A New Penitrem Analog with Antiinsectan Activity from the Sclerotia of *Aspergillus sulphureus*

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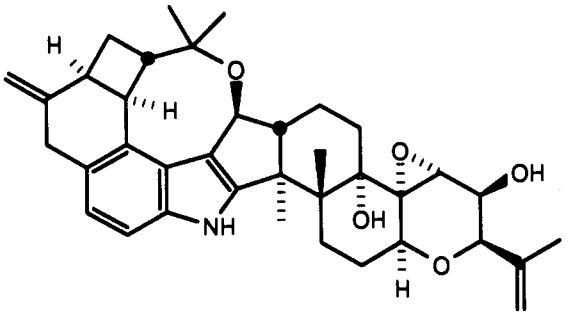
The new antiinsectan metabolite **2** (10-oxo-11,33-dihydropenitrem B) has been isolated from the sclerotia of *Aspergillus sulphureus*. Compound **2** is related to the penitrems, a known group of tremorgenic fungal metabolites. The structure of **2** was assigned primarily by NMR studies (e.g., COSY, HMQC, HMBC, and NOESY) and by comparison to data published for the penitrems. A known aflavinine analog (**3**; 10,23-dihydro-24,25-dehydroaflavinine) was also isolated from *A. sulphureus* sclerotia. This is the first report of any aflavinine analog from a member of the *Aspergillus ochraceus* taxonomic group.

INTRODUCTION

A wide array of bioactive secondary metabolites have been isolated from *Aspergillus* spp. (Cole and Cox, 1981; Turner and Aldridge, 1983). Despite these findings, the chemistry of *Aspergillus* sclerotia remained relatively unexplored until recently. Our chemical studies of these propagules indicate that *Aspergillus* sclerotial metabolites warrant thorough investigation. Specifically, *Aspergillus* sclerotia have proven to be prolific sources of new antiinsectan metabolites (Gloer et al., 1989; TePaske et al., 1990, 1992; Wicklow et al., 1988). Initial studies of the sclerotial metabolites of *Aspergillus sulphureus* (Fres.) Thom and Church (NRRL 4077) led to the isolation of four new indole diterpenoids called radarins A–D (Laakso et al., 1992a). More recently, four additional new antiinsectan metabolites, sulpinines A–C and secopenitrem B, as well as the known *Penicillium* metabolite penitrem B (**1**) were also isolated from *A. sulphureus* (Laakso et al., 1992b). Our continuing investigation of this species has led to the discovery of a new penitrem analog, 10-oxo-11,33-dihydropenitrem B (**2**). This compound exhibits potent activity in dietary assays against the corn earworm, *Helicoverpa zea*, and is also moderately effective as a feeding deterrent against the dried fruit beetle, *Carpophilus hemipterus*.



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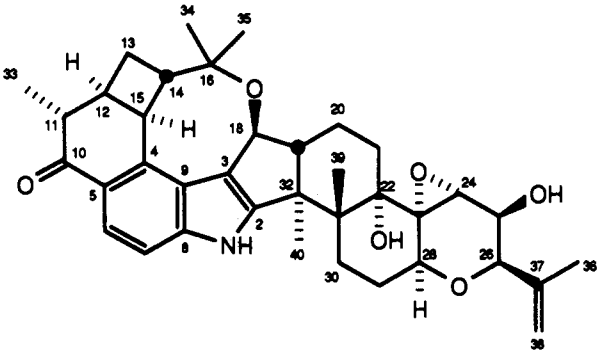

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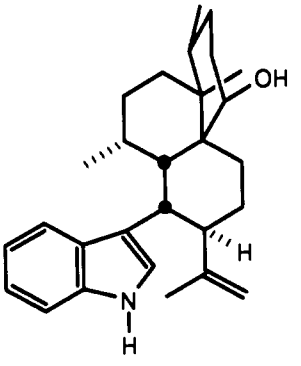

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EXPERIMENTAL PROCEDURES

General. General procedures and instrumentation employed in this work have been described previously (Dowd, 1988; Laakso et al., 1992b).

