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Antiinsectan Alkaloids: Shearinines A-C and a New Paxilline Derivative from the Ascostromata of *Eupenicillium Shearii*

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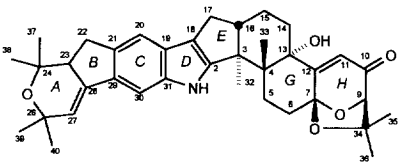
Abstract: Four new antiinsectan indole alkaloids (**1-4**) have been isolated from organic extracts of the sclerotioid ascostromata of *Eupenicillium shearii* (NRRL 3324). These extracts also afforded five known, related metabolites (**5-9**). The structures of the new compounds were determined through analysis of ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC experiments. Compounds **1-9** were isolated from fractions displaying activity in dietary assays against the corn earworm *Helicoverpa zea* and the dried-fruit beetle *Carpophilus hemipterus*, and most of the compounds show potent activity in these assays. Shearinine A (**1**) also exhibited activity in a topical assay against *H. zea*, and shearinine B (**2**) caused significant mortality in a leaf disk assay against the fall armyworm *Spodoptera frugiperda*.

Many fungi produce specialized propagational structures called sclerotia that are able to withstand extreme conditions of temperature, desiccation, and nutrient depletion. During their sometimes lengthy dormant phase in soil, fungal sclerotia may be exposed to predation by insects. Evolutionary considerations suggest that sclerotium-producing fungi may have developed the capacity to produce defensive compounds that protect these bodies against fungivorous insects. We have previously reported a variety of antiinsectan metabolites from the sclerotia of *Aspergillus* spp.[1-3] Our continuing interest in fungal sclerotia and similar resting bodies as sources of new antiinsectan metabolites prompted us to investigate the chemistry of sclerotioid ascostromata from *Eupenicillium* spp. Like sclerotia, ascostromata are hardened physiological structures that serve as sources of fungal inoculum upon germination. Chemical investigation of the ascostromata of *Eupenicillium shearii* Stolk and Scott (NRRL 3324) has led to the isolation of three new indole alkaloids of the janthitrem class,[4-6] which we have named shearinines A, B, and C (**1-3**). An additional new metabolite (**4**) related to paxilline (**5**), a known *Penicillium* metabolite,[7] was also isolated, along with four known members of the paxilline class (**6-9**), and paxilline itself. To our knowledge, ascostromata produced by *Eupenicillium* spp. have not been previously surveyed for unique bioactive metabolites. Details of this work are described here.



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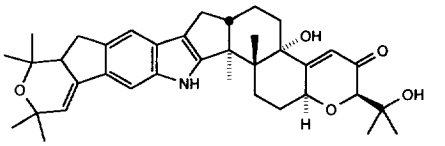

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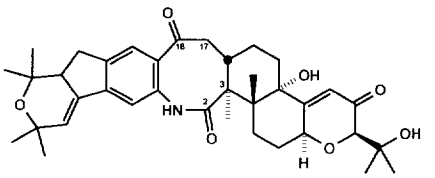

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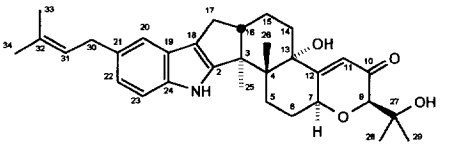

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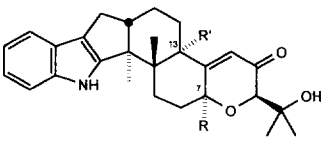
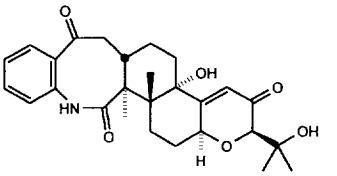


5 R = H, R' = OH
6 R = OH, R' = H
7 R = R' = H



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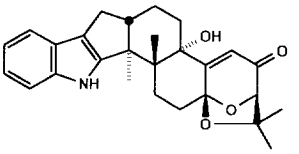

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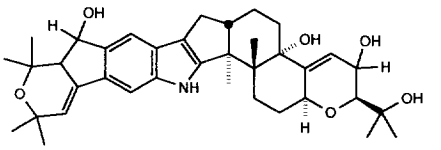

10

Compounds **1-9** were isolated by bioassay-guided fractionation of organic extracts of ascostromata produced by solid-substrate fermentation of *E. shearii* NRRL 3324. Fractions from chromatography on silica gel and reversed-phase HPLC were tested in feeding assays against larvae of *Helicoverpa zea* (corn earworm) and *Carpophilus hemipterus* (dried fruit beetle) in order to isolate constituents with antiinsectan activity. One of the major antiinsectan components (**1**) has the molecular formula $C_{37}H_{45}NO_5$, as deduced from mass spectral data (EIMS M^+ at *m/z* 583; HREIMS M - CH_3 at *m/z* 568.3063, Δ -2.9 mmu). The ^1H NMR spectrum contained eight aliphatic methyl singlets, two aromatic proton singlets, and an N-H singlet. DEPT, HMBC, and HMQC experiments permitted the corresponding proton and carbon assignments to be made. The NMR data (see Tables 1 and 2), particularly the number of methyl singlets, suggested that compound **1** is a representative of the janthitrem class. Detailed comparison of the data for compound **1** with those of the known compound

