DEPT experiment. One-bond C–H correlations were obtained using an HMQC experiment optimized for 120 Hz. Proton assignments were made by analysis of COSY, homonuclear decoupling, and HMQC experiments. Axial and equatorial orientations were determined where possible on the basis of coupling constants and NOESY interactions. Long-range C–H correlations were obtained either by selective INEPT experiments or by an HMBC experiment optimized for 8.5 Hz. All 2D NMR experiments were conducted at 600 MHz. Individual proton signals studied using the selective INEPT technique were subjected to as many as five separate experiments optimizing for 5, 7, 8, 10, or 12 Hz. Details of the *H. zea* bioassay have been reported elsewhere.[16]

Isolation and Properties of Aspernomine (3). Sclerotia of *A. nomius* (120.8 g) were ground with a mortar and pestle and then extracted with *n*-pentane in a Soxhlet apparatus for 54 h. Concentration of the resulting *n*-pentane extract afforded 297 mg of a yellow-orange oil. A portion of this extract (80 mg) was subjected to silica gel chromatography (26 × 1.5 cm column) using a hexane–ethyl acetate gradient, collecting 4-mL fractions. Aspernomine (**3**) was obtained as fine white needles (6.7 mg) upon evaporation of selected fractions eluted with 90% hexane. This procedure was repeated with the remaining extract to yield a total of 18.8 mg of **3**. Compound **3** could also be purified by reversed-phase HPLC separation of the pentane extract, eluting slightly before nominine with a t_R of 20.9 min, using 90:10 MeOH–H_2O on a Beckman Ultrasphere 5-μm C_{18} 10 × 250 mm column at 2.0 mL/min. Compound **3** decomposes slightly over time upon standing in organic solvents and forms a distinctive greenish spot on silica gel TLC plates upon exposure to I_2 (R_f 0.61; 85:15 $CHCl_3$–acetone). Aspernomine (**3**) has the following characteristics: $[\alpha]_D$ +225° (c = 0.12 g/dL; MeOH, 27 °C); ^1H NMR, ^{13}C NMR, NOESY, HMBC, and selective INEPT data, Table I; UV (MeOH) 333 (ϵ 1150), 302 (2510), 244 (5760), 222 (5460); IR (neat) 3500, 3360, 2970, 2930, 1698, 1606, 1490, 750 cm^{-1}; EIMS (70 eV) 421 (M^+, rel intensity 4), 184 (7), 156 (100), 143 (37), 130 (28); HREIMS obsd 421.3024, calcd for $C_{28}H_{39}NO_2$ 421.2981.

Acknowledgment. This work was conducted under Cooperative Research Agreements No. 58-5114-M-010 and No. 58-5114-O-1006 between the USDA Agricultural Research Service and the University of Iowa. We thank the University of Iowa NMR and MS Central Facilities for the use of instrumentation and the Purdue University Cancer Center for cytotoxicity data. We also thank the National Science Foundation (CHE-8905894) and Biotechnology Research and Development Corporation for financial support.

Supplementary Material Available: Proton and Carbon-13 NMR spectra for aspernomine (2 pages). Ordering information is given on any current masthead page.

(11) Gallagher, R. T.; McCabe, T.; Hirotsu, K.; Clardy, J.; Nicholson, J.; Wilson, B. J. *Tetrahedron Lett.* **1980**, *21*, 243.
(12) Cole, R. J.; Dorner, J. W.; Springer, J. P.; Cox, R. H. *J. Agric. Food Chem.* **1981**, *29*, 293.
(13) Nozawa, K.; Sekita, S.; Harada, M.; Udagawa, S.; Kawai, K. *Chem. Pharm. Bull.* **1989**, *37*, 626.
(14) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
(15) Wicklow, D. T.; Dowd, P. F.; TePaske, M. R.; Gloer, J. B. *Trans. Br. Mycol. Soc.* **1988**, *91*, 433.
(16) Dowd, P. F. *Entomol. Exp. Appl.* **1988**, *47*, 69.