Isolation and Characterization of 2. Sclerotia of *A. sulphureus* NRRL 4077 (150.0 g) were ground with a Tecator mill and sequentially extracted with pentane and CH_2Cl_2 using a Soxhlet apparatus. A portion (894 mg) of the total CH_2Cl_2 extract (1.59 g) was fractionated by silica gel column chromatography. A stepwise gradient from 0 to 10% (v/v) MeOH in $CHCl_3$ was employed, resulting in the elution of a distinct red band at 4% MeOH. The fraction that yielded compound **2** eluted immediately after this distinct band. This fraction (50.1 mg) showed antiinsectan activity and was therefore separated further by reversed-phase HPLC (92:8 MeOH–H_2O; Beckman Ultrasphere ODS column, 5-μm particles, 250 × 10 mm, 2.5 mL/min) to give 10-oxo-11,33-dihydropenitrem B (**2**; 9.7 mg) as a light yellow solid with the following properties: $[\alpha]_D$ −78.6° (*c* 0.2 g/dL); HPLC t_R 8.9 min; UV (MeOH) 261 nm (ϵ 28 900), 289

0021-8561/93/1441-0973$04.00/0

Table I. Spectral Data for 10-Oxo-11,33-dihydropenitrem B (2)[a] with ^{13}C NMR Data for Penitrem B (1)[b] Provided for Comparison

C/H	^1H	^{13}C for 1	^{13}C for 2	HMBC correlations	NOESY correlations[c]
2		153.0	155.1		
3		119.4	120.8		
4		128.8	136.5		
5		128.1	125.8		
6	7.38 (d; 8.5)	121.0	118.9	4, 7, 8, 9,[d] 10	
7	7.18 (d; 8.5)	110.3	111.4	4,[d] 5, 9	
8		139.3	143.4		
9		123.2	122.7		
10		38.8	203.8		
11	2.81 (br q; 6.4)	150.2	46.6	10, 12, 13, 33	
12	2.27 (m)	35.0	34.4	14, 33	33
13a	2.18 (m)	26.7	28.7	11, 12, 14, 16	
13b	1.91 (m)			11, 12, 15	
14	2.77 (m)	52.3	50.5	4, 15, 16, 34	35
15	3.83 (dd; 9.2, 9.2)	39.4	35.7	4, 5, 9, 11, 12, 14, 16	18
16		75.5	77.3		
18	4.89 (d; 8.2)	72.1	73.2	2, 3, 16, 19	15, 40
19	2.65 (m)	59.1	59.9	18, 21, 32, 40	39
20ax	1.93 (m)	18.6	18.8	19, 21, 32	40
20eq	1.77 (m)				
21ax	1.47 (ddd; 13.4, 12.7, 4.0)	30.6	30.5	19	24
21eq	1.74 (m)				
22		78.3	78.5		
23		66.2	66.7		
24	3.49 (br s)	62.0	62.2	25, 26	21ax, 36, 39
25	4.04 (br s)	66.3	66.5	28, 36, 37, 38	28
26	4.03 (br s)	74.7	75.1	23, 24, 26	
28	4.29 (dd; 9.2, 8.6)	72.0	72.7	23, 24, 29	25
29ax	2.10 (m)	29.0	29.0	28, 30	39
29eq	2.29 (m)			23, 28, 30, 31	
30ax	2.61 (ddd; 13.8, 13.3, 5.2)	26.9	27.0	22, 29, 31	40
30eq	1.62 (dd; 13.0, 6.1)			22, 28, 29, 31, 32	
31		43.6	44.0		
32		49.7	50.9		
33	1.14 (d; 6.4)	105.9	12.7	10, 11, 12	12
34	1.55 (s)	18.6	19.0	14, 16	15, 18, 35
35	1.20 (s)	28.8	28.7	14, 16	14, 34
36	1.71 (br s)	19.7	19.8	26, 37, 38	24, 38b
37		143.3	143.2		
38a	5.09 (br s)	111.6	111.9	26, 36, 37	
38b	4.91 (br s)			26, 36	36
39	1.23 (s)	18.9	19.0	22, 30, 31, 32	19, 24, 29ax
40	1.42 (s)	21.2	21.2	2, 19, 31, 32	18, 20ax, 30ax

[a] Data were recorded in CD_3OD at 600 and 75.6 MHz, respectively. [b] These data were published by de Jesus et al. (1983) and were recorded in acetone-d_6. [c] NOESY correlations between scalar coupled protons have been omitted. [d] These correlations represent four-bond couplings; all other HMBC correlations represent two- or three-bond couplings.