Table 1. ^{1}H NMR Data for Shearinines A-C in $CDCl_3$ (**1-3**)

Position	1	2	3
1	7.62 (s)	7.71 (s)	7.09 (s)
2	---	---	---
3	---	---	---
4	---	---	---
5	2.67 (m), 1.80 (m)	2.77 (m), 1.44 (dd; 13.2, 4.7)	2.50 (ddd; 14, 14, 4.4), 1.90 (m)
6	2.78 (m), 2.03 (m)	2.31 (m), 1.88 (m)	2.18 (m), 1.66 (m)
7	---	4.83 (br dd; 9.8, 8.3)	4.71 (dd; 10.2, 8.2)
9	4.30 (d; 1)	3.68 (d; 1.9)	3.59 (d; 2.1)
10	---	---	---
11	5.82 (d; 1)	5.83 (d; 1.8)	5.70 (d; 1.9)
12	---	---	---
13	---	---	---
14	1.97 (m), 1.87 (br d; 13.6)	2.00 (m), 1.59 (m)	1.90 (m), 1.48 (m)
15	2.04 (m), 1.76 (m)	2.00 (m), 1.75 (m)	1.74 (m), 1.48 (m)
16	2.77 (m)	2.77 (m)	3.04 (m)
17	2.68 (m), 2.39 (dd; 13.0, 10.7)	2.67 (m), 2.40 (dd; 13.0, 11.0)	3.04 (m), 2.42 (dd; 17.8, 3.2)
18	---	---	---
19	---	---	---
20	7.24 (s)	7.25 (s)	7.49 (s)
21	---	---	---
22	3.10 (dd; 15.7, 9.3), 2.66 (m)	3.10 (dd; 15.6, 9.4), 2.67 (m)	3.04 (m), 2.61 (dd; 16.3, 8.0)
23	2.91 (m)	2.91 (m)	2.87 (ddd; 8.6, 8.6, 3.0)
24	---	---	---
26	---	---	---
27	5.91 (d; 3.0)	5.90 (d; 3.0)	6.03 (d; 3.1)
28	---	---	---
29	---	---	---
30	7.32 (s)	7.33 (s)	7.00 (s)
31	---	---	---
32	1.36 (s)	1.31 (s)	1.59 (s)
33	1.21 (s)	0.98 (s)	0.95 (s)
34	---	4.10 (s; OH)	---
35	1.17 (s)	1.26 (s)	1.18 (s)
36	1.43 (s)	1.28 (s)	1.23 (s)
37	1.32 (s)	1.32 (s)	1.31 (s)
38	1.07 (s)	1.07 (s)	1.10 (s)
39	1.32 (s)	1.32 (s)	1.28 (s)
40	1.35 (s)	1.34 (s)	1.34 (s)

janthitrem E (**10**)[4] supported this conclusion, but also revealed several significant differences from the known members of this class. First, the hydroxyl group at C-10 in **10** is replaced by a ketone in **1**, for which a carbon signal appears at 197.0 ppm. The hydroxyl group at C-22 in **10** is absent in compound **1**, as evidenced by the lack of a corresponding proton signal at 4.90 ppm, and the presence of two new methylene proton signals at 3.10 and 2.66 ppm. This was further confirmed by long range correlations of H_2-22 with the neighboring indole aryl carbons and with C-23 and C-28 of the five-membered B-ring. Finally, C-7 of **1** is not protonated, and is relatively deshielded (104.4 ppm) when compared to the corresponding signal of janthitrem E. These features indicated the presence of an additional ring (the I-ring) in compound **1**, and accounted for the difference in elemental composition between **1** and **10**. The remaining ^{1}H and ^{13}C NMR assignments were very similar to those of **10**. The structure and the assignments were verified by analysis of COSY, NOESY, and HMBC data

(complete HMBC data for new compounds **1-4** are included in the Experimental section). The gross structure of shearinine A was therefore assigned as **1**, with relative stereochemistry proposed on the basis of ^{1}H NMR *J*-values, NOESY data, and structural analogy to **10**.

The ^{1}H NMR spectrum of shearinine B (**2**; $C_{37}H_{47}NO_5$) was nearly identical to that of **1** except for the presence of an additional exchangeable OH signal at 4.10 ppm (C-34-OH), and an additional doublet of doublets at 4.83 ppm, corresponding to H-7. These changes suggested that compound **2** lacks a closed I-ring. Furthermore, the C-7 carbon signal appears as a doublet at 72.6 ppm (as opposed to a singlet at 104.4 ppm in **1**), and C-9 is shifted upfield to 83.3 ppm (from 87.9 ppm in **1**). The above results, along with 1D- and 2D-NMR data (Tables 1-2 and Experimental section) are consistent with those observed for compounds **4**, **6**, **10**, and other metabolites with the analogous structural subunit.[8,9] The structure of shearinine B was thus assigned as **2**.

