Sulpinines A-C and Secopenitrem B: New Antiinsectan Metabolites from the Sclerotia of *Aspergillus sulphureus*

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Extracts from the sclerotia of *Aspergillus sulphureus* have yielded four new antiinsectan compounds of the paspaline/penitrem class (2-5). These metabolites were isolated by a combination of chromatographic techniques. The structures were determined primarily by analysis of various NMR experiments and also by comparison to the structurally related penitrems. Sulpinine C contains an unusual eight-membered-ring lactam and possesses a ring system previously unreported as part of a natural product. The three indole metabolites (2, 3, and 5) exhibit potent activity against the lepidopteran crop pest *Helicoverpa zea*.

Fungal bodies called sclerotia are produced by many species of fungi. These propagules are resistant to extremes of temperature, desiccation, and nutrient poor conditions and therefore foster the long-term survival of the fungal species.[1-3] Because these structures may remain dormant in the soil for years, they may be exposed to a variety of fungivorous insects.[2,4] Some flowering plants allocate defensive substances preferentially to metabolically costly but reproductively significant structures (i.e. seeds).[5] By analogy to seeds, sclerotia may also possess defensive compounds not found in the fungal mycelium. We have isolated a variety of antiinsectan metabolites from sclerotia of *Aspergillus* spp.[2,3,6] In some cases, these compounds are found only in the sclerotia.[3,6] We recently reported four new indole diterpenoids (e.g. radarin A; 1) from the sclerotia of *Aspergillus sulphureus* (Fres.) Thom and Church (NRRL 4077).[7] Four additional new antiinsectan metabolites, sulpinines A-C (2-4) and secopenitrem B (5), as well as the known *Penicillium* metabolite penitrem B (6) have now been isolated from *A. sulphureus*. Details of the isolation and characterization of 2-5 are presented here.

Sclerotia from *A. sulphureus* were produced by solid substrate fermentation on corn kernels.[2] The methylene chloride extract of the harvested, ground sclerotia possessed exceptional antiinsectan activity against the agriculturally important lepidopteran *Helicoverpa zea*. This extract was fractionated by silica gel column chromatography. All five compounds described here were found within a distinct red band that eluted first from the column using increasing concentrations of methanol in chloroform up to 4%. Although this fraction was complex, the mixture could be separated directly into pure compounds by reversed-phase HPLC.

The major component of this fraction, sulpinine A (2), exerted potent effects on *H. zea*. The molecular formula was determined to be $C_{32}H_{41}NO_4$ by analysis of ^{13}C NMR and HREIMS data. The ^1H NMR spectrum and homonuclear decoupling experiments revealed the presence of a 1,2,4-trisubstituted benzene ring, an isoprene unit, and a series of four downfield-shifted proton signals in the region from 3.4 to 4.3 ppm, three of which formed an individual spin system. Examination of selective INEPT[8] and HMQC[9] results allowed assignment of the aromatic ring as that of an indole prenylated at C-6 and alkyl-substituted at C-2 and C-3. Comparison of the DEPT results and the molecular formula indicated that three exchangeable protons were present. The indole NH proton accounted for one of these. The other two must be protons of hydroxyl groups. Further structure elucidation was hampered by slow decomposition of sulpinine A in $CDCl_3$ solution, failure of various methylene protons to reveal one-bond correlations in HMQC experiments, an inability to acetylate more than one hydroxyl group, and difficulty in unambiguously defining the spin system involving the four downfield-shifted protons.

A second compound from this same fraction exhibited biological activity and spectral properties similar to those of sulpinine A. Analysis of ^{13}C NMR and mass spectral data suggested a molecular formula of $C_{37}H_{45}NO_5$. This compound was identified as the known compound penitrem B (6) by comparison of its spectral data with published values.[10] Penitrem B was originally isolated from *Penicillium palitans* (NRRL 3468) in 1971,[11] though its structure was not determined until 1983 (isolated from *P. crustosum*).[10] Five other analogues (penitrems A and C-F) were also reported from the *P. crustosum* isolate which differ from penitrem B in the degree of oxygenation and/or chlorination. These metabolites are related to other known tremorgens, such as paspaline,[12] paspalicine,[12] and paspalinine[13] from *Claviceps paspali*. Comparison of the spectral data for 6 and 2 greatly facilitated the structure elucidation of 2. Of particular utility was the presence of a set of NMR signals corresponding to the four down-

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0022-3263/92/1957-2066$03.00/0