(7310); IR (neat) 3532, 3342, 1660, 1457, 1231, 1038, 934, 757 cm^{-1}; ^1H NMR, ^{13}C NMR, HMBC, and NOESY data, Table I; EIMS (70 eV) 599 (M^+; rel intensity 26), 493 (15), 469 (26), 400 (9), 265 (12), 264 (65), 134 (26), 133 (23), 131 (23), 130 (100), 119 (54); HREIMS obsd 599.3244, calcd for $C_{37}H_{45}NO_6$ 599.3247.

Isolation and Characterization of 3. Ground sclerotia of *A. sulphureus* NRRL 4077 (108 g) were extracted with hexane. Of the 361 mg of extract obtained, 200 mg was subjected to silica gel vacuum flash chromatography. Hexane–EtOAc and then $CHCl_3$–MeOH gradients were employed to yield 84.9 mg of an active fraction which eluted with 20% (v/v) EtOAc in hexane. This fraction was then subjected to reversed-phase HPLC (89:11 MeOH–H_2O) to yield 10,23-dihydro-24,25-dehydroaflavinine (3; 3.2 mg) as a yellow oil with HPLC t_R 22.3 min. This material coeluted with an authentic standard of 3 using HPLC and had physical and spectral properties (^1H NMR, MS) identical to those previously reported for 10,23-dihydro-24,25-dehydroaflavinine (TePaske et al., 1989). The standard of compound 3 was obtained from the sclerotia of *Aspergillus tubingensis* (NRRL 4700) and was provied by Dr. M. R. TePaske.

RESULTS AND DISCUSSION

Sclerotia of *A. sulphureus* were produced by solid substrate fermentation on corn kernels (Wicklow et al., 1988). The harvested sclerotia were ground and extracted first with pentane and then CH_2Cl_2 using a Soxhlet apparatus. The CH_2Cl_2 extract displayed potent activity against *H. zea*. This extract was fractionated on silica gel and further purified via reversed-phase HPLC to yield 2, in addition to other metabolites reported earlier (Laakso et al., 1992a,b). Preliminary examination of the ^1H NMR spectrum of 2 indicated many similarities to spectra obtained for penitrem B and secopenitrem B (e.g., signals corresponding to the two ortho aromatic protons, the highly oxygenated pyran ring, and the cyclobutane spin system). The molecular formula of 2 was determined to be $C_{37}H_{45}NO_6$ by HREIMS. This formula is identical to that of penitrem E (de Jesus et al., 1983). Penitrem E is hydroxylated at C-15 but otherwise identical to penitrem B. However, from a comparison of the ^{13}C NMR data for penitrems B and E (de Jesus et al., 1983) with those of 2, it was clear that 2 is not an hydroxylated version of penitrem B. Specifically, one aliphatic methylene and two vinylic carbon signals in the ^{13}C NMR spectrum of penitrem B appear to be replaced with new methyl, methine, and ketone carbon signals in the ^{13}C NMR spectrum of 2.

Table I provides ^{13}C and ^1H NMR data for 2. Carbon–proton one-bond correlations were made by analysis of an HMQC spectrum. By comparing HMBC data obtained

for 2 with the data published for 1, it was determined that the ring system remained intact. The location of the ketone carbonyl functionality at C-10 was readily established on the basis of an HMBC correlation (Table I) between H-6 and the ketone carbon (C-10). The new methyl doublet (H_3-33) showed HMBC correlations to the ketone carbonyl signal C-10 and two methine carbon signals (C-11, C-12), placing the methyl group alpha to C-10. In addition, the methine proton coupled to H_3-33 (H-11) showed correlations to C-10, C-12, C-13, and C-33, thereby confirming the location of the CH–CH_3 unit. HMBC data indicated that the remainder of the molecule is identical to the corresponding portion of penitrem B.