Table 2. ^{13}C NMR Data for Shearinines A-C in $CDCl_3$ (**1-3**)

Position	1	2	3	Position	1	2	3
1	---	---	---	21	137.0	137.0	144.2
2	153.2	152.9	176.2	22	33.0	33.0	33.1
3	51.6	50.9	57.2	23	48.8	48.8	48.5
4	39.9	43.2	44.0	24	74.5	74.5	74.2
5	27.0	27.2	25.1	26	72.6	72.5	72.6
6	28.2	28.5	28.2	27	119.9	120.0	126.3
7	104.4	72.6	72.6	28	139.5	139.5	137.5
9	88.0	83.3	83.0	29	133.5	133.7	144.6
10	197.0	199.2	199.2	30	102.9	102.9	118.5
11	117.7	119.7	120.1	31	139.9	139.7	136.0
12	169.9	168.1	168.2	32	16.2	16.1	16.5
13	77.7	77.2	76.6	33	23.6	19.7	19.9
14	33.9	34.3	32.0	34	78.8	72.6	72.6
15	21.1	20.9	25.2	35	23.1	24.2	24.0
16	48.5	49.4	35.5	36	28.9	26.6	26.5
17	27.5	28.0	47.7	37	30.0	30.0	29.9
18	117.0	117.3	203.0	38	22.0	22.0	22.3
19	126.7	126.4	134.2	39	31.9	31.9	31.4
20	114.0	114.1	125.9	40	30.1	30.1	29.7

Shearinine C (**3**) was shown by ^{13}C NMR and HRFABMS to have the molecular formula $C_{37}H_{47}NO_7$. Carbon signals at 203.0 ppm and at 176.2 ppm suggested the presence of two carbonyl carbons not present in **2**, accounting for the additional oxygen atoms in the molecular formula of **3**. Many of the ^{13}C NMR shifts of **3** were similar to those of **2**, but the pattern of ^{13}C signals associated with the C-15 through C-21 portion of the molecule suggested an alteration in the indole subunit. Comparison of the ^{1}H and ^{13}C NMR data for **3** (see Tables 1 and 2) with those for the known compounds 2,18-dioxo-2,18-seco-paxilline (**8**)[10] and sulpinine C[2] indicated the presence of an eight-membered keto-amide central ring in shearinine C, presumably formed via oxidation of the indole C-2-C-18 bond of shearinine B. This was further confirmed by the FAB mass spectrum of shearinine C, which showed a characteristic ion at *m/z* 270 ($C_{17}H_{19}NO_2$ + H) resulting from cleavage through the eight-membered ring between C-17 and C-18, and through the amide bond, as was reported for compound **8**.[10] The connectivity of **3** and all ^{1}H and ^{13}C NMR assignments were verified by analysis of HMBC, HMQC, and COSY experiments.

A new paxilline analog was also obtained from the organic extracts of *E. shearii* ascostromata. A molecular formula of $C_{32}H_{41}NO_4$ for this metabolite (**4**) was deduced from ^{13}C NMR, DEPT, and LRFABMS data. Examination of the NMR data indicated that **4** is similar in structure to the known indole alkaloids paxilline (**5**) and paspalinine (**9**). Comparison of the ^1H and ^{13}C NMR spectra of **4** with those of **5**[10,11] suggested that compound **4** differs from **5** by the addition of an isopentenyl group at C-21. The structure of **4** and the corresponding ^1H and ^{13}C NMR assignments were determined by analysis of HMBC and HMQC data (see Experimental section). Key HMBC correlations between H-20 (7.21 ppm) and C-30 (34.5 ppm), and between H_2-30 (3.38 ppm) and carbon signals at 117.7, 133.3, and 121.5 ppm (C-20, 21, and 22) allowed unambiguous placement of the isopentenyl unit on the aromatic ring at C-21.

Five related known compounds, paxilline (**5**), 7-hydroxy-13-dehydroxypaxilline (**6**), 13-dehydroxy-paxilline (**7**), 2,18-dioxo-2,18-seco-paxilline (**8**), and paspalinine (**9**) were also isolated from *E. shearii* ascostromata. Identification of these compounds was accomplished by analysis of LRFABMS, 1D- and 2D-NMR (COSY and/or HMBC) data, and comparison with literature values.[7, 10-13] The structure of **9** was further verified by comparison to a standard isolated from *A. nomius*. Of these, only paxilline (**5**) had been previously reported as a metabolite of *E. shearii*.[14]

Interestingly, all of the compounds containing intact indole subunits (**1-2**, **4-7**, and **9**) gave FAB mass spectra wherein the M^+ ion was significantly more intense than the expected $(M + H)^+$ ion. This was found to be the case in several different FAB matrices, including glycerol, thioglycerol, 1:1 dithioerythritol-dithiothreitol, and 3-nitrobenzyl alcohol.