Chart I

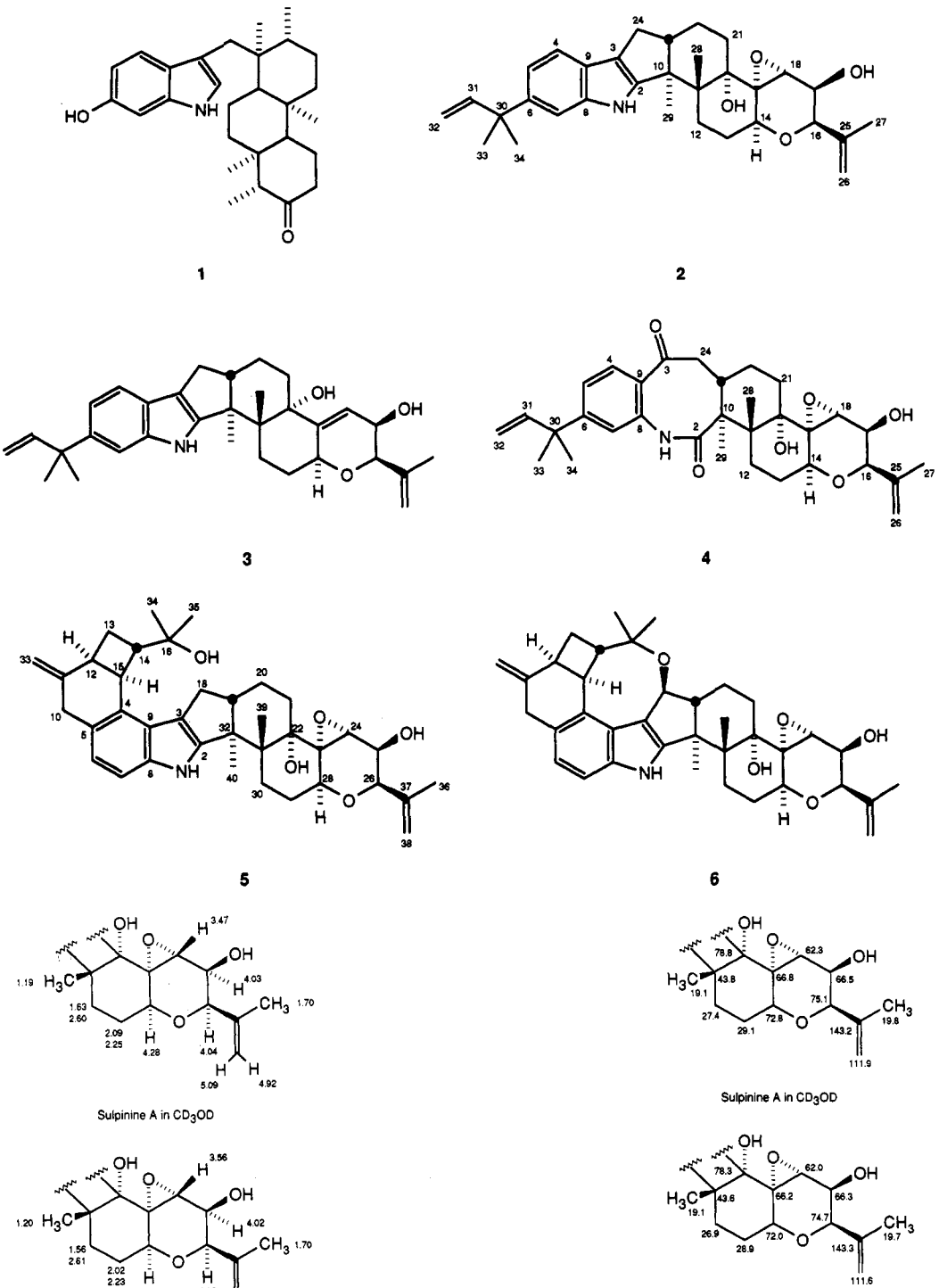

Figure 1. Comparison of proton NMR data of sulpinine A substructure with those of penitrem B.

Figure 2. Comparison of carbon NMR data of sulpinine A substructure with those of penitrem B.

field-shifted protons in sulpinine A. Proton and ^{13}C NMR assignments for sulpinine A are provided in Table I. Chemical shift data for the relevant portions of the two molecules are presented in Figures 1 and 2. The long-range correlation between H-14 and H-18 and the lack of any appreciable vicinal coupling between H-16 and H-17 rationalized the initial difficulties encountered in assigning the connectivity of this unit. The failure of the acetylation procedure to derivatize both hydroxyl group functionalities was explained by the hindered tertiary hydroxyl group at C-20. The remaining assignments (Table I) were straightforward due to the skeletal similarities between sulpinine A and the right-hand portion of penitrem B, establishing the structure of sulpinine A as **2**.