The relative stereochemistry of 2 (10-oxo-11,33-dihydropenitrem B) was deduced by examination of NOESY data and by analogy to penitrem B. Axial and equatorial proton dispositions were assigned on the basis of coupling constants, NOESY correlations, and comparisons to the data for penitrem B. All NOESY correlations are consistent with the relative stereochemistry previously proposed for penitrem B (de Jesus et al., 1983). The assignment of the new methyl group (CH_3-33) to a pseudoaxial position was based primarily on the fact that H-11 appears as a broad quartet in the 600-MHz ^{1}H NMR spectrum of 2. This indicates a near-zero vicinal coupling between H-11 and H-12, which would require a dihedral angle near 80°. On the basis of Dreiding molecular models, this bond angle can be achieved only if compound 2 has the stereochemistry shown with H-11 in a pseudoequatorial position. More rigorous analysis of the two possible C-11 epimers and their most stable conformations using SYBYL molecular modeling software (version 5.51) confirmed that only the stereochemistry shown, with H-11 in a pseudo-equatorial position, affords a dihedral angle consistent with the observed vicinal coupling (74.2°). Other low-energy conformations having either configuration at C-11 have vicinal bond angles that would yield an H-11/H-12 coupling constant greater than 4 Hz. Finally, a strong NOESY correlation between H_3-33 and H-12 appears in the NOESY spectrum. This correlation would be less likely if compound 2 had the opposite configuration at C-11.

The absolute stereochemistry of penitrem B (1) was originally determined by the partial resolution method of Horeau (de Jesus et al., 1983). Since the penitrem B isolated from *A. sulphureus* was found to have the same absolute stereochemistry as the compound in the original study (Laakso et al., 1992b), the new oxopenitrem analog most likely possesses the absolute stereochemistry depicted in 2.

A known aflavinine analog was also isolated from the extracts of the sclerotia of *A. sulphureus*. This metabolite, 10,23-dihydro-24,25-dehydroaflavinine (3), was originally isolated from *A. tubingensis* and subsequently from *Aspergillus flavus* and *Aspergillus parasiticus* (TePaske et al., 1989). Compound 3 was present in both the pentane and CH_2Cl_2 extracts of *A. sulphureus*. It is the first aflavinine analog to be isolated from *A. sulphureus* and, to our knowledge, is the first aflavinine reported from any member of the *Aspergillus ochraceus* taxonomic group.

Compound 2 possesses potent activity against the first instar larvae of the corn earworm, *H. zea*. A 95% reduction in weight gain relative to controls after 1 week was noted for this compound when incorporated into a standard *H. zea* test diet at 100 ppm (dry weight). This value is comparable to that obtained for the pesticide malathion at the same concentration, although malathion also causes ca. 50% mortality in the assay. A moderate feeding reduction of 33% was also induced by 2 in the adults of the fungivorous beetle *C. hemipterus* (Wicklow et al., 1988) at the same dietary level. Compound 3 is inactive in both assays at 100 ppm.

ACKNOWLEDGMENT

This work was conducted under Cooperative Research Agreements 58-5114-M-010 and 58-5114-O-1006 between the USDA Agricultural Research Service and the University of Iowa. We thank the National Science Foundation (CHE-8905894), Biotechnology Research and Development Corp., and the National Institutes of Health (K04 CA01571) for financial support. J.A.L. appreciates a University of Iowa Biocatalysis Predoctoral Fellowship. We also thank Boyd Knosp of the University of Iowa Image Analysis Facility for performing the molecular modeling studies.

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Received for review September 9, 1992. Revised manuscript received December 28, 1992. Accepted March 15, 1993.