The dioxoindole unit in shearinine C (**3**) and in compound **8** clearly arises through oxidation of the corresponding indole-containing precursor. Despite frequent reports of compounds from the paspalinine class, the only prior report to discuss this type of oxidation product in detail described a non-enzymatic oxidative transformation which occurred upon exposure of ^{14}C-labelled paxilline (**5**) to either fresh or thermally-inactivated sheep bile.[10] In fact, this is the only prior literature report of the resulting product (**8**). Although this report stated that paxilline (**5**) did not undergo oxidation in water or phosphate buffer, our results indicate that compound **3** probably forms from **2** by an autoxidation process. Certain simple 2,3-disubstituted indoles are known to undergo autoxidation via a process that has been investigated.[15,16] In fact, simply stirring a pure sample of **2** in methanol in air for three days resulted in approximately 15% conversion to **3** based on HPLC analysis. Although none of the corresponding oxidized analog of **1** was isolated from the extract, this is most likely due to the complexity of the mixture. Indeed, treatment of **1** under the same conditions resulted in a comparable percent conversion to the corresponding oxidation product based on HPLC, UV, and FABMS data. The increasing number of reports of structures of this type[2,13,17] suggests that this oxidation process may be an important degradation (and, to some degree, detoxification) pathway for the paspalinine class of compounds, and is probably common among members of this class. As an additional control experiment, a freshly purified sample of paspalinine (**9**) was subjected to the same set of conditions, once again leading to formation of the expected oxidation product. Presumably, the somewhat strained nature of the unsaturated 5-5 ring-fusion, and the presence of an alkyl substituent at the indole C-2,[15,16] contributes to the susceptibility to oxidation among members of this class. Monosubstituted indoles that we have isolated in similar studies do not undergo oxidative ring-opening under these conditions. Surprisingly, paxilline (**5**) does not seem to undergo this transformation as readily as **1**, **2**, and **9**. Paxilline has very limited water solublility, and it was felt that this may

have impacted on the absence of observed decomposition under the conditions employed in the earlier report.[10] However, under conditions identical to those affording *ca.* 15% conversion of compounds **1**, **2**, and **9**, the extent of conversion from **5** to **8** was negligible; only a trace of oxidation product **8** could be detected by HPLC.

As summarized in Table 3, most of the compounds showed significant antiinsectan activity. Based on the discussion above, the samples of indole-containing compounds tested may have contained small amounts of the ring-opened analogs, at least by the time the assays were completed, but the numbers are intended to be viewed as approximations. For example, incorporation of shearinine A (**1**) into a standard pinto bean test diet caused an 89% reduction in weight gain of *H. zea* larvae relative to controls at a dietary concentration of 100 ppm. The same concentration caused a 69% reduction in feeding rate by the fungivorous beetle *C. hemipterus*. The ring-opened analogs **3** and **8** were significantly less active than their indole-containing counterparts in both assays, and the only compound lacking an OH group at C-13 (**7**) was essentially inactive at 100 ppm. Shearinine A (**1**) also showed activity in a topical assay against first instar larvae of *H. zea*, causing an 80% reduction in weight gain relative to controls when applied to the dorsa at 2 μg/insect. Shearinine B (**2**) and paxilline (**5**) were also tested for activity in a leaf disk assay against the fall armyworm *Spodoptera frugiperda*. Addition of 50 μg of either compound to a 75-mg cotton leaf disk fed to *S. frugiperda* larvae resulted in 84% mortality and 85% reduction in leaf damage relative to controls. The commercial insecticide malathion gives similar results, albeit at one-tenth of this level.

Table 3. Antiinsectan Activity of Shearinines A-C (**1-3**) and Related Compounds from *E. Shearii*.[a]

Compound	*H. zea* larvae (% RGR)	*C. hemipterus* larvae (% RFR)	Compound	*H. zea* larvae (% RGR)	*C. hemipterus* larvae (% RFR)
1	89	69[b]	**6**	65	38
2	94	50	**7**	0	12
3	33	19	**8**	17	19
4	55	0	**9**	75	62
5	83	38			

[a]Results were obtained upon feeding standard test diets containing 100 ppm of the compound to be tested. Values given are % RGR (% reduction in growth rate relative to controls) and % RFR (% reduction in feeding rate relative to controls). [b]Shearinine A also caused a 48% RGR against *C. hemipterus* in this assay.

The isolated yields of compounds **1-9** obtained from the *E. shearii* ascostromata were limited by the complexity of the mixtures, but TLC and HPLC analysis indicated that **1** and **2** are the major active components of the ascostromatal extracts. Quantitative analysis of ascostromata-producing petri plate cultures by HPLC using pure compounds as standards indicated that shearinines A and B were present in the ascostromata at concentrations ranging from 1500 to 1700 ppm. Compounds **3-9** were present at levels ranging from 200 to 800 ppm. Based on the antiinsectan activities described above, these metabolites are clearly present at levels that would present an effective chemical defense against the fungivorous beetle *C. hempiterus*. Interestingly, by analogy to earlier results with metabolites isolated from the sclerotia of *Aspergillus* spp., the antiinsectan

compounds from *E. shearii* are heavily concentrated in the ascostromata. For example, the ascostromata collected from a single petri plate of *E. shearii* contained a combined total of approximately 1500 μg of shearinines A and B, while the mycelium and agar from the same petri plate contained no more than 10 μg.