A third component of the same column fraction, sulpinine B (**3**), possessed biological activity of the same magnitude as **2** and **6**. The molecular formula of this structurally similar compound was found to contain one less oxygen atom than sulpinine A by analysis of ^{13}C NMR and HREIMS data. The EI mass spectrum yielded a base

Table I. NMR Spectral Data for Sulpinine A (2)[a]

C/H no.	^{1}H	^{13}C	selective INEPT correlations
2	---	153.9	
3	---	116.7	
4	7.20 (d; 8.3)	118.1	3, 6, 8, 9
5	6.95 (dd; 8.3, 1.7)	118.6	7, 9, 30
6	---	142.0	
7	7.28 (d; 1.3)	110.0	5, 6, 8, 9, 30
8	---	141.2	
9	---	124.4	
10	---	52.0	
11	---	43.8	
12	1.63 (m), 2.60 (m)	27.4	
13eq	2.09 (m)	29.1	12, 14
13ax	2.25 (m)		
14ax	4.28 (br dd; 9.0, 8.8)	72.1	13, 18, 19
16ax	4.04 (br s)	75.1	
17eq	4.03 (br d; 3.1)	66.5	
18eq	3.47 (br d; 2.0)	66.3	16, 17, 19
19	---	66.8	
20	---	78.8	
21	1.37 (m), 1.60 (m)	27.2	
22eq	1.49 (dm; 10.0)	21.9	
22ax	1.89 (dddd; 12, 12, 12, 3)		10, 23
23ax	2.69 (m)	51.5	2, 10, 11, 22, 29
24	2.32 (m), 2.56 (m)	30.5	
25	---	143.2	
26	4.91 (br s), 5.10 (br s)	111.9	
27	1.70 (br s)	19.8	
28	1.14 (s)	19.1	10, 11, 12, 20
29	1.26 (s)	16.5	2, 10, 11, 23
30	---	42.1	
31	6.07 (dd; 17.5, 10.6)	150.5	6, 30, 33, 34
32	4.97 (dd; 10.6, 1.5)	110.2	
	5.07 (dd; 17.5, 1.5)		
33	1.41 (s)	29.3	
34	1.41 (s)	29.3	

[a] Data were recorded in methanol-d_4 at 600 and 75.6 MHz, respectively. All selective INEPT correlations represent 2- or 3-bond couplings.

Table II. NMR Spectral Data for Sulpinine B (3)[a]

C/H no.	^{1}H	^{13}C	selective INEPT correlations
1	7.72 (br s)	---	2, 3, 8, 9
2	---	152.2	
3	---	116.9	
4	7.34 (d; 8.3)	117.9	3, 6, 8, 9
5	7.07 (dd; 8.3, 1.5)	118.5	7, 9, 30
6	---	141.2	
7	7.27 (d; 0.8)	108.9	5, 6, 8, 9, 30
8	---	139.8	
9	---	123.1	
10	---	50.7	
11	---	42.9	
12	2.68 (dm; 6.0), 1.59 (m)	28.2	
13	2.23 (m), 1.84 (m)	29.3	
14	4.61 (br dd; 9.1, 8.8)	73.7	12, 18, 19
16	3.86 (br s)	79.1	14, 25, 26, 27
17	3.96 (br d; 5.6)	62.8	18, 19, 25
18	5.82 (br d; 4.7)	118.7	14, 16, 17, 20
19	---	148.1	
20	---	77.7	
21	1.71 (m), 1.34 (m)	27.3	
22	2.06 (m), 2.02 (m)	21.1	
23	2.80 (m)	49.7	
24	2.65 (dm; 4.4)	34.7	
	2.39 (dd; 12.9, 12.9)		2, 3
25	---	141.6	
26	5.20 (br s)	111.7	16, 25, 27
	5.03 (br s)		
27	1.79 (br s)	19.8	16, 25, 26
28	1.10 (s)	20.2	10, 11, 12, 20
29	1.28 (s)	16.2	
30	---	41.1	
31	6.05 (dd; 17.4, 10.6)	148.8	6, 30, 33, 34
32	5.06 (dd; 17.3, 1.2)	110.0	
	4.99 (dd; 10.6, 1.2)		
33	1.41 (s)	28.6	
34	1.41 (s)	28.6	

[a] Data were recorded in $CDCl_3$ at 600 and 75.6 MHz, respectively. All selective INEPT correlations represent 2- or 3-bond couplings.