Several compounds related to shearinines A-C, the janthitrems, were previously reported from liquid cultures of *Penicillium janthinellum*, an organism associated with toxic ryegrass pastures.[4-6] None of the known janthitrems were isolated from *E. shearii* ascostromata in the present investigation. Recently, a janthitrem-like compound lacking the OH group at C-22, together with its oxidized (i.e., dioxoindole) analog, were reported in the patent literature as metabolites of another *Penicillium* sp.[17]

The occurrence of these antiinsectan metabolites in *Eupenicillium* ascostromata is analogous to the presence of unique antiinsectan compounds in the sclerotia of *Aspergillus* spp.[1-3] These results suggest that fungal ascostromata may be an equally promising source of new bioactive natural products.

EXPERIMENTAL

General. A culture of *E. shearii* Stolk and Scott (NRRL 3324; anamorph state = *Penicillium shearii* Stolk and Scott) was obtained from the Agricultural Research Service (ARS) Collection at the National Center for Agricultural Utilization Research in Peoria, IL. The ARS collection received this strain in 1968 as CBS 488.66 from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. The fungus was originally isolated by J. L. Renard from savannah soil collected near Abidjan, Ivory Coast, and was identified by A. C. Stolk.[18] Production of ascostromata was accomplished by solid substrate fermentation on autoclaved corn kernels using procedures described elsewhere for sclerotium production.[19] The ascostromata were harvested, ground to a powder using a Tecator mill (Perstorp Instrument Co.), and stored at 4°C until extraction. Analytical HPLC separations were accomplished using a Rainin Microsorb MV C_{18} column (5 μm particles, 4.6 mm x 25 cm) at 1.2 mL/min with UV detection at 254 nm. Preparative HPLC separations were accomplished using a Rainin Dynamax-60A, C_{18} column (8 μm particles, 21.4 mm x 25 cm) at 10 mL/min with UV detection at 254 nm. TLC employed silica gel (Merck Kieselgel 60 F254, 0.25 mm) eluting with 9:1 CH_2Cl_2-MeOH. TLC spots were visualized by exposure to UV light at 254 nm or to a vanillin/H_2SO_4 (1% w/v) spray reagent. ^1H NMR data was obtained at 300 or 600 MHz on Bruker AC-300 and AMX-600 spectrometers, respectively, and ^{13}C NMR data were obtained at 75.5 MHz. HMBC and HMQC data were obtained at 600 MHz (^1H dimension), and experiments were optimized for $^nJ_{CH}$ = 8.3 Hz and $^1J_{CH}$ = 150.2 Hz, respectively. All spectra were recorded in $CDCl_3$ and chemical shifts were referenced using the corresponding solvent signals at 7.24 ppm or 77.0 ppm. EIMS data were recorded at 70 eV using a VG Trio 1 quadrupole mass spectrometer, and HREIMS and FABMS (matrix = 3-NBA) experiments were performed on a VG ZAB-HF mass spectrometer. Details of bioassay procedures have been described previously.[20]

Isolation of Compounds 1-9. A sample of powdered *E. shearii* ascostromata (39 g) was extracted by stirring successively in hexane, CH_2Cl_2, EtOAc, and MeOH. The combined crude EtOAc and MeOH extracts (1.4 g) were preadsorbed onto 5 g of silica gel in a solution of 2:1 CH_2Cl_2-MeOH. This mixture was concentrated under vacuum and the resulting powder was subjected to silica gel vacuum liquid chromatography over a prepacked column bed (3.3 x 4 cm). The column was eluted using a stepwise gradient of MeOH (0 - 100%) in CH_2Cl_2, and a total of twelve 100 or 200 mL fractions were collected. The six fractions that eluted with 0% to 15% MeOH were combined (390 mg) and fractionated on Sephadex LH-20 (2 x 50 cm) with 1:1

CH_2Cl_2-MeOH. The resulting active fraction (125 mg) was separated further by silica gel chromatography (2 x 13 cm) using a linear gradient of MeOH (0 - 10%) in CH_2Cl_2. Fractions of similar composition as determined by TLC were pooled. The second and third fractions (11 mg and 10 mg, respectively) contained mixtures of less polar compounds with R_f values between 0.5 and 0.9. These two fractions were further purified by preparative reversed-phase HPLC (CH_3CN-H_2O) to afford compounds **1** (3.3 mg), **2** (3.5 mg), and **4** (4.1 mg).

The CH_2Cl_2 extract of the same 39 g ascostromatal sample (272 mg) was fractionated on Sephadex LH-20 (2 x 50 cm) with 3:1:1 hexane-toluene-MeOH. Fractions of similar composition as determined by TLC were combined. The resulting active fraction (28 mg) was further purified by preparative reversed-phase HPLC to yield additional amounts of compounds **1** (6.4 mg), **2** (6.6 mg), and **4** (3.4 mg).

A second powdered ascostromatal sample (320 g) was extracted by stirring successively in hexane, CH_2Cl_2, EtOAc, and MeOH. The combined crude CH_2Cl_2, EtOAc, and MeOH extracts (13.4 g) were subjected to fractionation procedures similar to those described above. Gel filtration on Sephadex LH-20 (3:1:1 hexane-toluene-MeOH) yielded compounds **6** (11.6 mg) and **9** (26.5 mg). After gel filtration and silica gel chromatography, other fractions of similar composition as determined by TLC were subjected to preparative reversed-phase HPLC (CH_3CN-H_2O and MeOH-H_2O) to yield compounds **3** (18.1 mg), **5** (65.1 mg), **7** (14.0 mg), and **8** (8.7 mg), as well as additional amounts of **1** (40.3 mg) and **2** (48.5 mg).

Shearinine A (1): white solid; mp >250° (dec.); $[\alpha]_D$ +16° (*c* = 0.0020 g/mL, $CHCl_3$); analytical HPLC t_R 17.2 min (80:20 CH_3CN:H_2O); UV (MeOH) 255 (ε 15000), 330 nm (12000); IR 3581, 3474, 3020, 2976, 2934, 2857, 1689, 1455, 1365 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations (H# => C#) H-1 => C-2, 18, 19, 31; H-5 => C-4, 6, 7, 13, 33; H-6 => C-4, 5, 7, 12; H-9 => C-7, 10, 11, 36; H-11 => C-7, 9, 12, 13; H-15 => C-13; H-17 => 2, 3, 16, 18; H-20 => C-18, 19, 22, 29, 31; H-22 => C-20, 21, 23, 24, 28, 29; H-23 => C-24, 28; H-27 => C-23, 26, 29; H-30 => C-19, 20, 21, 29, 31; H-32 => C-2, 3, 4, 16; H-33 => C-3, 4, 5, 13; H-35 => C-9, 34, 36; H-36 => C-9, 34, 35; H-37 => C-23, 24, 28; H-38 => C-23, 24, 37; H-39 => C-26, 27, 40; H-40 => C-26, 27, 39. FABMS *m/z* 583 (M^+, rel. int. 78), 568 (100), 526 (18), 510 (25); EIMS (70 eV) *m/z* 583 (M^+, rel. int. 28), 568 (100), 553 (2.6), 526 (34), 511 (53), 452 (23); HREIMS obsd. 568.3034 (M - CH_3), calcd. for $C_{37}H_{45}NO_5$ - CH_3, 568.3063; HRFABMS, obsd. 583.3250 (M^+), calcd. for $C_{37}H_{45}NO_5$, 583.3298.

Shearinine B (2): white solid; mp 165-170° (dec.); $[\alpha]_D$ -76° (*c* = 0.0020 g/mL, $CHCl_3$); HPLC t_R 16.0 min; UV (MeOH) 257 (ε 15000), 334 nm (12000); IR 3477, 2976, 2929, 2853, 1663, 1460, 1357 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations (H# => C#) H-1 => C-2, 18, 19, 31; H-5 => C-3, 4, 6, 7, 13, 33; H-6 => C-4, 5, 7, 12; H-7 => C-6, 11, 12; H-9 => C-7, 35, 36; H-11 => C-7, 9, 13; H-14 => C-4, 13, 15, 16; H-15 => C-3, 13, 14; H-16 => C-4; H-17 => C-2, 3, 15, 16, 18; H- 20 => C-18, 19, 22, 29, 31; H-22 => C-20, 21, 23, 24, 28, 29; H-23 => C-22, 24, 27, 28, 37, 38; H-27 => C-23, 26, 29, 40; H-30 => C-19, 21, 28; H-32 => C-2, 3, 4; H-33 => C-3, 4, 5, 13; H-35 => C-9, 34, 36; H-36 => C-9, 34, 35; H-37 => C-24, 38; H-38 => C-23, 24, 37; H-39 => C-26, 27, 40; H-40 => C-26, 27, 39; FABMS *m/z* 585 (M^+, rel. int. 27), 570 (79) 526 (8.7), 512 (18), 482 (12), 454 (6.0), 436 (4.9); EIMS (70 eV) *m/z* 527 (M - C_3H_6O, rel. int. 5.3), 512 (31), 494 (19), 454 (4.1), 436 (5.1); HRFABMS (thioglycerol), obsd. 585.3425 (M^+), calcd. for $C_{37}H_{47}NO_5$, 585.3454.

Shearinine C (3): pale yellow solid; mp 180-190° (dec.); $[\alpha]_D$ -146° (*c* = 0.0020 g/mL, $CHCl_3$); HPLC t_R 4.3 min; UV (MeOH) 246 (ε 14000), 299 nm (5900); ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations (H# => C#) H-1 => C-2, 3, 19, 30; H-5 => C-4, 6, 7, 13, 33; H-6 => 4, 5, 7; H-7 => 6, 11, 12; H-9

=> C-34, 35, 36; H-11 => C-7, 9, 13; H-14 => C-4, 13, 15; H-15 => C-13, 16; H-16 => C-3, 18; H-17 => C-3, 15, 16, 18; H-20 => C-18, 22, 29, 31; H-22 => C-21, 23, 24, 27, 28, 29; H-23 => C-22, 24, 27, 28, 37, 38; H-27 => C-23, 26, 29; H-30 => C-19, 21, 28, 31; H-32 => C-2, 3, 4, 16; H-33 => C-3, 4, 5, 13; H-35 => C-9, 34, 36; H-36 => C-9, 34, 35; H-37 => C-23, 24, 38; H-38 => C-23, 24, 37; H-39 => C-26, 27, 40; H-40 => C-26, 27, 39; FABMS *m/z* 618 [(M + H)$^+$; rel. int. 100], 602 (51), 582 (9), 558 (17), 542 (27), 531 (8.6), 490 (7.5), 339 (13); HRFABMS (glycerol), obsd. 618.3425 (M + H)$^+$, calcd. for $C_{37}H_{47}NO_7$ + H, 618.3431.