peak of m/z 250 which was the same base peak found in the mass spectrum for sulpinine A. This fragmentation arises from cleavage of the C-21/C-22 and the C-10/C-11 bonds. The intensity and identity of this ion has been noted for other compounds with similar skeletons, such as aflatrem.[14] Comparison of the ^{1}H NMR spectrum of sulpinine B to that of sulpinine A confirmed that the spin systems for the substituted indole and isoprene units were intact. The three other methyl singlets and two other terminal methylene protons were also still present. Sulpinine B contained only three proton signals in the region from 3.25 to 4.75 ppm, rather than four. There was also a new doublet at 5.82 ppm. This information, coupled with the changes that were evident in the carbon spectrum—two oxygenated carbon signals were replaced by two vinylic carbon signals—suggested that the epoxide functionality was replaced by a carbon–carbon double bond. This was confirmed by COSY, HMQC, and a series of selective INEPT experiments (Table II), which permitted assignment of the structure of sulpinine B as **3**. In support of this spectral evidence, the known penitrems C and D also possess a similar unit with a C–C double bond in place of the epoxide.[10] Comparison of the ^{13}C NMR chemical shifts for the pyran ring of penitrem C to those of the corresponding carbons of sulpinine B yielded values which were all within 1.0 ppm of each other. The only discrepancy was that the shift assignments for carbons 14 and 16 were reversed. In penitrem C, the chemical shifts for the carbons corresponding to positions 14 and 16 of **3** were reported as 80.38 and 74.36 ppm, respectively. However, in selective INEPT experiments optimized for 7 Hz, irradiation of H_3-27 and H_a-26 afforded polarization transfer to 79.1. Thus, this signal must correspond to C-16. Furthermore, irradiation of H-16 showed a correlation to 73.7, confirming the assignment for this signal as C-14.

A fourth metabolite from this column fraction, sulpinine C ($C_{32}H_{41}NO_6$), differed from **2** and **3** only in the presence of additional oxygen atoms. However, the EI mass spectrum did not contain an intense peak at m/z 250. Cursory examination of the ^{1}H and ^{13}C NMR spectra revealed many similarities to sulpinine A, including the familiar subunit depicted in Figures 1 and 2, a 1,2,4-trisubstituted benzene ring, the isoprene unit, and the three other methyl groups. However, the eight carbon signals expected for an indole were not all present. Instead, two new carbonyl signals appeared at 176.6 and 202.4 ppm and the NH proton shift was significantly different from the values for **2** and **3**.

An explanation for these differences was obtained upon examination of the spectral data presented in Table III. The assignments for the benzene ring including the 6-prenyl unit were easily made through analysis of HMQC and HMBC[15] experiments. In addition, H-4 displayed a correlation to the signal at 202.4 ppm (C-3). This ketone carbon must therefore be connected to the benzene ring at C-9. The NH proton (H-1) was correlated to C-7 and

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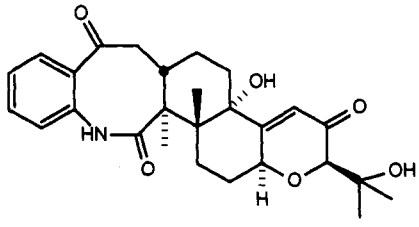
C-9 of the benzene ring, confirming attachment of the nitrogen to C-8. This proton was also correlated to the 176.6 ppm carbon and to a quaternary carbon at 57.5 ppm (C-10). The carbonyl signal at 176.6 ppm cannot be a ketone and all other oxygen atoms in the molecule are assigned elsewhere, eliminating the possibility of an ester or acid functionality. Therefore, this carbonyl (C-2) must be attached directly to the nitrogen to form an amide group, and C-10 must be linked to C-2. The presence of an amide group was confirmed by a new IR absorption at 1647 cm^{-1}. The methyl group at 1.55 ppm (H_3-29) must be attached to the quaternary carbon (C-10) adjacent to the amide group, as it showed a correlation to the amide carbonyl carbon C-2. This methyl also correlated with C-23. The proton attached to C-23 shows correlations to every carbon that is two or three bonds away from it, including the two carbonyl carbons. The connection of C-24 to C-3 was based on HMBC correlations of the protons on C-24 to C-3 and C-9. The final structure could then be assigned as the unusual eight-membered-ring lactam **4**. This metabolite appears to be a biological oxidation product of sulpinine A. Autoxidation seems less likely because subjecting sulpinine A to the extraction and isolation conditions does not result in any discernible conversion to sulpinine C. Because of this relationship, and to facilitate spectral comparisons, sulpinine C bears the same numbering system as sulpinines A and B even though C-2 and C-3 are no longer connected. Interestingly, a recent publication reported the isolation of a compound from the incubation of paxilline and sheep bile that is structurally similar to sulpinine C.[16] This biotransformation product, 2,18-dioxo-2,18-*seco*-paxilline (**7**), is believed to be the result of a biological detoxification mechanism.