21-Isopentenylpaxilline (4): colorless oil; $[\alpha]_D$ = -12° (*c* = 0.0030 g/mL, $CHCl_3$); HPLC t_R 35.4 min; UV (MeOH) 236 (ε 9800), 283 nm (2500); ^{1}H NMR ($CDCl_3$) H-1, 7.63 (s); H_2-5, 2.75 (ddd; 13.8, 13.8, 5.0), 1.45 (m); H_2-6, 2.30 (m), 1.83 (m); H-7, 4.84 (m); H-9, 3.71 (br d; 1.8); H-11, 5.87 (d; 2.0); H_2-14, 2.05 (m), 1.64 (br d; 12.0); H_2-15, 2.03 (m), 1.77 (m); H-16, 2.82 (m); H_2-17, 2.70 (dd; 13.2, 6.3), 2.42 (dd; 13.2, 10.9); H-20, 7.21 (br s); H-22, 6.90 (dd; 8.3, 1.6); H-23, 7.18 (d; 8.3); H_3-25, 1.30 (s); H_3-26, 1.01 (s); H-27 (OH), 4.07 (br s); H_3-28, 1.26 (s); H_3-29, 1.28 (s); H_2-30, 3.38 (d; 7.3); H-31, 5.35 (m); H_3-33, 1.71 (s); H_3-34, 1.70 (s); ^{13}C NMR ($CDCl_3$) C-2, 151.8; C-3, 50.1; C-4, 43.2; C-5, 28.0; C-6, 28.5; C-7, 72.6; C-9, 83.3; C-10, 199.2; C-11, 119.6; C-12, 168.1; C-13, 77.0; C-14, 34.4; C-15, 20.9; C-16, 49.4; C-17, 27.2; C-18, 117.2; C-19, 124.6; C-20, 117.7; C-21, 133.3; C-22, 121.5; C-23, 111.3; C-24, 138.3; C-25, 16.2; C-26, 19.8; C-27, 72.4; C-28, 24.2; C-29, 26.6; C-30, 34.5; C-31, 124.6; C-32, 131.3; C-33, 17.8; C-34, 25.8; HMBC correlations ($CDCl_3$) (H-# => C-#) H-1 => C-2, 18, 19, 24; H_2-5 => C-4, 6, 7, 13, 26; H_2-6 => C-4, 5, 7, 12; H-7 => C-6, 12; H-9 => C-7, 28, 29; H-11 => C-7, 9, 13; H_2-14 => C-4, 13, 15, 16; H_2-15 => C-3, 13, 16; H-16 => C-14, 25; H_2-17 => C-2, 3, 15, 16, 18; H-20 => C-18, 22, 24, 30; H-22 => C-20, 24, 30; H-23 => C-19, 21; H_3-25 => C-2, 3, 4, 16; H_3-26 => C-3, 4, 5, 13; H_3-28 => C-9, 27, 29; H_3-29 => C-9, 27, 28; H_2-30 => C-20, 21, 22, 31, 32; H-31 => C-30, 33, 34; H_3-33 => C-31, 32, 34; H_3-34 => C-31, 32, 33; FABMS *m/z* 503 (M$^+$, rel. int. 8.6), 488 (7.2), 358 (5.4), 356 (8.8), 330 (6.5).

Paxilline (5): white solid; HPLC t_R 9.7 min; FABMS *m/z* 435 (M$^+$, rel. int. 100), 420 (63), 402 (10), 376 (11), 362 (11), 344 (15), 258 (21); other properties of **5** have been previously reported.[7,8,10]

7-Hydroxy-13-dehydroxypaxilline (6): white solid; $[\alpha]_D$ -20° (*c* = 0.0020 g/mL, $CHCl_3$); HPLC t_R 11.6 min; UV (MeOH) 240 (ε 10000), 282 nm (5300); ^{13}C NMR ($CDCl_3$) C-2, 149.3; C-3, 50.5; C-4, 42.8; C-5, 31.7; C-6, 36.9; C-7, 93.9; C-9, 76.9; C-10, 198.6; C-11, 122.2; C-12, 165.1; C-13, 41.9; C-14, 24.0; C-15, 25.5; C-16, 48.9; C-17, 27.2; C-18, 118.4; C-19, 124.9; C-20, 118.5; C-21, 119.7; C-22, 120.7; C-23, 111.5; C-24, 140.0; C-25, 14.6; C-26, 15.5; C-27, 72.5; C-28, 24.3 C-29, 26.3; FABMS *m/z* 435 (M$^+$, rel. int. 100), 420 (49), 395 (10), 376 (8.0), 360 (30), 346 (22), 332 (7.0), 272 (11), 259 (11); EIMS data and ^{1}H NMR assignments have been previously reported.[13]