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A final new compound isolated from this mixture, secopenitrem B (**5**), had a molecular formula indicating one less unsaturation than penitrem B. By examination of ^{13}C and ^{1}H NMR data, it was clear that the right-hand three-ring subunit was intact in secopenitrem B as in penitrem B and sulpinines A and C. Evidence for one less oxygenated carbon atom in the ^{13}C NMR spectrum suggested that the ether linkage between C-16 and C-18 was severed. Specifically, the oxygenated methine carbon at 72.1 ppm was apparently replaced by a new methylene carbon at 29.4 ppm. Analysis of the results of an HMBC experiment confirmed this hypothesis. For example, the methine proton H-19 shows correlations in HMBC experiments to three methylene carbons: C-18, 20, and 21. The correlation of H-19 to the new methylene carbon signal at 29.8 ppm (C-18) together with the fact that one proton attached to C-18 shows correlations to indole carbons C-2 and C-3 as well as to C-32 provided corroboration for the assignment of secopenitrem B as **5**. The numbering system reported for penitrem B[10] was retained in the numbering of secopenitrem B.

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The relative stereochemistries assigned for **2–5** were deduced partly by comparison to the reported stereochemistry of penitrem B and also by analysis of NOESY data. Because the proton and carbon chemical shifts of sulpinines A and B and secopenitrem B are in such close agreement with those published for the penitrems, it is proposed that these new compounds have the same stereochemistry as the penitrems. NOESY data for these compounds are in agreement with this proposed stereochemistry. The additional oxygenation present in sulpinine C precluded direct chemical shift comparisons for atoms in the vicinity of the oxygenation. However, NOESY data for sulpinine C (Table III) suggest that the relative stereochemistry of the three intact rings is the same as that proposed for sulpinine A. A correlation between H-23ax and H_3-28 represents a 1,3-diaxial interaction and supports the presumption that the eight-membered ring is trans fused to the six-membered ring. A NOESY interaction between H_3-29 and H-24a suggests that H-24a is in a pseudoaxial position below the face of the ring. H-24b has a NOESY correlation with H-22eq which would be unlikely were it not in a pseudo-equatorial disposition. The NH proton (H-1) shows a NOESY correlation to H_3-29 and therefore must be below the face of the ring. Thus, the relative stereochemistry of sulpinine C was assigned as depicted in **4**. The NMR data and Dreiding molecular models suggest that compound **4** most likely adopts a conformation in which the nitrogen and carbons 3, 8, 9, and 24 are nearly coplanar, with C-2, C-10, and C-23 forming a parallel plane.

The absolute stereochemistry of penitrem B was originally determined by the partial resolution method of Horeau.[10] Because the CD data for the penitrem B isolated from *A. sulphureus* matched the published data for penitrem B, the absolute stereochemistry must be the same. The four new compounds isolated from *A. sulphureus* most likely possess the same absolute stereochemistry, as depicted in **2–5**.

Aside from the unusual ring-opened structure of sulpinine C, all three sulpinines differ from the paspaline family of metabolites in the pattern of oxygenation of the right-hand portion of the molecule, and in the presence of a prenyl substituent at C-6. All five compounds are active against the first instar larvae of the corn earworm, *H. zea*. Sulpinine A possesses the most potent activity. A 96.0% reduction in weight gain relative to controls after one week was noted for this compound when incorporated into a standard *H. zea* test diet at 100 ppm. A 10% mortality rate was also observed in this assay. Penitrem B, sulpinine B, and secopenitrem B brought about similar weight gain reductions (87.4%, 87.2%, and 87.0%, respectively), while secopenitrem B also caused 32.0% larval mortality. These values are comparable with those obtained for nominine[6] and the commercial pesticide permethrin. Interestingly, the ring-opened sulpinine C had significantly less impact in reducing larval weight gain (26.5%). However, sulpinine C induced a moderate feeding reduction of 32.0% in the adults of the fungivorous dried fruit beetle *Carpophilus hemipterus* at 100 ppm.[2] None of the other compounds deterred feeding in *C. hemipterus* at this level. Sulpinine A and secopenitrem B also possess significant topical activity against *H. zea*. In these assays, 2 μg of the metabolite in 0.2 μL of acetone per 2 mg larva was administered to the dorsum of the larvae, which were then fed the standard pinto bean diets. Under these conditions, sulpinine A induced a 54.5% reduction in weight gain relative to controls, and secopenitrem B caused a 66.2% reduction in weight gain. Sulpinine A exhibits

Table III. NMR Spectral Data for Sulpinine C (4)[a]

	^{1}H	^{13}C	HMBC correlations	NOESY correlations
1	7.13 (br s)	--	2, 7, 9, 10	7, 29
2	---	176.6		
3	---	202.4		
4	7.62 (d; 8.1)	129.4	3, 5, 6, 8	
5	7.32 (dd; 8.1, 1.8)	125.3	4, 9, 30	33, 34
6	---	154.5		
7	6.99 (d; 1.7)	125.0	3,[b] 5, 8, 9, 30	1, 31, 33, 34
8	---	136.8		
9	---	131.6		
10	---	57.5		
11	---	43.4		
12ax	2.41 (m)	24.1	11, 13, 14, 28	14ax, 29
12eq	1.89 (d m; 11.4)		11, 13, 14, 20	28
13ax	1.70 (m)	27.3	12, 14	28
13eq	2.25 (m)		11, 14, 19	
14ax	4.19 (dd; 9.3, 9.0)	71.1	13, 18, 19	16ax
16ax	4.04 (br s)	73.4	14, 25, 26, 27	14ax, 17eq, 27
17eq	3.96 (br d; 1.9)	64.6	18, 19	16ax, 18, 27
18	3.47 (br d; 2.3)	61.8	16, 17	21ax, 21eq, 28
19	---	65.5		
20	---	77.1		
20(OH)	1.45 (br s)	--		22ax, 29
21ax	1.21 (m)	28.1		18, 28
21eq	1.34 (m)			18
22ax	1.76 (dd m; 13.4, 3.4)	24.4	10, 21, 23	20(OH), 29
22eq	1.34 (m)			
23ax	2.99 (m)	36.1	2, 3, 10, 11, 21, 22, 24, 29	28
24a	3.05 (dd; 17.8, 4.4)	47.7	3, 9, 10, 22, 23	29
24b	2.40 (dd; 17.8, 4.0)		3, 9, 10, 22, 23	22eq
25	---	140.9		
26a	5.13 (br s)	112.5	16, 25, 27	
26b	5.00 (d; 1.6)		16, 27	27
27	1.68 (br s)	19.4	16, 25, 26	16ax, 17eq, 26b
28	0.99 (s)	18.7	10, 11, 12, 20	12eq, 13ax, 18, 21ax, 23ax
29	1.55 (s)	16.5	2, 10, 11, 23	1, 12ax, 20(OH), 22ax, 24a
30	---	41.3		
31	5.93 (dd; 17.4, 10.6)	146.4	6, 30, 33, 34	7, 33, 34
32a	5.09 (dd; 10.6, 0.9)	112.1	30, 31	33, 34
32b	5.06 (dd; 17.4, 0.9)		30, 31	33, 34
33	1.37 (s)	28.0	6, 30, 31, 34	5, 7, 31
34	1.37 (s)	28.0	6, 30, 31, 33	5, 7, 31

[a] Data were recorded in $CDCl_3$ at 600 and 75.6 MHz, respectively. [b] This correlation represents a 4-bond coupling; all other HMBC correlations represent 2- or 3-bond couplings. NOESY correlations between measurably scalar coupled protons have been omitted.

cytotoxicity towards human lung carcinoma A549, breast adenocarcinoma MCF7, and colon adenocarcinoma HT-29 cells with ED_{50} values of 25.7, 58.1, and 3.7 μg/mL, respectively.[17]

Experimental Section

General. The culture of *A. sulphureus* (NRRL 4077) was obtained from the Agricultural Research Service (ARS) Collection at the National Center for Agricultural Utilization Research in Peoria, IL. Production of sclerotia was accomplished by solid substrate fermentation on autoclaved corn kernels using procedures described elsewhere.[2] The sclerotia were harvested, ground to a powder using a Tecator mill (Perstorp Instrument Co.), and stored at 4 °C until extraction. Proton NMR and HMBC data were obtained at 600 MHz. Carbon-13 NMR data were obtained at 75.0 or 90.7 MHz. HMQC experiments were recorded at 300 or 600 MHz (^{1}H dimension). All spectra were recorded in $CDCl_3$, acetone-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, or 3.30 ppm/49.0 ppm, respectively. Multiplicities of carbon signals were determined through DEPT experiments, and all carbon assignments made are consistent with the DEPT results. 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 were accomplished using a Beckman Ultrasphere ODS column (5-μm particles, 250 × 10 mm) at a flow rate of 2.5 mL/min with UV detection at 215 nm. Details of bioassays and other experimental procedures have been described previously.[3,6,18]

Isolation and Characterization of 2–6. Powdered sclerotia of *A. sulphureus* (150.0 g) were 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 and collection of a distinct red band at 4% MeOH. This active fraction (105.2 mg) was further separated by reversed-phase HPLC (89:11 MeOH–H_2O) to yield **2** (18.0 mg), **3** (4.1 mg), **4** (4.0 mg), **5** (10.2 mg), and **6** (4.3 mg).