13-Dehydroxypaxilline (7): white solid; HPLC t_R 15.8 min; FABMS *m/z* 419 (M$^+$, rel. int. 17), 406 (6.5), 382 (3.6), 360 (4.0), 339 (18), 257 (5.9); other properties of **7** have been previously reported.[11]

2,18-Dioxo-2,18-seco-paxilline (8): white solid; HPLC t_R 3.2 min; UV (MeOH) 238 (ε 9800), 367 nm (700); FABMS *m/z* 468 [(M + H)$^+$, rel. int. 11)], 450 (2.8), 408 (1.7), 382 (1.9), 339 (7.5), 219 (8.5); other properties of **8** have been previously reported.[10,11]

Paspalinine (9): white solid; $[\alpha]_D$ +112° (c = 0.0020 g/mL, $CHCl_3$); HPLC t_R 9.5 min; FABMS *m/z* 433 (M$^+$, rel. int. 100), 418 (36), 400 (7.8), 376 (17), 360 (6.6), 342 (8.0), 329 (9.9), 273 (31), 258 (2.8); other properties of **9** have been previously reported.[8,12]

Oxidation of Shearinine B (2) to Shearinine C (3). A sample of freshly purified shearinine B (**2**; 0.8 mg) was dissolved in 4.0 mL of MeOH, and the solution was stirred in an open vial for 72 h at rt. Analysis of the stirred solution by HPLC revealed the presence of a new, more polar component. This component was collected and identified as shearinine C (**3**) after ^{1}H NMR, FABMS, and UV comparison to an authentic sample.

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REFERENCES

1. Gloer, J. B.; Rinderknecht, B. L.; Wicklow, D. T.; Dowd, P. F. *J. Org. Chem.* **1989**, *54*, 2530.
2. Laakso, J. A.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *J. Org. Chem.* **1992**, *57*, 2066.
3. Staub, G. M.; Gloer, K. B.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Tetrahedron Lett.* **1993**, *34*, 2569.
4. deJesus, A. E.; Steyn, P. S.; vanHeerden, F. R.; Vleggaar, R. *J. Chem. Soc. Perkin Trans. I* **1984**, 697.
5. Wilkins, A. L.; Miles, C. O.; Ede, R. M.; Gallagher, R. T.; Munday, S. C. *J. Agric. Food Chem.* **1992**, *40*, 1307.
6. Penn, J.; Swift, R.; Wigley, L. J.; Mantle, P. G.; Bilton, J. N.; Sheppard, R. N. *Phytochemistry* **1993**, *32*, 1431.
7. Springer, J. P.; Clardy, J.; Wells, J. M.; Cole, R. J.; Kirksey, J. W. *Tetrahedron Lett.* **1975**, *30*, 2531.
8. Cole, R. J.; Dorner, J. W.; Lansden, J. A.; Cox, R. H.; Papa, C.; Confer, B. M.; Nicholson, S.S.; Bedell, D. M. *J. Agric. Food Chem.* **1977**, *25*, 1197.
9. Dorner, J. W.; Cole, R. J.; Cox, R. H.; Cunfer, B. M. *J. Agric. Food Chem.* **1984**, *32*, 1069.
10. Mantle, P. G.; Burt, S. J.; MacGeorge, K. M.; Bilton, J. N.; Sheppard, R. N. *Xenobiotica* **1990**, *20*, 809.
11. Nozawa, K.; Nakajima, S.; Kawai, K.; Udagawa, S. *J. Chem. Soc. Perkin Trans. I* **1988**, 2607.
12. Gallagher, R. T.; Finer, J.; Clardy, J.; Leutwiler, A.; Weibel, F.; Acklin, W.; Arigoni, D. *Tetrahedron Lett.* **1980**, *21*, 235.
13. Mantle, P. G.; Weedon, C. M. *Phytochemistry* **1994**, *36*, 1209.
14. Stolk, A. C.; Samson, R. A., Frisvad, J. C.; Filtenborg, O. In *Modern Concepts in Penicillium and Aspergillus Classification.* Samson, R. A. and Pitt, J. I., Eds.; Plenum: New York, 1990, pp. 121-137.
15. Witkop, B.; Patrick, J. B.; Rosenblum, M. *J. Am. Chem. Soc.* **1951**, *73*, 2641.
16. Leete, E. *J. Am. Chem. Soc.* **1961**, *83*, 3645.
17. Takagi, M.; Yoshida, S.; Yaguchi, T.; Imamura, K.; Okada, T. Jpn. Kokai Tokkyo Koho JP 06 65,246 [94 65,246], **1994**, Appl. 92/215,892 (*Chem. Abstr.* **1994**, *121*, 155915t).
18. Stolk, A. C.; Scott, D. B. *Persoonia*, **1967**, *4*, 391.
19. Wicklow, D. T.; Dowd, P. F.; TePaske, M. R.; Gloer, J. B. *Trans. Br. Mycol. Soc.* **1988**, *91*, 433.
20. Dowd, P. F. *Entomol. Exp. Appl.* **1988**, *47*, 69.

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