Sulpinine A (**2**) was isolated as a yellow-orange solid with the following properties: mp 149–152 °C dec; $[\alpha]_D$ −29.6° (*c* 0.005 g/mL, $CHCl_3$); HPLC t_R 16.0 min; UV (MeOH) 235 (ε 22 600), 283 nm (5600); IR 3473, 2933, 2873, 1457, 1371, 1043, 911, 756 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and selective INEPT data, Table I; EIMS (70 eV) 503 (M^+, rel int 32), 488 (43), 467 (12), 452 (13), 434 (10), 397 (10), 373 (24), 358 (29), 250 (100), 222 (35), 198 (33), 182 (72), 167 (21); HREIMS obsd 503.3039, calcd for $C_{32}H_{41}NO_4$ 503.3036.

Sulpinine B (**3**): orange oil; $[\alpha]_D$ −43.2° (*c* 0.003 g/mL, $CHCl_3$); HPLC t_R 13.8 min; ^{1}H NMR, ^{13}C NMR and selective INEPT data, Table II; EIMS (70 eV) 487 (M^+, rel int 21), 472 (16), 469 (10), 451 (26), 436 (11), 250 (100), 198 (34), 182 (71), 167 (23), 129 (45), 115 (28), 105 (21); HREIMS obsd 487.3104, calcd for $C_{32}H_{41}NO_3$ 487.3086.

Sulpinine C (**4**): yellow oil; $[\alpha]_D$ +17.1° (*c* 0.003 g/mL, $CHCl_3$); HPLC t_R 6.0 min; UV (MeOH) 229 (ε 25 600), 262 nm (18 200); IR 3483, 2965, 2872, 1685, 1647, 1603, 1391, 756 cm^{-1}; ^{1}H NMR, ^{13}C NMR, HMBC, and NOESY data, Table III; EIMS (70 eV) 535 (M^+, rel int 13), 254 (3), 242 (3), 228 (3), 214 (11), 200 (4), 188 (100), 172 (5), 145 (9), 130 (9), 115 (5), 105 (6); HREIMS obsd 535.2945, calcd for $C_{32}H_{41}NO_6$ 535.2934.

Secopenitrem B (**5**): orange-brown solid; mp 185–189 °C dec; $[\alpha]_D$ −5.2° (*c* 0.005 g/mL, $CHCl_3$); HPLC t_R 8.5 min; EIMS (70 eV) 585 (M^+, rel int 4), 567 (11), 552 (17), 531 (6), 516 (7), 499 (84), 484 (40), 437 (33), 422 (35), 369 (32), 354 (26), 314 (70), 301 (31), 246 (100), 233 (37), 194 (38); ^{1}H NMR ($CDCl_3$) H-6, 6.88 (d; 8.1); H-7, 7.07 (d; 8.1); H-10, 3.90 (br d; 15.1), 3.40 (d; 15.3); H-12, 3.15 (dd; 8.9, 8.8); H-13, 2.33 (m), 1.85 (m); H-14, 2.48 (m); H-15, 4.08 (dd; 8.5, 8.7); H-18, 2.99 (dd; 13.0, 6.1), 2.47 (m); H-19, 2.75 (m); H-20, 1.89 (m), 1.62 (m); H-21, 1.53 (m), 1.50 (m); H-24, 3.64 (d; 2.3); H-25, 4.03 (br s); H-26, 4.10 (br s); H-28, 4.30 (dd; 9, 9); H-29, 2.36 (m), 1.93 (m); H-30, 2.67 (ddd; 13.6, 13.6, 5.1), 1.33 (m); H-33, 4.83 (br s), 4.72 (br s); H_3-34, 1.16 (s); H_3-35, 1.07 (s); H_3-36, 1.72 (br s); H-38, 5.16 (br s), 5.02 (br s); H_3-39, 1.163 (s); H_3-40, 1.175 (s); ^{13}C NMR ($CDCl_3$) C-2, 152.0; C-3, 116.3; C-4, 131.3; C-5, 129.7; C-6, 121.1; C-7, 109.3; C-8, 123.4; C-9, 138.9; C-10, 37.6; C-11, 149.2; C-12, 36.5; C-13, 25.8; C-14, 52.3; C-15, 37.0; C-16, 71.4; C-18, 29.4; C-19, 49.9; C-20, 20.5; C-21, 30.2; C-22, 78.0; C-23, 65.8; C-24, 61.8; C-25, 64.7; C-26, 73.6; C-28, 71.5; C-29, 27.7; C-30, 27.4; C-31, 42.4; C-32, 50.2; C-33, 108.3; C-34, 26.5; C-35, 27.3; C-36, 19.5; C-37, 141.1; C-38, 112.3; C-39, 18.6; C-40, 15.6; HRFABMS obsd 586.3589, calcd for $C_{37}H_{47}NO_5$ + H 586.3532.

Penitrem B (**6**): $[\alpha]_D$ −4.9° (*c* 0.005 g/mL, $CHCl_3$); HPLC t_R 20.1 min; other properties of penitrem B have been previously reported.[10]

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Supplementary Material Available: ^1H NMR spectra for sulpinines A and C and secopenitrem B and a ^{13}C NMR spectrum of sulpinine B (4 pages). Ordering information is given on any current masthead page.

Synthesis and Structural Determination of (±)-Neplanocin F

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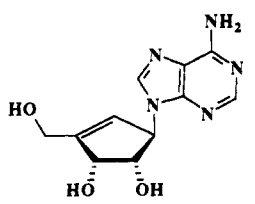
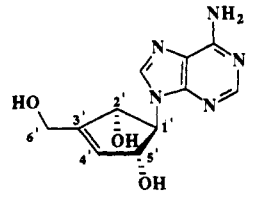
Neplanocin F (**2**), a minor constituent of the family of neplanocin antibiotics, was synthesized as a racemate in 12 steps from cyclopentenone **3**/**4**, which in turn was available from D-ribonolactone. The carbocyclic ring of neplanocin F corresponds to the allylic rearranged isomer of the biologically active agent neplanocin A. Regiospecific reduction of the racemic cyclopentenone **3**/**4** and protection of the resulting α-alcohol as a benzyl ether **6** produced, after removal of the isopropylidene moiety, a compound (**7**) having allylic and homoallylic secondary alcohol functionalities. Differences in the reactivity of these two secondary alcohols were successfully manipulated to prepare the homoallylic substituted azide **14**, which was then reduced and converted to the desired adenine ring by conventional methods. The spectral properties of the synthesized material [(±)-neplanocin F] were identical to those of the natural product, except for its lack of optical rotation. X-ray crystallographic analysis helped corroborate the structure. In contrast to its bioactive isomer, neoplanocin A, neplanocin F was devoid of cytotoxicity and in vitro antiviral activity.

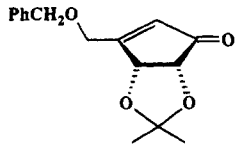
Synthetic modifications of the naturally occurring carbocyclic nucleoside neplanocin A (**1**) have, in some instances, resulted in analogues with superior antitumor and antiviral activities relative to the parent compound.[1-3] Of the various neplanocin analogues isolated from *ampullariella regularis* A11079,[4] neplanocin A has received wide attention because of its interesting biological properties.[5-7] In contrast, the other minor metabolites of the neplanocin family have received less attention, perhaps due to the lack of adequate amounts for testing. Based on this premise, we embarked on a project aimed at providing a suitable synthetic route to one of these minor metabolites, neplanocin F (**2**), which is an allylic rearranged isomer of neplanocin A.[8] Some of these results have been briefly reported in a previous communication from this laboratory,[9] and in the present paper we present a detailed account of this work. In addition, we provide additional crystallographic evidence for the correct structure of neplanocin F in order to resolve a discrepancy that exists between two published communications[8,10] and the patent literature[11] regarding the stereochemistry of the 2′-hydroxyl group.



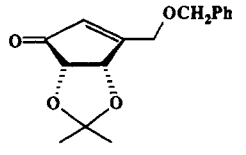
1 (Neplanocin A)



2 (Neplanocin F)



3



4

Results and Discussion

During the scale-up synthesis of (−)-neplanocin A, the desired cyclopentenone enantiomer **3** was efficiently separated from smaller quantities of the racemate which consists of a mixture of **3** and **4**.[12] As this process was repeated several times for the syntheses of other cyclopentenyl nucleosides, significant quantities of the racemate were accumulated. For the synthesis of natural neplanocin F, the mirror image enantiomer **4** appeared to be a most suitable starting material since the double bond would appear moved to the desired rearranged position after appropriate addition of the base to the carbocyclic moiety

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